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Structural specialities, curiosities and record-breaking features of crustacean reproduction

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ABSTRACT

Crustaceans are a morphologically, physiologically and ecologically highly diverse animal group and correspondingly diverse are their reproductive characteristics. They have evolved structural specialities with respect to penis construction, sperm form, sperm storage, mode of fertilization and brood care. Unique in the animal kingdom are safety lines that safeguard hatching and first moulting of the offspring. Further curiosities are dwarf males in parasitic and sessile species and the induction of feminization, castration and gigantism by parasites and bacteria. Record-breaking features are relative penis length, clutch size, sperm size, chromosome number, viability of resting eggs and fossil ages of penis, sperm and brooded embryos. This article reviews and illustrates these structural reproductive peculiarities and curiosities and discusses implications for basic and applied biological topics including sperm competition, posthumous paternity, fitness enhancement of the young, reconstruction of pristine ecosystems prior to man-made pollution, population change by infectious feminization and sustainable male-based fishery.

Key words: Crustacea, penis, sperm, multiple paternity, brood care, safeguarding of hatching, infectious feminization, gigantism, dwarf males, diapause, fossils.

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I. INTRODUCTION

Crustacea is a phylogenetically very old animal group that is renowned for its exceptional diversity in morphology, physiology, life history and ecology. The crustaceans date back to the Early Cambrian some 520 million years ago and thus had ample time for experimentation with form and function (Sepkoski, 2000; Harvey *et al.*, 2012). They include six classes, 42 orders, 849 extant families and more than 52,000 described species (Martin & Davis, 2001). Maximum body length of the adults ranges from about 0.1 mm in a tantulocarid ectoparasite to more than 1 m in the American lobster (including chelae). Crustaceans occur in marine, limnic and terrestrial habitats from the polar regions to the tropics and from lowlands to high mountains. Most crustaceans are free living but some taxa are sessile or parasitic (Martin & Davis, 2001). Because of this enormous diversity there are a lot of special adaptations in this animal group.

The most curious adaptations in the animal kingdom have evolved in context with reproduction, particularly with respect to behaviour. Examples are the sexual cannibalism in praying mantis females, which sometimes eat the males during mating (Lawrence, 1992), and

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brooding in megapodid birds, which incubate their eggs by heat generated from microbial decomposition or volcanism (Dekker & Brom, 1992). However, there are also reproductive specialities and curiosities related to morphology and anatomy such as the antlers of deer (Price *et al.*, 2005), the love darts of gastropods (Chase & Blanchard, 2006) or the occurrence of three vaginas in kangaroos (Dawson, 1995).

The present article highlights structural specialities, curiosities and record-breaking features of reproduction in crustaceans and emphasizes their impact on general biological issues. The topics discussed include inflatable and permanently stiff copulatory organs, giant sperm and explosion sperm, safeguarding of hatching and first moult by safety lines, manipulation of sex and body size by parasites, and records in relative penis length, chromosome number, viability of resting eggs and fossil ages of reproductive structures.

II. EXTENSIBLE VERSUS PERMANENTLY STIFF COPULATORY ORGANS

Most crustaceans transfer sperm by copulatory organs. This evolutionary legacy has led to some curiosities in sessile and parasitic groups as explained further down. In this chapter, I want to compare two different construction principles of copulatory organs, the extensible hydraulic penis in sessile Cirripedia and the form-invariant and permanently stiff copulatory organs in Decapoda.

Cirripedia are among the few sessile animals that use a penis for sperm transfer (Fig. 1A-C) (Barnes, 1992). They are hermaphrodites but usually do not fertilize themselves. Their unpaired penis is greatly extensible and well suited for sweeping movements to search for functional females in the surroundings (Fig. 1B). It is extended and retreated by modulation of the turgor pressure in the longitudinally running inflatable haemolymph channels (Fig. 1D) (Klepal *et al.*, 1972). The cuticle of the penis is thin and annulated (Fig. 1C, D) facilitating length variation and its structure is highly complex combining mechanical strength with

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flexibility (Klepal *et al.*, 2010). Searching movements are enabled by a compact layer of longitudinal musculature underneath the epidermis (Fig. 1D) (Klepal *et al.*, 1972). Circular musculature is only found around the central ductus ejaculatorius, which serves for sperm ejection (Klepal *et al.*, 1972). The anterior portion of the penis is studded with rows of sensory setae (Fig. 1C), which are assumed to help in the identification of females.

Most intertidal barnacles have a brief reproductive season and only fully develop a functional penis during this period (Barnes, 1992; Klepal *et al.*, 2010). For example, in New York populations of *Semibalanus balanoides,* which reproduce in late October and early November, the penis grows rapidly to its maximum size over September and October and degenerates during November (Horch, 2009). Interestingly, intertidal barnacles can change the size and shape of their penises to suit local hydrodynamic conditions as shown for *Balanus glandula* and *Semibalanus balanoides* (Neufeld & Palmer 2008; Horch, 2009). On wave-exposed shores, they develop shorter penises with greater diameters than in wave-protected sites. In *Balanus glandula*, penis mass corrected for body size was 16% higher in the most exposed site when compared with the most protected site (Neufeld & Palmer, 2008), suggesting that exposed barnacles invest more energy and resources in penis development and function. Transplant experiments between wave-exposed and protected sites revealed phenotypic plasticity as the cause of penis variation rather than differential settlement or selective mortality (Neufeld & Palmer 2008; Horch, 2009). The unusual plasticity of barnacle penises identifies an environmental factor as driver of genital diversification in animals aside of female choice, sexual conflict and male-male competition (Neufeld & Palmer, 2008).

Cirripeds are the record holders in relative penis length. For example, the penis of *Cryptophialus minutus* is eight times longer than its body size (Neufeld & Palmer, 2008). The resting penis of *Semibalanus balanoides* is 8–13 mm long, depending on size of the animal, and can be stretched to about four times its resting length (Klepal *et al.*, 1972).

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A totally different construction of the copulatory organs is found in the Decapoda. Most members of this order of vagile and rather big-sized crustaceans have permanent and form-invariant copulatory organs (Factor, 1995; Holdich, 2002; Becker *et al.*, 2012). In freshwater crayfish and brachyuran crabs each of the paired copulatory organs is composed of the first and second gonopods (Fig. 1E, F), which serve different functions. The first gonopod forms a tube-like structure (Fig. 1G) that takes the sperm from the genital opening. Unlike in the cirriped penis, there is no ductus ejaculatorius in these copulatory organs. For sperm transfer, the second gonopod is inserted into the tube of the first gonopod (Fig. 1F) and acts like a plunger that produces spermatophores of appropriate size and pushes them into the female storage site (Holdich, 2002; Becker *et al.*, 2012). The form constancy and permanent stiffness of both gonopods is achieved by particularly thick cuticles (Fig. 1G) (Brandis *et al.*, 1999; Ewers-Saucedo *et al.*, 2015). The complex structure and form-invariance qualify the gonopods for taxonomic purposes. For example, in cambarid crayfish the morphology of the gonopods is so specific that it is the main criterion for species identification (Hobbs, 1989).

III. GIANT SPERM AND EXPLOSION SPERM

Crustacea have evolved an enormous variety of sperm forms and fertilization mechanisms (Jamieson, 1991). A few groups show the classical sperm morphology consisting of a head and a flagellum but most groups have a derived sperm structure. Here, I want to present the two most curious types, the giant sperm of Ostracoda and the explosion sperm of Decapoda.

Ostracods of the superfamily Cypridoidea have some of the longest sperm in the animal kingdom, surpassed only by a few insects. Sperm length in cypridoidean species ranges from 268 µm to 11.8 mm (Fig. 2A, B) and from 0.33 to 4.3 times the carapace length of the producing male (Smith *et al.*, 2015). The record holder in the animal kingdom is the fly *Drosophila bifurca*, which produces sperm of 58.2 mm, which is 20 times longer than the

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specimens that manufacture them (Pitnick *et al.*, 1995). However, in contrast to insect giant sperm, in which most of the length is accounted for by exceptionally long flagella, ostracod sperm are aflagellate and represent an extremely elongated sperm-head. The nucleus runs from one end to the other and is partially encased by two enormous, spiralling mitochondria (Fig. 2C, D). Although tailless, these spermatozoa are motile because contractile elements can produce longitudinal rotation of the entire sperm (Matzke-Karazs *et al.*, 2014). During fertilization the entire spermatozoon enters the egg although even the shortest giant sperm is five times as long as the diameter of the corresponding egg (Matzke-Karazs, 2005).

The spermatozoa of the Decapoda Reptantia, which include freshwater crayfish, lobsters and crabs, are called "Explosionsspermien" (explosion sperm) in the German literature. Like the sperm of other decapods they are aflagellate and nonmotile (Jamieson, 1991; Tudge & Koenemann, 2009) but can achieve an abrupt short-term motility by a special acrosomal reaction. Reptantian sperm are composed of a compact main body consisting of the nucleus and acrosome and radial arms (Fig. 2E) (Brown, 1966; Talbot & Chanmanon, 1980a; López-Camps *et al.*, 1981; Vogt, 2002). The radial arms are rich in microtubules and become free shortly before fertilization when the spermatozoa are released from the spermatophore (Niksirat *et al.*, 2014).

The acrosomal reaction is characterized by an abrupt eversion of the acrosome, which causes a leap-like forward movement (Fig. 2F, G) (Talbot & Chanmanon, 1980b). In vitro induction of sperm reaction in the lobster *Homarus americanus* and video microscopy revealed a reaction time from 0.66 to 5.16 seconds (Tsai & Talbot, 1993). The acrosomal reaction occurred in two phases, eversion of the acrosome and forward tearing of the nucleus. It propelled the entire sperm forward about 18 µm (Fig. 2F) (Tsai & Talbot, 1993). The force generated by the acrosome reaction is sufficient to push the sperm nucleus through the egg coat as shown for lobster (Fig. 2H) and crabs (Brown, 1966; Goudeau, 1982; Talbot *et al.*, 1991).

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IV. SPERM STORAGE AND PATERNITY

Multiple paternity seems to be widespread in Crustacea. It is mainly known for Decapoda but there are also examples for Isopoda, Cirripedia and Copepoda (Avise *et al.*, 2011; Dennenmoser & Thiel, 2015). Avise *et al.* (2011) have compiled data on paternity in 11 decapod species including shrimps, crayfish, lobsters and crabs. In nine species, multiple paternity was proven with the microsatellite technique for between 20-100% of clutches. The mean number of fathers per clutch and species ranged from 1 to 5.3. The maximum number of fathers per clutch was 11 in *Caridina ensifera* (Yue & Chang, 2010). Multiple paternity requires promiscuous behaviour of the females and sperm storage until egg-laying and fertilization, which can occur weeks, months and even years after the last mating (Jensen & Bentzen, 2012).

Usually, the multiple sires contribute differently to the broods (Dennenmoser & Thiel, 2015). For example, in crayfish clutches fertilized by more than three males, one sire always dominated the brood by sharing his genes with 50-80% of the offspring (Walker *et al.*, 2002; Yue *et al.*, 2010). The skewed offspring numbers are the result of cryptic female choice and sperm competition, which is, among others, dependent on the mode of sperm storage and sperm mobilization in the females (Birkhead & Pizzari, 2002; Wigby & Chapman, 2004; Dennenmoser & Thiel, 2015). In decapods, sperm can be stored either externally by the attachment of spermatophores to the ventral side of the female or internally in specialized spermathecas and seminal receptacles of the female. In the following, I will present examples for both types and discuss consequences for paternity.

In freshwater crayfish, the spermatophores are either externally attached as in Astacidae and Parastacidae or stored in an unpaired cuticular spermatheca without luminal connection to the oviducts as in Cambaridae. Fertilization is generally external (Holdich, 2002). In astacids,

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the spermatophores are glued to the sternal plate between the genital openings (Fig. 3A) and to the underside of the tail fan. In multiply mated females, late males may leave the spermatophores of earlier males untouched or may remove them partly (Fig. 3B, C) or completely, which significantly influences paternity in the clutch. For example, in *Austropotamobius italicus*, 33% of the males completely removed the spermatophores of previous rivals, 63% left intact some of the rivals' spermatophores and 3% simply added their own sperm (Galeotti *et al.*, 2007). Paternity in species with external sperm storage may also be incidentally biased by mechanical abrasion of spermatophores during locomotion (Galeotti *et al.*, 2007). In cambarid crayfish, males transfer the sperm into the annulus ventralis and then close the opening with a sperm plug (Galeotti *et al.*, 2012). Such plugs seem to secure the sperm in the spermatheca rather than block insemination by later males as may be deduced from the high frequency of multipaternity in clutches of cambarids (Walker *et al.*, 2002; Yue *et al.*, 2010; Kahrl *et al.*, 2014).

In brachyuran crabs, some species are primarily unipaternal and others are primarily multipaternal (Avise *et al.*, 2011; Dennenmoser & Thiel, 2015). This feature seems to depend mainly on the species-specific mode of sperm storage and sperm competition. In eubrachyurans, the sperm is deposited in paired ventrally located seminal receptacles that are continuous with the oviducts and the vaginas (Fig. 3D) (Becker *et al.*, 2011; McLay & López Greco, 2011; Pardo *et al.*, 2013). The oocytes are fertilized in a fertilization area close to the opening of the oviduct (Fig. 3D) (Becker *et al.*, 2011; McLay & López Greco, 2011). Since the spermatozoa are non-motile the sperm stored closest to the fertilization area have the highest chance to fertilize the eggs. Principally, males have two possibilities aside of female guarding to ensure dominance over the next clutch. They could either remove the sperm of preceding rivals from the seminal receptacle by the intromittent organ or displace it to an unfavourable position and seal it off. Beninger *et al.* (1991) have advocated for the first alternative because the male gonopods of some crabs have recurved spoon-like tips with

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swap-like bundles of setae (Fig. 3G) resembling the male genitalia of insects for which sperm removal is proven (Waage, 1979; Tsuchiya & Hayashi, 2014). However, until now there is no experimental proof for active sperm removal of predecessors in brachyurans.

In contrast, the alternative possibility to gain last-male precedence by ejaculate stratification and sealing (Fig. 3E, F) is proven for several brachyuran species (Diesel, 1990; Urbani *et al.*, 1998; Sainte-Marie *et al.*, 2000; Pardo *et al.*, 2013). For example, in spider crab *Inachus phalangium* the last male to mate displaces the ejaculate of his predecessor dorsally towards the apex of the receptacle and seals it off with a hardening sperm gel. He then places his own sperm closest to the oviduct opening. Similar observations were made in the laboratory in multiply mated snow crab *Chionoecetes opilio*, in which the last male was mostly the only father of the clutch (Urbani *et al.*, 1998). Stratification and sealing of the sperm packages of rivals seems to be more effective in some species than in others (Sainte-Marie *et al.*, 2000; Pardo *et al.*, 2013; Rojas-Hernandez *et al.*, 2014) but even in the most effective species stratification occasionally fails, particularly if the fresh ejaculate is large in volume, resulting in multiple paternity in otherwise unipaternal species (Sainte-Marie *et al.*, 2000; Jensen & Bentzen, 2012).

The mode of sperm storage and related sperm competition has considerable consequences for population biology, ecology and fisheries. In long-lived species like clawed lobsters and some crabs, sperm can be stored across moults and be utilized for years (Factor, 1995; Jensen & Bentzen, 2012; Pardo *et al.*, 2013). This is made possible by sperm storage in mesodermal sectors of the seminal receptacles that are not shed during ecdysis (Becker *et al.*, 2011; Pardo *et al.*, 2013). For example, in the Tanner crab *Chionoecetes bairdi* females isolated from males after copulation produced viable eggs by 100% in the mating year and 97% and 71% in the following two years (Paul, 1984). In such species, males that die or are captured soon after copulation can contribute to the gene pool of the population even years later (posthumous paternity). In exploited species, in which males are selectively fished, long-term storage of

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sperm from multiple mates is thought to contribute to the viability and genetic diversity of populations and may even be essential for a sustainable fishery (Sainte-Marie *et al.*, 2008; Pardo *et al.*, 2013).

V. GELATINOUS FERTILIZATION TENT

Freshwater crayfish produce a gelatinous tent-like structure for fertilization of the eggs and their attachment to the pleopods. This tent is formed by the females immediately before egg laying by a secretion from the glair glands and disrupted and shed several hours later when the fertilized eggs are firmly glued to the pleopods. Glair glands and fertilization tent are shared by all crayfish families, the Astacidae, Cambaridae and Parastacidae.

Glair glands develop in the weeks before spawning (Thomas & Crawley, 1975) and are good indicators of forthcoming egg-laying. They appear as creamy-white patches on the last thoracic sternal plates, the sterna of the abdomen, the inner sides of the pleura, the pleopods and the uropods of the female. In the final stage of development they are so prominent that they can even be seen from the dorsal side (Fig. 4A). The glair glands are structurally different from other integumental glands and terminate with many pores on the underside of the thorax and pleon (Andrews, 1904; Thomas & Crawley, 1975).

Prior to egg laying, crayfish form a pouch by bending the pleon towards the underside of the cephalothorax. This pouch is then filled with a gelatinous secretion from the glair glands (Fig. 4B), which lasts about 30 minutes in *Orconectes limosus* (Andrews, 1906). Lying on her back, the female then releases the eggs into the glair and fertilizes them by sperm that is mobilized from the externally attached spermatophores or the *annulus ventralis* (Mason, 1970; Gherardi, 2002). Egg laying takes about 10-30 min in *Orconectes limosus* but may last for hours in other species (Andrews, 1904). The inner mass of the glair is rather fluid but at the contact zones with the water it forms a more rigid parchment-like structure resembling the

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flysheet of a tent. Within this tent-like structure the eggs are transferred backwards by gravity and paddling movements of the pleopods to be fastened to the oosetae of the pleopods. The attachment process then requires repeated sideward turnings, which usually lasts several hours (Andrews, 1904, 1906; Mason, 1970).

The function of the fertilization tent is probably manifold. It may protect the freshly laid eggs that are soft and highly labile from the osmotic stress of fresh water and may provide an optimal milieu for fertilization. The eggs achieve their typical round shape and rigid consistency only after formation of the outermost shell layer during attachment to the pleopods (Andrews 1904, 1906). Another possible function of the fertilization tent is the mechanical confinement of the eggs to the brood pouch during the complicated and time-consuming attachment procedure. This idea is corroborated by the generally very low loss of eggs at this time and the observation that the eggs are not properly attached and decay rapidly if the fertilization tent is damaged.

Glair glands and fertilization tents occur in all crayfish species independent of whether they store sperm externally or internally. They are absent in the marine lobsters, the closest relatives of freshwater crayfish (Factor, 1995) and may therefore represent a special adaptation to reproduction in fresh water. However, the other freshwater decapods lack this fertilization tent too, even the freshwater shrimps and aeglids that have external fertilization like the crayfish (Chow *et al.*, 1982; Almerão *et al.*, 2010). Therefore, the fertilization tent has to be regarded as a special characteristic of freshwater crayfish, which has evolved in the stem group that invaded fresh water from the marine environment.

VI. BROODING COMPARTMENTS FOR POSTHATCHING STAGES

The simplest pattern of reproduction in Crustacea is the release of eggs and sperm into the water (broadcasting strategy) and larval development in the plankton. However, most

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crustacean groups have evolved mechanisms to brood their embryos, larvae or even the first juvenile stages on or in the body of the female (Gruner, 1993; Thiel, 2000, 2003). In some clades, for instance in the Peracarida, parental care is obligatory and morphologically so specific that it can be used as a character for phylogenetic analysis (Richter & Scholtz, 2001). In other lineages like the Euphausiacea, brood care is limited to a fraction of the species only (Gómez-Gutiérrez, 2003). Here, I focus on brooding of the offspring in or on the body of the mother requiring specific structural adaptations. Information on brood care in dwellings that is based more on behavioural interactions of mother and offspring is found in reviews by Thiel (2003, 2007) and Vogt (2013).

The simplest type of brood care is carrying of the eggs on the mother until hatching of the nauplius larva. Examples are the Euphausiacea among the higher crustaceans (Malacostraca) and the Copepoda among the lower crustaceans ("Entomostraca"). Both groups include broadcasting and sac-spawning species. Species of the krill genera *Nyctiphanes* and *Nematoscelis* brood their eggs in a membranous sac on the thoracopods (Fig. 5A) (Gómez-Gutiérrez, 2003) whereas cyclopoid and some calanoid copepods carry the eggs in membranous sacs on the urosome (Fig. 10I) (Kiørboe & Sabatini, 1994).

Brooding of embryos and posthatching stages in dorsal brood chambers is typical of some entomostracan groups like the Cladocera (Fig. 5B) and Ostracoda (Fig. 11G). In cladocerans, the brood chamber is the space between the dorsal side of the trunk and the carapace. The eggs are laid in this pouch (Fig. 10A) and the embryos develop until a miniature adult is released (Fig. 5B) (Mittmann *et al.*, 2014). Some cladocerans with small eggs secrete a fluid rich in nutrients into this chamber, which may be absorbed by the eggs and developing embryos (Schminke, 2007). Interestingly, a dorsal brood pouch is also found in the Thermosbaenacea, which raise the embryos and four posthatching stages in this chamber (Olesen *et al.*, 2015). This type of brood care is exceptional among the Malacostraca, which normally brood their offspring on the ventral side of the body (Fig. 5C, E).

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Like all Peracarida, the isopods have a ventral brood chamber that is delimited by the oostegites, which are specialized parts of the thoracopods (Fig. 5C) (Csonka *et al.*, 2015). This marsupium probably evolved for mechanical protection of eggs and developing embryos in the sea but has become particularly important for colonization of the land (Hoese & Janssen, 1989). In terrestrial Isopoda, this chamber serves as a micro-aquarium to raise the developmental stages. It contains cotyledons (Fig. 5C) that secrete the marsupial fluid and probably supply the young with nutrients. The latter possibility was inferred from the presence of lipid globules in the cotyledons (Fig. 5C) and the continuity of these organs with the lipid storing fat body of the mother (Hoese & Janssen, 1989).

In the Decapoda, the simplest type of brood care is carriage of the eggs until hatching. It is rare in Dendrobranchiata, the basal subclass of the Decapoda, but obligatory in the derived Pleocyemata. Dendrobranchiate shrimps usually broadcast eggs and sperm and only the pelagic *Lucifer* species carry their eggs on the 3rd thoracopods until the nauplii emerge (Lee *et al.*, 1992). The pleocyemates brood their eggs on the pleopods at least until hatching of the zoea larva. Egg carriage can be as long as 16 months as shown for the American lobster *Homarus americanus* (Goudeau *et al.*, 1987), and therefore, egg attachment must be very firm. This requirement is fulfilled by a special egg attachment system that consists of a rigid egg envelope, a firm but elastic egg stalk and the oosetae that arise laterally from the pleopods. This highly effective egg attachment system presumably originated in the common ancestor of the Pleocyemata some 430 million years ago (Porter *et al.*, 2005).

In many Pleocyemata, carriage of the young is extended to the larval stages or even to the juveniles. For example, in freshwater crayfish the juveniles are brooded on the pleopods (Fig. 5E) at least until the first feeding stage, which is juvenile stage 3 in cambarids. However, if shelters are scarce the brooding period is prolonged (Vogt, 2013). Brood care in freshwater crayfish includes special structural adaptations that secure the young to the mother during hatching and the first moult as discussed in detail in the next chapter. Other decapod examples

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of brood care extension until the juvenile stage are the freshwater crabs and aeglid anomurans. In these freshwater inhabitants, the brooded stages are carried on the pleopods and are protected in a ventral pouch that is formed by forward folding of the pleon under the cephalothorax. An exceptional case of brood care in the Decapoda is the terrestrial crab *Geosesarma notophorum*, which carries the juveniles on top of the carapace covered by a film of water (Fig. 5D) (Ng & Tan, 1995).

Brooding decapods provide protection and grooming but usually do not feed the young, although the offspring may sometimes participate in the maternal meal. The main advantage of brood care is the increased survival and growth of the young (Clutton-Brock, 1991). Disadvantages are the higher energy expenditure of the caring mothers and the pronounced reduction of egg number in brooding species (Anger, 2001; Vogt, 2013). Therefore, this life history strategy should be favoured only under specific circumstances. The Decapoda are particularly suitable to examine this issue in depth because they include non-brooders and brooders of different degrees and have evolved extended brood care independently in several lineages. Interestingly, brooding until the juvenile stage was convincingly demonstrated for less than 30 species of the ~12,000 marine decapods (mainly from polar regions and harsh coastal environments) but is supposed to occur in about 70% of the ~3.000 species of freshwater decapods (Vogt, 2013). This imbalance suggests that extended brood care was mainly selected for in environments with heavily fluctuating nutritional and abiotic conditions (Anger, 2001; Vogt, 2013).

VII. SAFETY LINES FOR SAFEGUARDING OF HATCHING AND FIRST MOULT

Freshwater crayfish have evolved effective safety lines, the telson thread and anal thread, to secure the brooded offspring during the immobile and helpless phases of hatching and first moult, respectively. At other times the juveniles can actively hold on to their mother with

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terminal hooks on the chelipeds (Vogt & Tolley, 2004; Vogt, 2008). Such safety lines are unique in the animal kingdom and have probably evolved during colonization of fresh water by the stem group of crayfish (Scholtz & Kawai, 2002; Vogt, 2013).

The telson thread occurs in all crayfish families (Andrews, 1907; Scholtz, 1995; Vogt & Tolley, 2004). It emerges during hatching and probably originates from two sources, a secretion and the detaching inner layer of the egg case. The telson thread extends from the posterior end of the telson of the hatchling to its egg case, which persists on the pleopods after hatching (Fig. 6A, B). During eclosion it keeps the helpless hatchling passively secured to the mother (Fig. 6A) preventing it from being dislodged by the water current. Thereafter, it secures the hatchling during its attempts to hook into pleopodal structures of the mother (Vogt, 2008).

The anal thread occurs only in the Cambaridae and Parastacidae. It is composed of cuticular material and originates from delayed moulting of the hindgut (Scholtz, 1995; Rudolph & Rojas, 2003; Vogt, 2008). During moulting of the hatchling it keeps the emerging stage-2 juvenile passively linked to its exuvia (Fig. 6C), which itself remains hooked into pleopodal structures of the mother. In vitro tests revealed that this curious anal thread-exuvia-pleopod connection is firm enough to protect the freshly moulted juveniles from being washed away (Vogt, 2008). After some hours, the anal thread is disconnected by flapping movements of the juveniles, which are now attached to the maternal pleopods by their peraeopodal hooks (Vogt, 2008). A special situation is found in the parastacid species *Astacopsis gouldi* and *Astacopsis franklinii* which secure three juvenile stages by anal threads (Hamr, 1992).

VIII. DWARF MALES

Dimorphism between females and males is common in crustaceans and results from secondary sex characteristics related to sperm transfer and brood care. The larger sex is

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sometimes the female and sometimes the male. An extreme form of sexual dimorphism is the reduction of males to dwarf males, which has independently evolved in sessile cirripeds and parasitic isopods, copepods and cirripeds. Dwarf males are much smaller than females of the same species and are usually equipped with special structures to hold on the female. Their internal organs except of the gonads are reduced to different degrees, depending on taxon. The advantage of dwarf males is the permanent availability of males at a relatively low physiological cost under conditions of limited mating opportunities (Vollrath, 1998).

In isopods, dwarf males are typical of the ten families of the Bopyroidea and Cryptoniscoidea, which parasitize on other crustaceans from shallow waters to the deep sea. These families comprise 795 species or 7.7% of all isopods and have a shared evolutionary history with their hosts dating back to the Jurassic (Williams & Boyko, 2012). The males reside on the females but their feeding biology is largely unknown. An example is the bopyrid *Pseudione overstreeti* (Fig. 7A) that infests the branchial chamber of the Mexican ghost shrimp *Callichirus islagrande* (Adkison & Heard, 1995). The greatest total length of the females is 19.1 mm and the greatest width is 14.4 mm. In males, the corresponding values are 4.9 mm and 2.3 mm, respectively. The male usually attaches to the ventral side of the female's pleon. In the dajid *Zonophryxus quinquedens*, which parasitizes on the carapace of the deep sea Antarctic shrimp *Nematocarcinus longirostris,* the females and males have maximum lengths of 20 mm and 4.5 mm, respectively (Brandt & Janssen, 1994). The dwarf males live on the underside of the attached female (Fig. 7B).

In sessile Cirripedia, which are basically hermaphrodites, dwarf males have evolved several times probably because of the limited opportunity to find sexual partners and to reach them with the penis for insemination (Yusa *et al.*, 2012). An example is the pedunculate gooseneck barnacles, which show a broad diversity of sexual systems including simultaneous hermaphroditism, the coexistence of hermaphrodites and dwarf males (androdioecy) and the combination of pure females and dwarf males (dioecy) (Yusa *et al.*, 2012). Androdioecy

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occurs in shallow-water species that live in large groups and dioecy occurs in the deep sea and in symbiotic species. In the androdioecious *Scalpellum scalpellum* two to five dwarf males are usually attached to a large hermaphrodite. In the deep sea species *Trianguloscalpellum regium* that depends entirely on the presence of dwarf males for fertilization the dwarf males are very small and are attached to the female in groups located in a specific receptacle inside the scutal edge (Fig. 7C).

The rhizocephalan cirripeds are endoparasites in marine Decapoda. The body of the female is composed of two parts, a root-like interna that penetrates the host organs and serves for the absorption of nutrients and a sac-like externa that is located on the host's pleon and serves for reproduction. The eggs in the externa are fertilized by the sperm of dwarf males. These can invade the mantel cavity of the female as a very small trichogon of only 200 µm in length that develops from the cypris larva. The trichogons migrate to specific receptacles close to the ovaries, moult at the entrance of the receptacle and seal it with the shed cuticle. Therefore, each of the two receptacles of a female is inhabited by a single trichogon only, which produces sperm and remains together with the female until the end of life (Hoeg, 1991; Schminke, 2007). In species with closed mantles, dwarf males attach to the outside of the mantle and inject spermatogonia through their antennae into the mantle cavity. Depending on species, these spermatogonia settle then in the receptacles or form balls of spermatogonia that float in the mantle cavity to produce sperm (Hoeg, 1991; Schminke, 2007).

Several families of parasitic copepods have evolved dwarf males, which are many times smaller than their corresponding females (Østergaard & Boxshall, 2004). In the Chondracanthidae, a family parasitic on marine fishes, the males attach to immature females at the second copepodite stage, complete their development on the female and remain there until they die. A maximum of eight males on a single female has been recorded but in most members of the family adult females rarely have more than one male attached. An example is *Chondracanthus lophii*, in which tiny males adhere near the female genital apertures to so-

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called nuptial organs (Fig. 7D), which are special structures for holdfast and probably nourishment. The males use the claws on the transformed antennae (Fig. 7E) for attachment. The pinecone-like nuptial organs (Fig. 7F) contain glandular tissue and are assumed to produce a secretion to nourish the males. Adult males have well developed mouthparts (Fig. 7E) and a functional oesophagus and midgut but no anus, suggesting that they feed on easily digestible stuff like mucous produced by the female (Østergaard, 2004; Østergaard & Boxshall, 2004).

IX. FEMINIZATION, CASTRATION AND GIGANTISM

Parasitic crustaceans and microorganisms can significantly change the reproductive biology of crustacean hosts by manipulating sex or causing infertility. The latter is sometimes paralleled by gigantism promoting multiplication of the parasite.

Parasitic isopods and cirripeds are well known for their ability to cause feminization or infertility of their crustacean hosts (Schminke, 2007; Williams & Boyko, 2012). For example, in the ghost shrimp *Callichirus islagrande* the reproductive activity is obviously suppressed when infected with the bopyrid *Pseudione overstreeti.* In all of the more than 100 host-parasite associations investigated, the gonads of the hosts were greatly reduced (Adkison & Heard, 1995). Moreover, since only female shrimp were found to be infested some female morphotypes may represent primary males that have changed sex after parasitic manipulation (Adkison & Heard, 1995). Among the parasitic isopods some bopyrids and dajids and all entoniscids and Cryptoniscoidea are assumed to be parasitic castrators of their hosts (Williams & Boyko, 2012).

A well-known bacterial manipulator of crustacean reproduction is the intracellular alpha-proteobacterium *Wolbachia pipientis*. Bouchon *et al.* (1998) detected this bacterium in 19 species from eight terrestrial isopod families and in one limnic and two estuarine isopod

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species. Meanwhile, it was also found in a limnic amphipod and a marine gooseneck barnacle (Cordaux *et al.*, 2012). *Wolbachia* was shown to be able to induce feminization in isopods by converting genetic males into functional females (Juchault *et al.*, 1992; Rigaud *et al.*, 1997, 2001). However, not all infected males are transformed into females. Transinfection experiments established that the susceptibility or resistance to sex conversion depends much on the combination of host species and *Wolbachia* strain (Cordaux *et al.*, 2004). *Wolbachia* is usually vertically transmitted via the egg cytoplasm but some horizontal transmission has also occurred as demonstrated by genetic analysis. This mode of transmission may explain the occurrence of *Wolbachia* in highly diverse crustacean groups and "infectious feminization" may threaten the genetic diversity of wild populations.

A good example of castration and the induction of gigantism in a crustacean host is the *Daphnia magna – Pasteuria ramosa* system (Ebert *et al.*, 1996, 2004; Cressler *et al.*, 2014). *Daphnia* females continue to produce eggs within the first 12 days after infection by the bacterial manipulator (Ebert *et al.*, 1996) but thereafter, most of the infected individuals are castrated (Fig. 8A) to direct nutrients and energy towards reproduction of the parasite. By manipulating food levels during the infection Ebert *et al.* (2004) showed that both antagonists are resource-limited and that there is a negative correlation between host and parasite reproduction, indeed. Curiously, a certain proportion of the saved energy is channelled into growth of the host resulting in gigantism (Fig. 8A). Although illogical at first glance, the parasite benefits from this resource allocation because it can produce more spores in a bigger host (Ebert *et al.*, 2004). *Pasteuria ramosa* has a polymorphic life cycle beginning with cauliflower-like rosettes and ending with individual spores (Fig. 8B). It is horizontally transmitted through spores that are released from dead host bodies into the water.

X. RECORD-BREAKING CLUTCH SIZE AND CHROMOSOME NUMBER

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Crustaceans are among the animals with the highest clutch sizes. Particularly high numbers of eggs per clutch are produced by brachyuran decapods. Examples are *Metacarcinus anthonyi* and *Scylla tranquebarica*, which can have 3.8 and 5 million eggs per clutch, respectively (Hines, 1991; Srinivasagam *et al.*, 2000). In the blue crab *Callinectes sapidus*, the eggs are brooded until hatching of the zoea larvae in a so-called sponge under the pleon (Fig. 9A, B). The average sponge contains about three million eggs but in large females it was shown to include up to 8 million eggs (Prager *et al.*, 1990). In some *Cancer* species, the number of eggs produced per female and lifetime was estimated to more than 20 million (Hines, 1991) but in *Callinectes sapidus*, which has up to 18 broods (Graham *et al.*, 2012), this value may be exceeded.

The record in chromosome number in animals is hold by the freshwater crayfish *Pacifastacus leniusculus trowbridgii* with a diploid set of 376 chromosomes (Fig. 9C) corresponding to a chromosome number of 188 in the gametes (Fig. 9D) (Niiyama, 1962). The second highest chromosome number was recently found in the triploid crayfish *Procambarus virginalis* (3n=276, Fig. 9E) (Martin *et al.*, 2015). *Procambarus virginalis* is the only obligatory parthenogenetic decapod known (Martin *et al.*, 2015; Vogt *et al.*, 2015). It reproduces by apomictic parthenogenesis, i. e. without meiosis, and therefore, the eggs should include 276 chromosomes as well, making *Procambarus virginalis* the new animal world record holder with respect to chromosome number of gametes.

The high chromosome numbers in freshwater crayfish are explained by whole genome duplication events in their early evolution. The arguments for this assumption come from the observation that some crayfish species have the double and four-fold chromosome numbers of others (Lécher *et al.*, 1995; Martin *et al.*, 2015) and the generally exceptionally high polyploid index in Decapoda (Otto & Whitton, 2000). The decapods have haploid chromosome numbers ranging from 27 to 188 and a polyploid index of 41.7%. Ancient polyploidization is particularly supposed for the Astacidea, Palinuridae and Paguroidea (Otto & Whitton, 2000).

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However, the increase in chromosome number within a lineage is not paralleled by an increase in genome size, which would contradict the polyploidization hypothesis. For example, *Astacus astacus* has fewer chromosomes (2n=176) than *Orconectes virilis* (2n=200) (Martin *et al.*, 2015) but has a much larger genome size (19.64 pg versus 4.69 pg) (Jeffery, 2015), suggesting that high chromosome numbers may at least partly result from evolutionary chromosome fragmentation.

XI. RECORD-BREAKING VIABILITY OF DIAPAUSING EGGS

Short-lived members of the limnic and coastal marine plankton and ephemeral water bodies often produce resting eggs that can survive adverse environmental conditions for years (Radzikowski, 2013). In the Crustacea, such diapausing eggs and cysts are produced by the Branchiopoda, Copepoda and Ostracoda. Under laboratory conditions resting eggs of the anostracan *Branchinecta packardi*, the notostracan *Triops longicaudatus* and the conchostracan *Caenestheriella gynecia* remained viable for a minimum of 16, 14 and 8 years, respectively (Radzikowski, 2013). Eggs of the cladoceran *Daphnia pulicaria* (Fig. 10A) and the copepods *Boeckella poppei* and *Onychodiaptomus sanguineus* (Fig. 10I) isolated from accurately dated lake sediments even hatched after 125, 196 and 332 years, respectively (Hairston *et al.*, 1995; Cáceres, 1998; Jiang *et al.*, 2012). The adaptation of short-lived animals to adverse and changing environments by resting eggs has apparently evolved in the earliest metazoans already (Cohen *et al.*, 2009).

Crustacean resting eggs can be the result of parthenogenetic or bisexual reproduction, depending on taxon (Radzikowski, 2013). Cladocerans like *Daphnia* usually produce subitaneous summer eggs (Fig. 10A) by parthenogenesis and resting winter eggs (Fig. 10B-D) by sexual reproduction. Resting eggs are usually highly tolerant to environmental stress like drying, freezing, UV radiation and mechanical damage. For example, the cysts of the

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anostracan *Artemia franciscana*, a common model of dormancy, can survive prolonged exposure to temperatures of -271°C and +100°C (Radzikowski, 2013). This stress tolerance is achieved by protecting coverings that are often shaped and ornamented in a group or species-specific manner (Fig. 10E-H) (Thiery & Gasc, 1991; Fryer, 1996). These coverings also permit passage through the guts of birds facilitating dispersal and colonization of isolated water bodies (Radzikowski, 2013). In some cladocerans, the resting eggs are additionally enveloped by a cuticular ephippium, which is derived from the carapace (Fig. 10C). Stress resistance is further provided by cryoprotectants like trehalose and glycerol and molecular chaperones like heat shock proteins (MacRae, 2010; Radzikowski, 2013).

The production of long-lived diapausing eggs in crustaceans is a bet-hedging strategy that constitutes an ecological and evolutionary reservoir. Mobilization of this reservoir can help the actual population to respond to environmental changes by enhancing the genetic variation and species richness (Hairston, 1996; Cáceres, 1998). In Oneida Lake, New York, diapausing eggs accumulate in the sediments to densities of 2.5 x 10^4 eggs/m^2 for *Daphnia galeata* and 8.0 x 10^4 eggs/m^2 *for Daphnia pulicaria* (Cáceres, 1998). ^{210}Pb dating of sediments suggests that these eggs can remain viable for >125 years and that the two *Daphnia* populations have persisted in the lake for >200 years. Annual emergence rates back to the water column range between 0 and 25 *Daphnia*/m^2. Because annual variation in the size of the overwintering water-column population ranges between 0 and 2.5 individuals/L, the contribution of emergence to the development of the spring population is considerable in some years and negligible in others (Cáceres, 1998). In some copepod species, the density of diapausing eggs is >10^6/m^2 and their annual mortality rate is approximately 1% (Hairston *et al.*, 1995; Hairston, 1996).

Egg banks of crustaceans are in many ways analogous to the seed banks of terrestrial plant species (Cáceres, 1998) and are valuable tools for ecological, biogeographical and evolutionary research. For example, they are useful to reconstruct ancient plankton

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communities and to study their change over time (Ohtsuki *et al.*, 2015). They also reflect invasion histories as shown for the introduction and spreading of a parthenogenetic American *Daphnia pulex* clone in Africa and the subsequent displacement of native *Daphnia pulex* populations by this clone (Mergeay *et al.*, 2006). Crustacean egg banks were also used to investigate the influence of man-made eutrophication on plankton communities (Brede *et al.*, 2008). Short-term evolutionary changes of the genotype were studied on the example of the increasing resistance of *Daphnia galeata* in Lake Constance, Central Europe, to nutritionally poor and toxic cyanobacteria that drastically proliferated after eutrophication (Hairston *et al.*, 1999).

XII. RECORD-BREAKING FOSSIL AGES OF REPRODUCTIVE STRUCTURES

Ostracod crustaceans provide one of the most complete and consistent fossil records of any animal group including tens of thousands fossil species dating back to the Ordovician (Rodriguez-Lazaro & Ruiz-Muñoz, 2012). Usually, only their shells are preserved but in the last decade some exceptional fossils with soft body parts have been detected. They revealed, among other things, record-breaking ages of copulatory organs, sperm and brooded embryos.

Most ostracods are sexually reproducing and transfer sperm with complex paired copulatory organs called hemipenes (Fig. 11A) (Mc Gregor & Kesling, 1969; Karanovic, 2012). In an exceptionally well-preserved specimen of the myodocopid *Colymbosathon ecplecticos* from the Lower Silurian of Herefordshire, England, 3-D reconstruction revealed amazing details of the soft body including a copulatory organ (Siveter *et al.*, 2003). This ostracod was preserved as a three-dimensional calcite infill in nodules hosted within volcanic ash. The copulatory organ detected is relatively large and stout (Fig. 11B), projects anteriorly and has lobe-like distal flanks. With an age of 425 million years it is the oldest penis documented for any animal.

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Ostracods of the suborder Cypridocopina are famed for having some of the longest sperm in the animal kingdom as discussed above. Matzke-Karasz *et al.* (2014) have recently discovered fossil giant sperm by X-ray synchrotron microtomography in five ostracods (one male and four females) from the early Miocene of Queensland, Australia. The 16 million years old specimens belong to the species *Heterocypris collaris* and *Newnhamia mckenziana*. Giant sperm bundles were found in the seminal vesicles and vasa deferentia of the male (Fig. 11C) and the sperm receptacles of the females. These bundles included spermatozoa of excellent three-dimensional preservation showing subcellular features such as longitudinal coiling (Fig. 11D) and spiraling of the sperm nucleus. Other well-preserved reproductive structures in the male were the paired Zenker organs (Fig. 11E, F), which are chitinous and muscular pumps that help to transfer the sperm into the females (Yamada & Matzke-Karasz, 2012).

The sperm of *Heterocypris collaris* and *Newnhamia mckenziana* are the oldest giant sperm on record and the third oldest sperm of any animal. Older fossil sperm were found in a 50 million years old annelid cocoon from Antarctica (Bomfleur *et al.*, 2015) and a spring tail trapped 40 million years ago in Baltic amber (Poinar, 2000). The giant sperm of ostracods are exceptional in as far as they are considered to have originated only once some 100 million years ago and have been retained since then, which stands in contrast to the rapid sperm evolution in other taxa (Smith *et al.*, 2015). Indirect evidence of their occurrence already in the Cretaceous comes from the detection of Zenker organs (Fig. 11F), which are restricted to taxa with giant sperm (Matzke *et al.*, 2009). The long history and persistence of giant sperm in ostracods makes them a unique model to study the evolutionary significance and function of this unusual sperm type in animals (Matzke-Karasz *et al.*, 2014; Smith *et al.*, 2015).

Structures indicative of brood care are only rarely found in invertebrate fossils (Wang et *al.*, 2015). In 2007, Siveter and colleagues detected a 425 million years old myodocopid ostracod, *Nymphatelina gravida*, in Herefordshire, England, which included 20 ovoid and two

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valve-shaped structures of a mean length of 558 mm in the posterior domiciliar area. These globules were interpreted as eggs and juveniles in a marsupium-like brood chamber, which is a unique combination in fossil invertebrates (Siveter *et al.*, 2007). Later, Siveter *et al.* (2014) found a pyritized ostracod, *Luprisca incuba*, with well preserved embryos in the Upper Ordovician of central New York State, USA (Fig. 11H). This discovery provides conclusive evidence of a conserved brood-care strategy within the myodocopid Ostracoda for at least 450 million years (Fig. 11G, H).

The oldest crustacean fossil showing structural features of brood care is *Kunmingella maotianshanensis* from the Lower Cambrian Chengjiang Lagerstätte in China aged to 525-530 million years ago. This specimen belongs to the Bradoriida, an early derivative of the stem line Crustacea. *Kunmingella* was dorsoventrally flattened and crawling on the surface of the sediment (Shu *et al.*, 1999). Clusters of rounded bodies of 150 µm in diameter were found in a ventral chamber of the fossil delimited by the post-antennular appendages and their distal setae (Fig. 11I). These globular bodies are interpreted as brooded embryos, resembling brood care in the recent phyllocarid *Nebalia bipes*, which has a similar basket-like brood pouch created by intersecting setae of the thoracic appendages (Shu *et al.*, 1999). Brood care in *Kunmingella maotianshanensis* suggests that this reproductive strategy dates back to the early radiation of the Metazoa.

XIII. CONCLUSIONS

(1) Most crustaceans transfer sperm with copulatory organs. These can be either extensible as in cirripeds or form-invariant as in decapods. The construction of the former type resembles vertebrate penises. Permanent stiffness in the latter type is achieved by thick cuticles. In sessile cirripeds, penis length and width can be adapted to population density and habitat. Their penises are the record-holders with respect to relative size.

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(2) Sperm structure is very variable in crustaceans. Particularly interesting are the aflagellate giant sperms of ostracods and the explosion sperms of decapods. The ostracods are among the record holders with respect to sperm size in animals. The immobile explosion sperm of lobsters generate leap-like short-term motility by abrupt eversion of the acrosome, which is sufficient to penetrate the ovarian envelope.

(3) Multiple paternity is widespread in decapods. Dominance of the clutch in multiply mating species is much dependent on the mode of female sperm storage and male strategies to remove or seal the ejaculates of predecessors (sperm competition). Long-term storage of sperm in female receptacles for months and years is of particular relevance in commercially exploited populations, in which gender proportions fluctuate heavily due to selective fishery of males.

(4) Prior to egg-laying, the females of freshwater crayfish produce a unique gelatinous tent on the underside of their body, which facilitates external fertilization and attachment of the eggs to the pleopods. Such a fertilization tent is lacking in clawed lobsters, the closest relatives of crayfish, but is also absent from other freshwater decapods with external fertilization.

(5) Brood care has independently evolved in many groups of the Crustacea. Brooded embryos, larvae and juveniles are carried either in internal brood pouches or on the thoracopods and pleopods of the mothers. Unique brooding structures are the cotyledons of terrestrial isopods that secrete a nutrient-rich fluid and the telson thread and anal thread of freshwater crayfish that safeguard hatching and the first moult.

(6) A most curious phenomenon related to crustacean reproduction is the feminization, castration and induction of gigantism by parasitic crustaceans and bacteria. These gender manipulators and reproduction inhibitors can significantly change the dynamics of populations by transmitting "infectious feminization" among population members.

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(7) A further curiosity is the occurrence of dwarf males in ecto-parasitic isopods, copepods and cirripeds and sessile cirripeds from extreme environments. In some copepods the males are obviously nourished by a secretion from specialized female organs.

(8) The highest chromosome numbers in diploid and polyploid animals and animal gametes were found in freshwater crayfish with 376, 276 and 276 chromosomes, respectively. This phenomenon is probably the result of polyploidisation and chromosome fragmentation events during crayfish evolution. The clutch sizes of up to eight million eggs in marine crabs are record-breaking as well.

(9) Some cladocerans and copepods produce resting eggs with record-breaking viabilities of up to 332 years, providing excellent examples of posthumous parenthood. Egg banks with dormant eggs enable the investigation of plankton fluctuation over time, the reconstruction of pristine aquatic ecosystems before man-made pollution, invasion histories, and short-term genotype changes within species.

(10) Crustaceans also hold some records with respect to fossil ages of reproductive structures. A 425 million years old ostracod copulatory organ is the oldest penis ever found, 16 million years old ostracod sperm is the oldest giant sperm on record and the third oldest sperm of any animal, and eggs in a marsupium-like chamber of a 525 million years old crustacean stem line derivative is the oldest evidence of brood care in animals.

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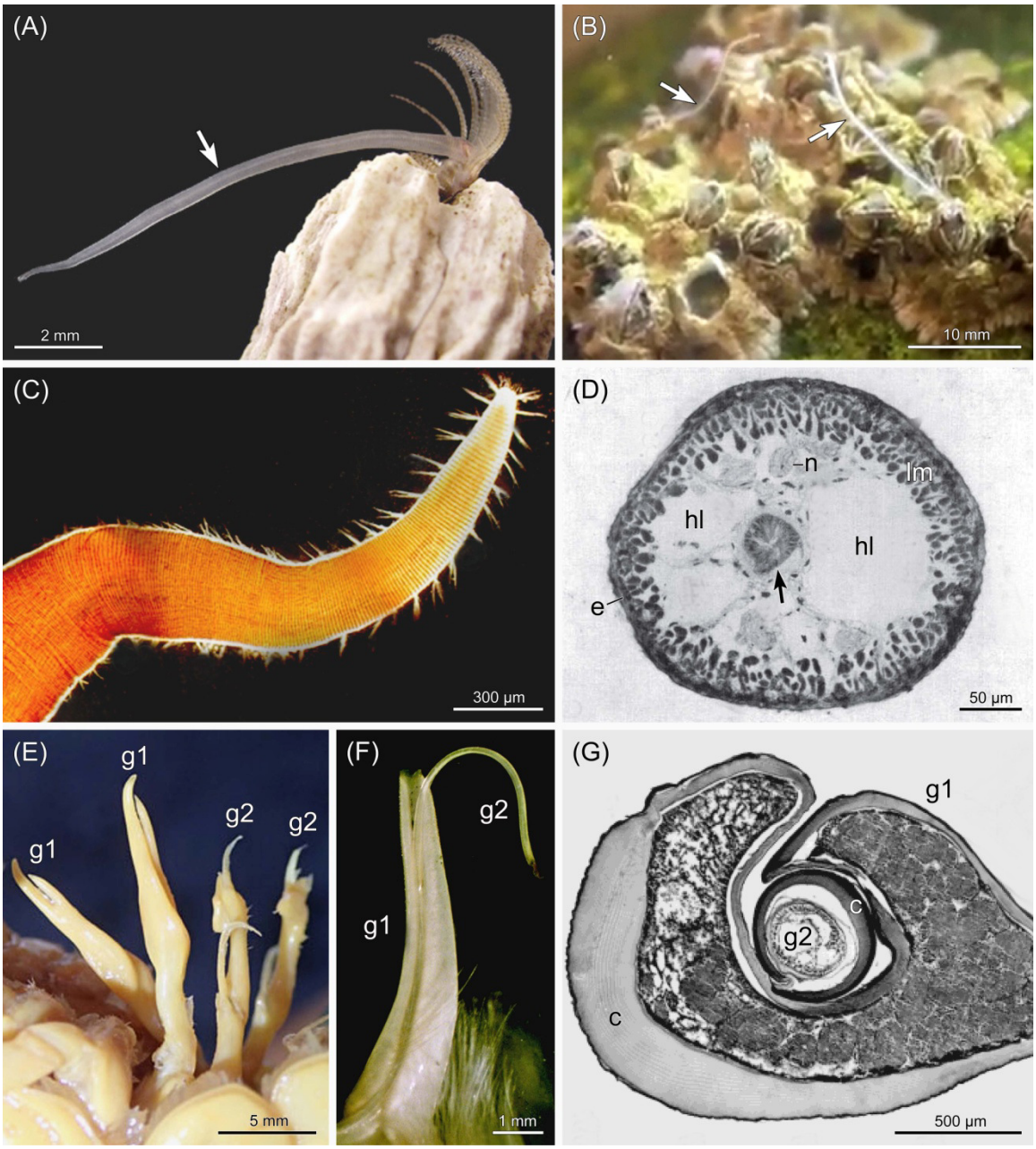

Fig. 1. Extensible versus form-invariant copulatory organs. (A-D) Hydraulic penis of Cirripedia. (A) Relaxed penis (arrow) of *Balanus glandula*. Photo: Christopher J. Neufeld. (B) Extended penises (arrows) of *Semibalanus balanoides*. From a video by Casey Dunn, filmed by Stefan Siebert: https://vimeo.com/7461478. (C) Apical part of penis of *Semibalanus balanoides* showing annulations and groups of setae. Photo: J. Matthew Horch. (D) Cross section through penis of *Balanus balanus* showing inflatable haemolymph lacunae (hl). Arrow, ductus ejaculatorius; e, epidermis with thin cuticle; lm, longitudinal musculature; n, nerve. From Klepal *et al.* (1972). (E-G) Permanently stiff copulatory organs of Decapoda. (E) Paired copulatory organs of crayfish *Orconectes cristavarius* consisting of first (g1) and second gonopods (g2). Photo: North Carolina Wildlife Resources Commission. (F) Copulatory organ of box crab *Calappula saussurei* with second gonopod inserted in first gonopod. From Ewers-Saucedo *et al.* (2015). (G) Cross section through functional state of copulatory organ of freshwater crab *Potamon gedrosianum* showing thick stabilizing cuticles (c) in both gonopods. From Brandis *et al.* (1999).

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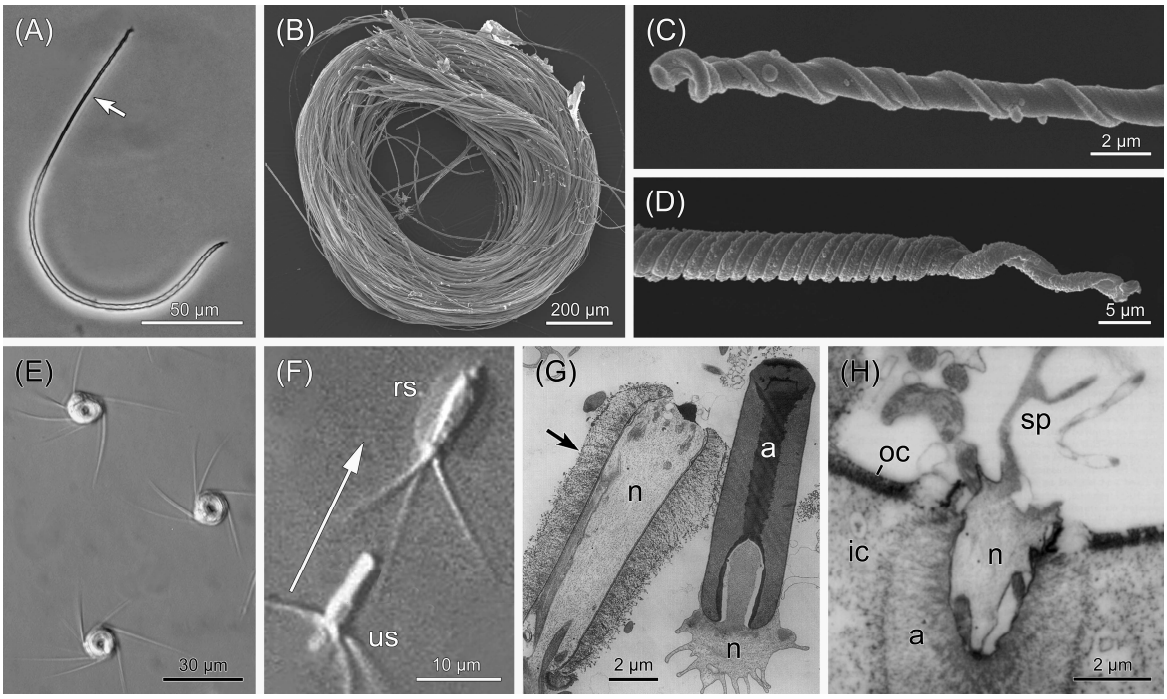

Fig. 2. Spermatozoa of exceptional form and function. (A-D) Giant sperm of cypridoidean Ostracoda. From Smith *et al.* (2015). (A) Shortest giant sperm from *Fabaeformiscandona velifera*. Arrow denotes anterior region. (B) Sperm bundle from seminal vesicle of *Australocypris robusta*, the ostracod with the longest sperm. (C) Drilled anterior tip of sperm of *Pseudocandona marchica*. (D) End piece of sperm of *Eucypris virens*. (E-H) Explosion sperm of Decapoda. (E) Spermatozoa of crayfish *Austropotamobius italicus* showing compact body and extended radial arms. From Galeotti *et al.* (2012). (F-H) Spermatozoa of lobster *Homarus americanus*. (F) Movement of sperm (arrow) by abrupt acrosome eversion. Montage of two pictures from a video. rs, reacted sperm; us, unreacted sperm. From Tsai & Talbot (1993). (G) Ultrastructural aspects of unreacted (right) and reacted (left) sperm. Note relative position of the nucleus (n). Arrow denotes eversed material of the reacted acrosome. a, unreacted acrosome. From Talbot & Chanmanon (1980b). (H) Penetration of spermatozoon through envelope of oocyte. The acrosome is in the process of eversion and the nucleus is thereby torn into the envelope. ic, inner chorion layer; oc, outer chorion layer; sp, spike. From Talbot *et al.* (1991).

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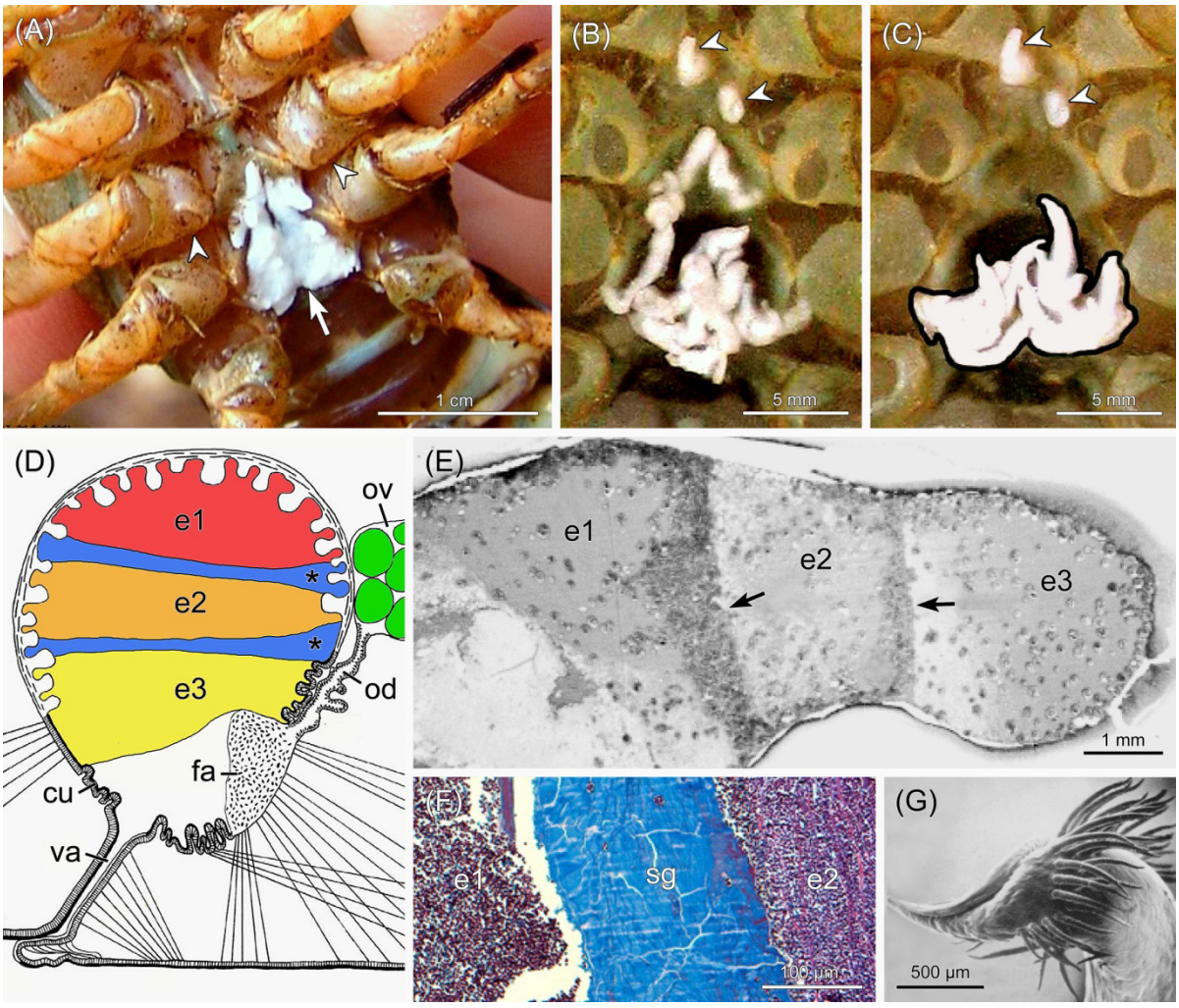

Fig. 3. Storage of sperm from multiple matings in Decapoda. (A-C) External attachment of spermatophores in astacid crayfish. (A) Preferred sperm attachment site (arrow) between female gonopores (arrowheads) in *Astacus astacus*. Photo: Lukáš Konečný. (B, C) Partial removal of spermatophores of earlier mate (B) by following mate (C) in *Austropotamobius italicus*. Arrowheads denote spermatophores remaining from earlier mate. Newly added sperm is black-rimmed. From Galeotti *et al.* (2007). (D-F) Internal sperm storage in eubrachyuran crabs. (D) Scheme of stratification of multiple ejaculates (e1-e3) in seminale receptacle of female. Mixing of sperm is prevented by hardened sperm gel (asterisks) between ejaculates. The last male deposits his sperm closest to the fertilization area (fa). cu, cuticle; od, oviduct; ov, ovary; va, vagina. Modified after Becker *et al.* (2011). (E) Histological section through seminal receptacle of *Chionoecetes opilio* showing sperm stratification. Arrows denote layers of densely packed sperm. From Sainte-Marie *et al.* (2000). (F) Older (e1) and fresh (e2) sperm packages separated by sperm gel (sg) in sperm receptacle of *Metacarcinus edwardsii*. From Pardo *et al.* (2013). (G) Tip of first gonopod of *Chionoecetes opilio* with recurved spoon-like structure and setal brushes suspected to serve for the removal of rivals' sperm from the seminal receptacle. From Beninger *et al.* (1991).

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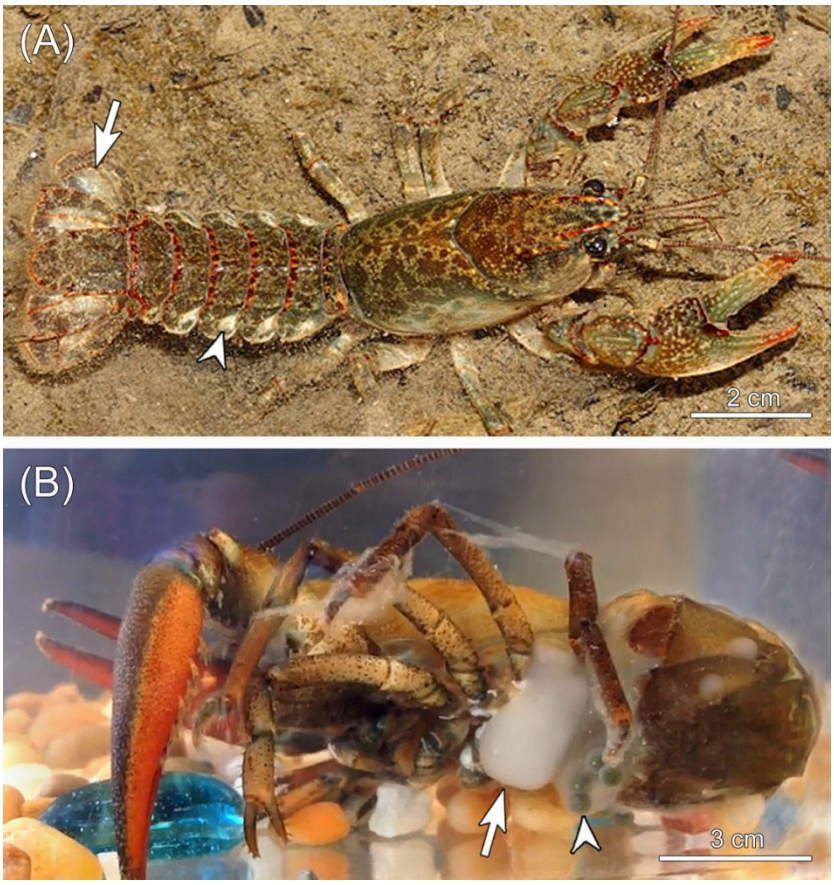

Fig. 4. Glair glands and fertilization tent in crayfish. (A) Whitish glair glands in uropods (arrow) and pleonal pleura (arrowhead) of female of *Cambarus polychromatus*. Photo: Andrew Hoffman. (B) Fertilization pouch in egg-laying female of *Pacifastacus leniusculus* filled with gelatinous mass from glair glands (arrow). A tarpaulin-like sheet is formed at the contact zone between gelatinous mass and water. Arrowhead denotes eggs confined within the tent. From a video by Bat Won: https://www.youtube.com/watch?v=Ur3iU1sbsIY.

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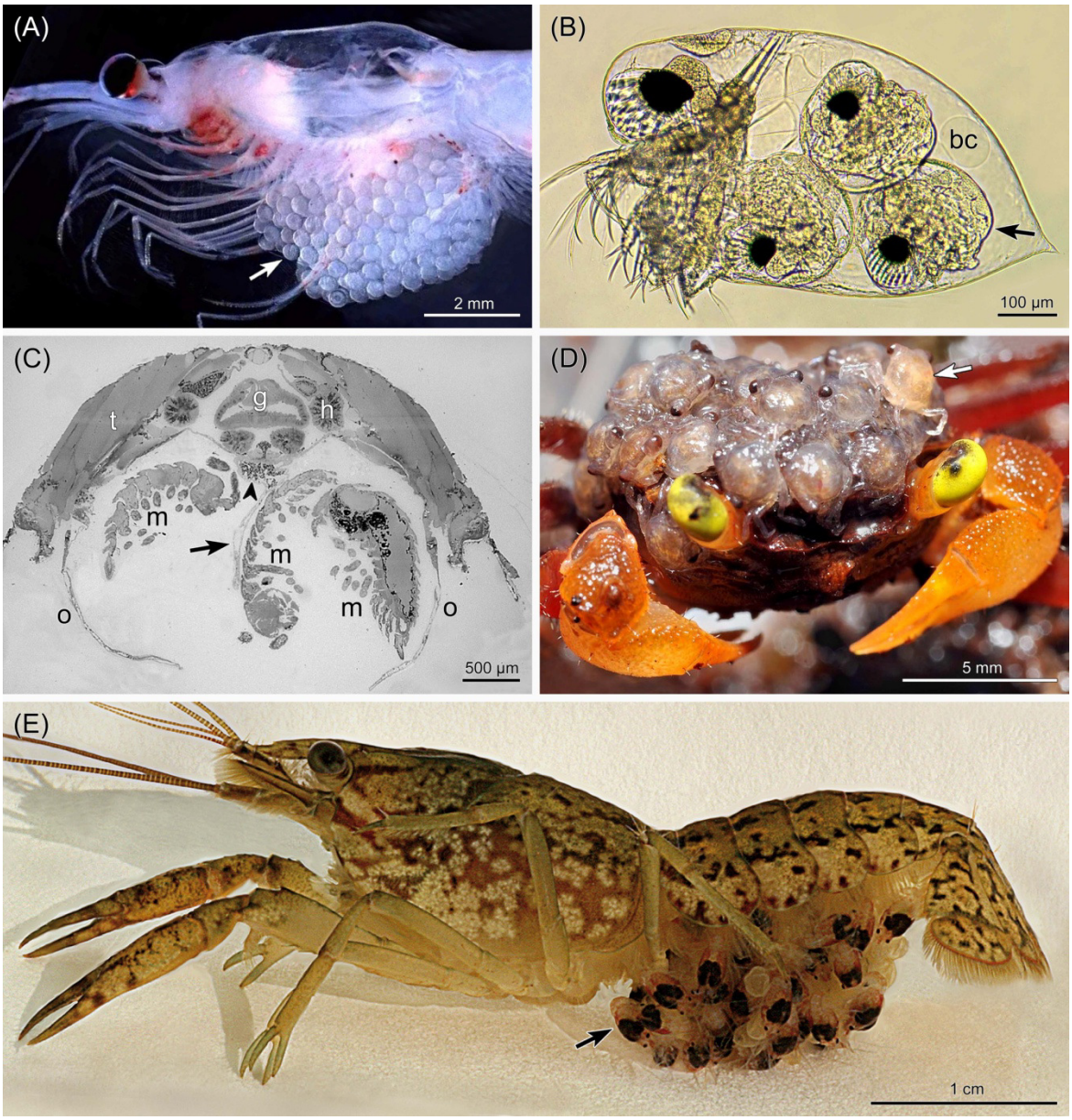

Fig. 5. Brooding of eggs and posthatching stages in specialized body compartments. (A) Krill *Nyctiphanes australis* with egg sac (arrow) between thoracopods. Photo: Anita Slotwinski. (B) Water flea *Evadne nordmanni* with advanced embryos (arrow) in dorsal brood chamber (bc). Photo: Maurice Loir. (C) Cross section through marsupium of isopod *Cylisticus convexus* with brooded mancas (m). The marsupium is delimited by oostegites (o) and includes liquid and nutrient secreting cotyledons (arrow). Arrowhead denotes lipid droplets in upper part of cotyledon. From Csonka *et al.* (2015). (D) Terrestrial crab *Geosesarma notophorum* carrying juveniles (arrow) on top of carapace. From Vogt (2013); photo: Oliver Mengedoht. (E) Crayfish *Procambarus virginalis* carrying stage-2 juveniles (arrow) on pleopods. From Vogt & Tolley (2004).

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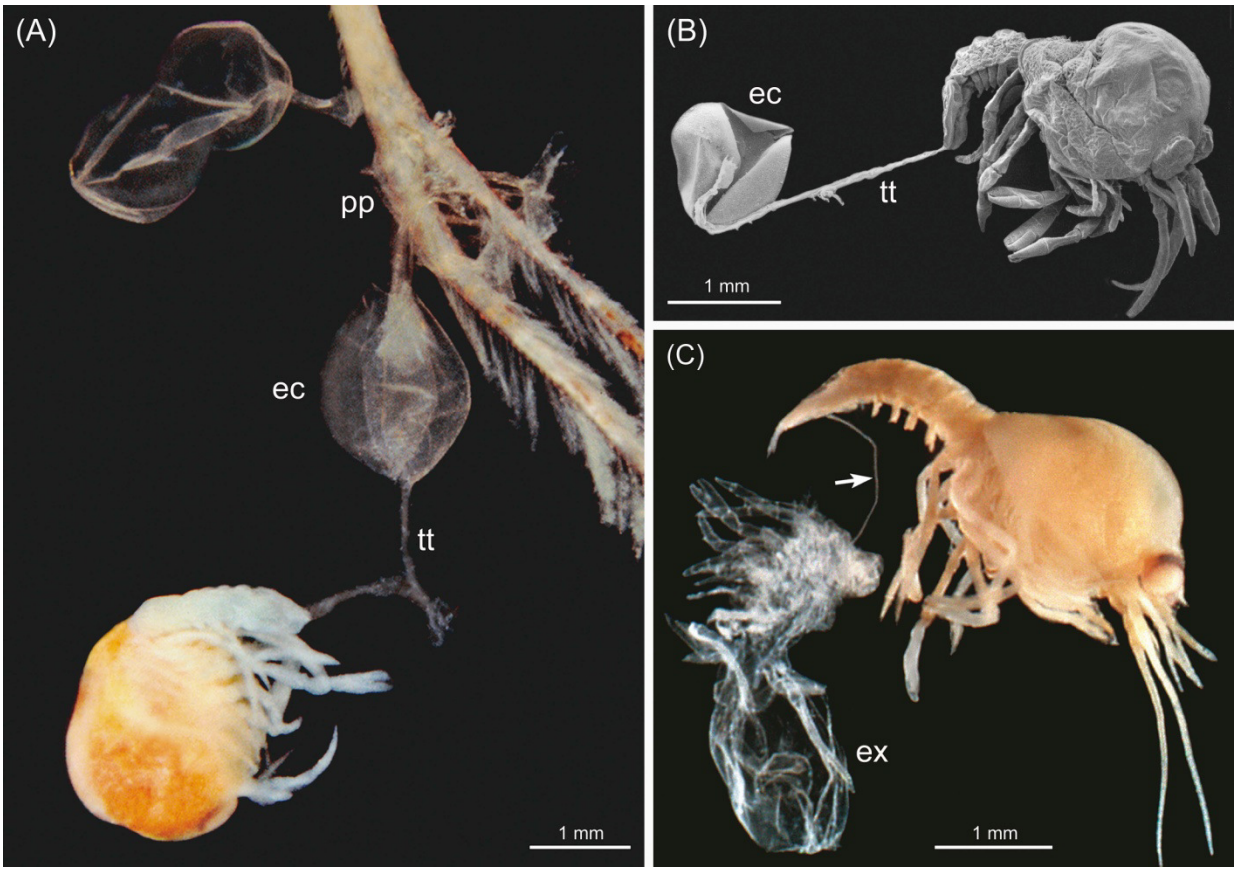

Fig. 6. Safeguarding of brooded crayfish juveniles by safety lines. (A) Safeguarding of hatching by telson thread in *Procambarus virginalis*. The hatchling is secured to the maternal pleopod (pp) via telson thread (tt) and egg case (ec). From Vogt & Tolley (2004). (B) Scanning electron micrograph of telson thread connection between hatchling and egg case. From Vogt & Tolley (2004). (C) Safeguarding of first moult by anal thread in *Procambarus virginalis*. The anal thread (arrow) secures the newly emerged stage-2 juvenile to its exuvia (ex) which in situ is hooked in pleopodal structures of the mother. From Vogt (2008).

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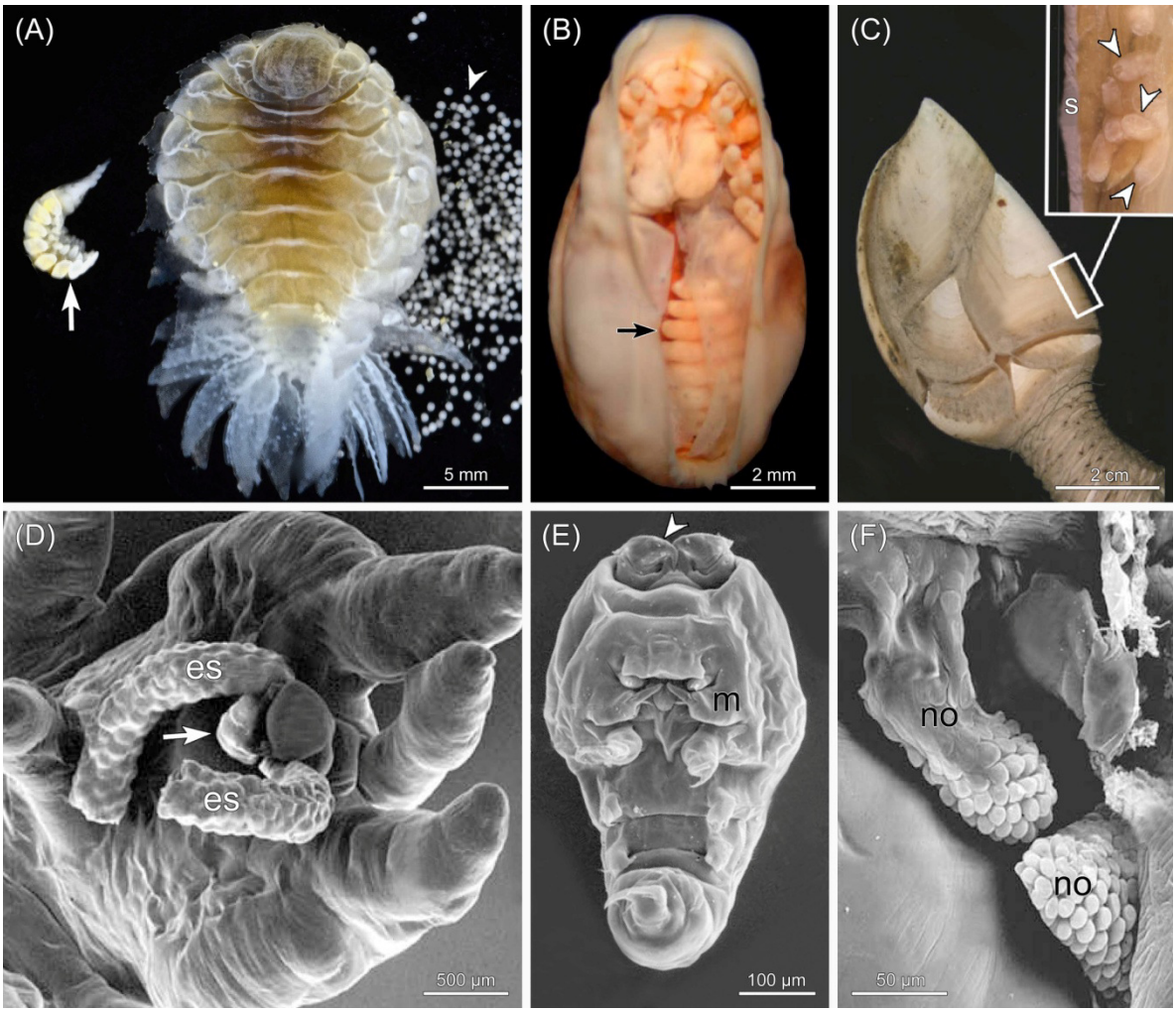

Fig. 7. Dwarf males in isopods (A, B), cirripeds (C) and copepods (D-F). (A) Ovigerous female and male (arrow) of bopyrid *Pseudione overstreeti*. Arrowhead denotes detached eggs. From crabbytaxonomists.com. (B) Ventral view of female dajid *Zonophryxus quinquedens* with attached male (arrow). From Raupach & Thatje (2006). (C) Female gooseneck barnacle *Trianguloscalpellum regium* with several dwarf males (arrowheads) attached to receptacle inside scutal edge (frame). s, scutum. From Yusa *et al.* (2012). (D) Ventral aspect of posterior body part of female *Chondracanthus lophii* with male (arrow) attached between egg sacs (es). From Østergaard & Boxshall (2004). (E) Close-up of male showing hooks on antennae (arrowhead) and well developed maxillae (m). From Østergaard (2004). (F) Pinecone-like nuptial organs (no) of female serving as holdfast and nutrient source for the male. From Østergaard & Boxshall (2004).

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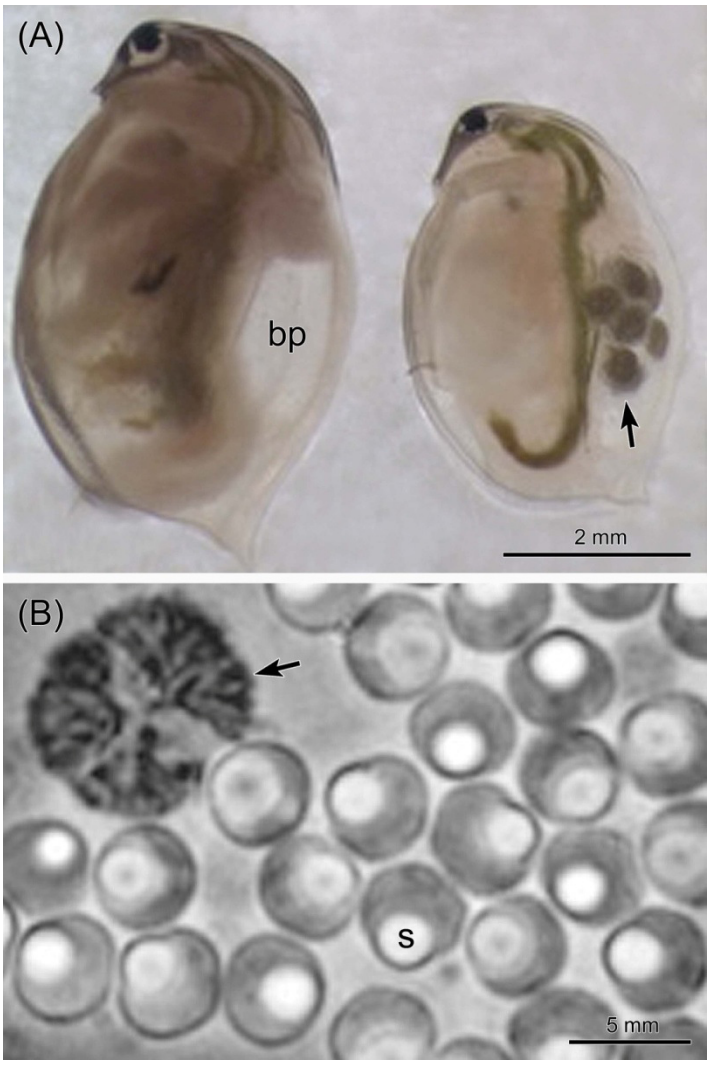

Fig. 8. Castration and gigantism in bacteria-infected water flea. (A) Comparison of healthy (right) and *Pasteuria ramosa*-infected (left) clutch-mates of *Daphnia magna*. The healthy specimen carries a clutch of eggs (arrow) in the brood pouch. The infected specimen is much larger and its brood pouch (bp) is empty indicating gigantism and castration. Its haemolymph spaces are full of bacterial spores. Photo: William A. Nelson. (B) Different life stages of *Pasteuria ramosa.* Arrow: cauliflower-type microcolonies; s, spores. Photo: Matthew D. Hall.

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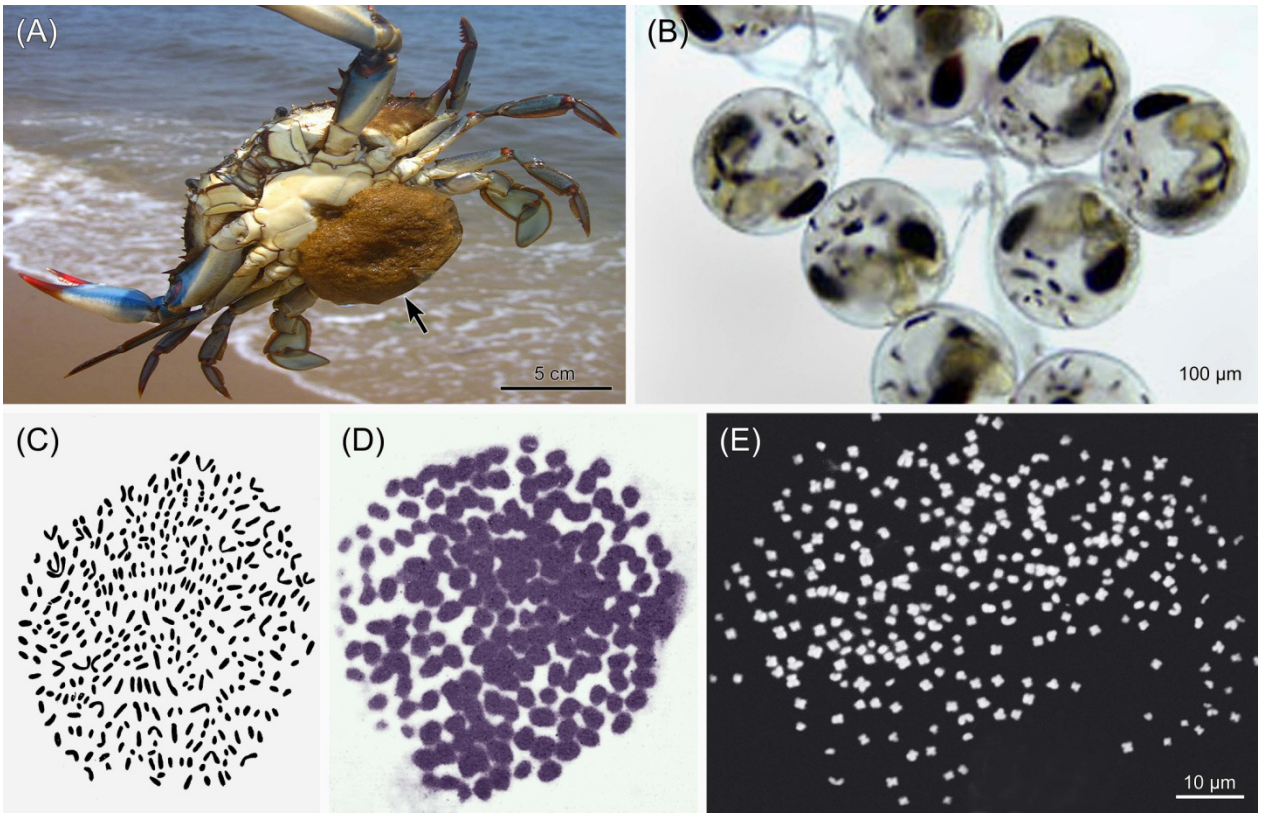

Fig. 9. Record-breaking clutch size and chromosome number in Decapoda. (A) Blue crab *Callinectes sapidus* with "sponge" (arrow) including up to 8 million eggs. Photo: Joe Reynolds. (B) Late eggs from sponge of blue crab. Photo: Thomas H. Shafer. (C) Drawing of spermatogonial metaphase of crayfish *Pacifastacus leniusculus trowbridgii* (2n=376 chromosomes). From Niiyama (1962). (D) Metaphase of primary spermatocyte of *Pacifastacus leniusculus trowbridgii* (n=188 chromosomes). From Niiyama (1962). (E) Metaphase spread of embryonic cell of triploid crayfish *Procambarus virginalis* (3n= 276 chromosomes). From Martin *et al.* (2015).

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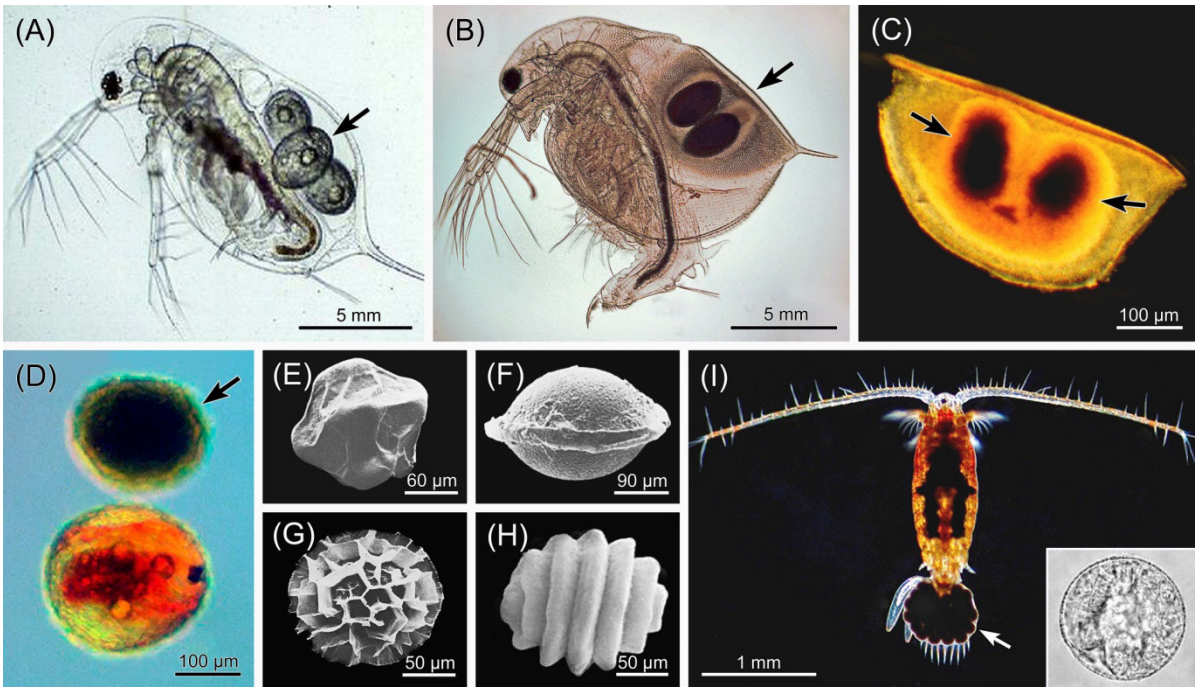

Fig. 10. Resting eggs with record breaking viability in short-lived Crustacea. (A-D) Resting eggs and cysts and their producers in Cladocera. (A) *Daphnia galeata*, the branchiopod record holder with a resting egg viability of 125 years. Arrow denotes brood chamber with subitanous eggs. From novaquatis.eawag.ch. (B) *Daphnia pulex* with almost mature ephippium (arrow) in brood chamber. From forum.mikroscopia.com; photo: Jean-Pierre Claes. (C) Chitinous daphnid ephippium including two resting eggs (arrows). Photo: Nelson Hairston lab. (D) Dormant (arrow) and resurging daphnid egg. Photo: Colleen Kearns. (E-H) Examples of specially shaped and ornamented resting eggs of Branchiopoda. From Thiery & Gasc (1991). (E) *Branchipus schaefferi*. (F) *Tanymastix stagnalis*. (G) *Chirocephalus diaphanus*. (H) *Imnadia yeyetta*. (I) Copepod *Onychodiaptomus sanguineus*, the record holder of dormant egg viability with 332 years. Arrow denotes egg sac. Photo: Ian Gardiner. Inset: resting egg of copepod *Acartia tonsa*. From Diodato *et al.* (2006).

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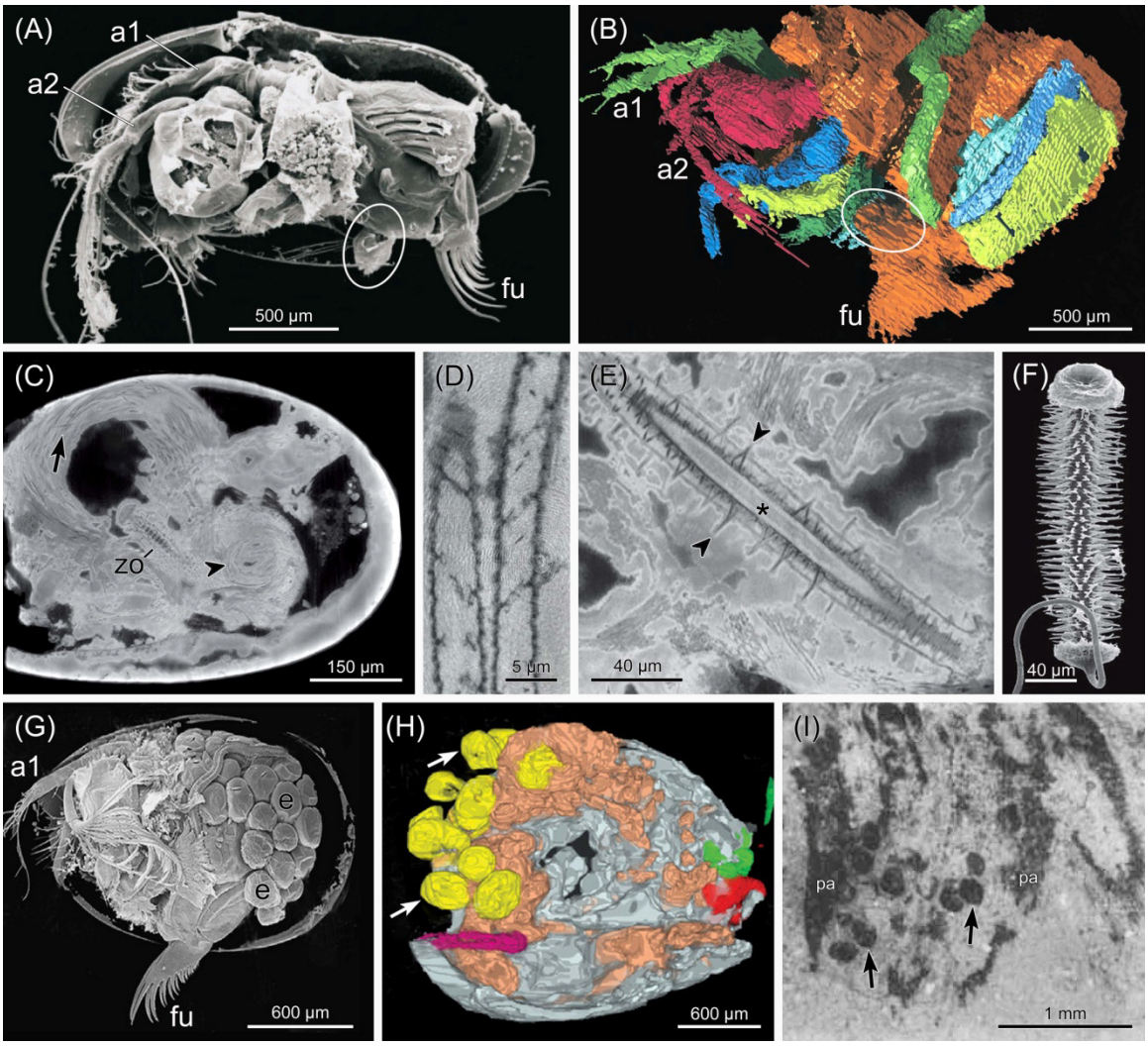

Fig. 11. Record-breaking fossil ages of reproductive structures. (A, B) Copulatory organ of Ostracoda. From Siveter *et al.* (2003). (A) Extant myodocopid *Xenoleberis yamadai* male showing ventrally located copulatory organ (circle). Left lateral view. a1, first antenna; a2, second antenna; fu, furca. (B) Virtual reconstruction of 425 million years old myodocopid *Colymbosathon ecplecticos* male showing soft body parts including the copulatory organ (circle). Left lateral view. (C-F) Sperm and sperm pump of Ostracoda. From Matzke-Karasz *et al.* (2014). (C) Lateral tomographic slice through 16 million years old *Heterocypris collaris* male showing bundles of giant sperm in seminal vesicle (arrow) and vas deferens (arrowhead). zo, Zenker organ acting as sperm pump. (D) Close-up of giant spermatozoa showing longitudinal spiralisation. (E) Close-up of Zenker organ with chitinous spines (arrowheads) around central tube (asterisk). (F) Scanning electron micrograph of Zenker organ of extant *Heterocypris barbara.* (G, H) Brooded embryos in Ostracoda. From Siveter *et al.* (2014). (G) Extant *Vargula hilgendorfii* female showing embryos (e) in brood chamber. Left lateral view. (H) Volume-rendered image of 450 million years old *Luprisca incuba* with brooded embryos (arrows). Oblique ventral view. (I) Ventral body part of 525 million years old crustacean stem line derivative *Kunmingella* showing brood chamber with embryos (arrows). The chamber is delimited by the post-antennular appendages (pa) and their setae. From Shu *et al.* (1999).