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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.

CONTENTS

48

49   **I.   INTRODUCTION**

50

51   Crustacea is a phylogenetically very old animal group that is renowned for its exceptional

52   diversity in morphology, physiology, life history and ecology. The crustaceans date back to

53   the Early Cambrian some 520 million years ago and thus had ample time for experimentation

54   with form and function (Sepkoski, 2000; Harvey *et al.*, 2012). They include six classes, 42

55   orders, 849 extant families and more than 52,000 described species (Martin & Davis, 2001).

56   Maximum body length of the adults ranges from about 0.1 mm in a tantulocarid ectoparasite

57   to more than 1 m in the American lobster (including chelae). Crustaceans occur in marine,

58   limnic and terrestrial habitats from the polar regions to the tropics and from lowlands to high

59   mountains. Most crustaceans are free living but some taxa are sessile or parasitic (Martin &

60   Davis, 2001). Because of this enormous diversity there are a lot of special adaptations in this

61   animal group.

62   The most curious adaptations in the animal kingdom have evolved in context with

63   reproduction, particularly with respect to behaviour. Examples are the sexual cannibalism in

64   praying mantis females, which sometimes eat the males during mating (Lawrence, 1992), and

65   brooding in megapodid birds, which incubate their eggs by heat generated from microbial

66   decomposition or volcanism (Dekker & Brom, 1992). However, there are also reproductive

67   specialities and curiosities related to morphology and anatomy such as the antlers of deer

68   (Price *et al.*, 2005), the love darts of gastropods (Chase & Blanchard, 2006) or the occurrence

69   of three vaginas in kangaroos (Dawson, 1995).

70   The present article highlights structural specialities, curiosities and record-breaking

71   features of reproduction in crustaceans and emphasizes their impact on general biological

72   issues. The topics discussed include inflatable and permanently stiff copulatory organs, giant

73   sperm and explosion sperm, safeguarding of hatching and first moult by safety lines,

74   manipulation of sex and body size by parasites, and records in relative penis length,

75   chromosome number, viability of resting eggs and fossil ages of reproductive structures.

76

77   **II.   EXTENSIBLE VERSUS PERMANENTLY STIFF COPULATORY ORGANS**

78

79   Most crustaceans transfer sperm by copulatory organs. This evolutionary legacy has led to

80   some curiosities in sessile and parasitic groups as explained further down. In this chapter, I

81   want to compare two different construction principles of copulatory organs, the extensible

82   hydraulic penis in sessile Cirripedia and the form-invariant and permanently stiff copulatory

83   organs in Decapoda.

84   Cirripedia are among the few sessile animals that use a penis for sperm transfer (Fig. 1A-

85   C) (Barnes, 1992). They are hermaphrodites but usually do not fertilize themselves. Their

86   unpaired penis is greatly extensible and well suited for sweeping movements to search for

87   functional females in the surroundings (Fig. 1B). It is extended and retreated by modulation of

88   the turgor pressure in the longitudinally running inflatable haemolymph channels (Fig. 1D)

89   (Klepal *et al.*, 1972). The cuticle of the penis is thin and annulated (Fig. 1C, D) facilitating

90   length variation and its structure is highly complex combining mechanical strength with

91   flexibility (Klepal *et al.*, 2010). Searching movements are enabled by a compact layer of

92   longitudinal musculature underneath the epidermis (Fig. 1D) (Klepal *et al.*, 1972). Circular

93   musculature is only found around the central ductus ejaculatorius, which serves for sperm

94   ejection (Klepal *et al.*, 1972). The anterior portion of the penis is studded with rows of

95   sensory setae (Fig. 1C), which are assumed to help in the identification of females.

96   Most intertidal barnacles have a brief reproductive season and only fully develop a

97   functional penis during this period (Barnes, 1992; Klepal *et al.*, 2010). For example, in New

98   York populations of *Semibalanus balanoides,* which reproduce in late October and early

99   November, the penis grows rapidly to its maximum size over September and October and

100   degenerates during November (Horch, 2009). Interestingly, intertidal barnacles can change

101   the size and shape of their penises to suit local hydrodynamic conditions as shown for

102   *Balanus glandula* and *Semibalanus balanoides* (Neufeld & Palmer 2008; Horch, 2009). On

103   wave-exposed shores, they develop shorter penises with greater diameters than in wave-

104   protected sites. In *Balanus glandula*, penis mass corrected for body size was 16% higher in

105   the most exposed site when compared with the most protected site (Neufeld & Palmer, 2008),

106   suggesting that exposed barnacles invest more energy and resources in penis development and

107   function. Transplant experiments between wave-exposed and protected sites revealed

108   phenotypic plasticity as the cause of penis variation rather than differential settlement or

109   selective mortality (Neufeld & Palmer 2008; Horch, 2009). The unusual plasticity of barnacle

110   penises identifies an environmental factor as driver of genital diversification in animals aside

111   of female choice, sexual conflict and male-male competition (Neufeld & Palmer, 2008).

112   Cirripeds are the record holders in relative penis length. For example, the penis of

113   *Cryptophialus minutus* is eight times longer than its body size (Neufeld & Palmer, 2008). The

114   resting penis of *Semibalanus balanoides* is 8–13 mm long, depending on size of the animal,

115   and can be stretched to about four times its resting length (Klepal *et al.*, 1972).

116   A totally different construction of the copulatory organs is found in the Decapoda. Most

117   members of this order of vagile and rather big-sized crustaceans have permanent and form-

118   invariant copulatory organs (Factor, 1995; Holdich, 2002; Becker *et al.*, 2012). In freshwater

119   crayfish and brachyuran crabs each of the paired copulatory organs is composed of the first

120   and second gonopods (Fig. 1E, F), which serve different functions. The first gonopod forms a

121   tube-like structure (Fig. 1G) that takes the sperm from the genital opening. Unlike in the

122   cirriped penis, there is no ductus ejaculatorius in these copulatory organs. For sperm transfer,

123   the second gonopod is inserted into the tube of the first gonopod (Fig. 1F) and acts like a

124   plunger that produces spermatophores of appropriate size and pushes them into the female

125   storage site (Holdich, 2002; Becker *et al.*, 2012). The form constancy and permanent stiffness

126   of both gonopods is achieved by particularly thick cuticles (Fig. 1G) (Brandis *et al.*, 1999;

127   Ewers-Saucedo *et al.*, 2015). The complex structure and form-invariance qualify the

128   gonopods for taxonomic purposes. For example, in cambarid crayfish the morphology of the

129   gonopods is so specific that it is the main criterion for species identification (Hobbs, 1989).

130

131   **III.   GIANT SPERM AND EXPLOSION SPERM**

132

133   Crustacea have evolved an enormous variety of sperm forms and fertilization mechanisms

134   (Jamieson, 1991). A few groups show the classical sperm morphology consisting of a head

135   and a flagellum but most groups have a derived sperm structure. Here, I want to present the

136   two most curious types, the giant sperm of Ostracoda and the explosion sperm of Decapoda.

137   Ostracods of the superfamily Cypridoidea have some of the longest sperm in the animal

138   kingdom, surpassed only by a few insects. Sperm length in cypridoidean species ranges from

139   268 µm to 11.8 mm (Fig. 2A, B) and from 0.33 to 4.3 times the carapace length of the

140   producing male (Smith *et al.*, 2015). The record holder in the animal kingdom is the fly

141   *Drosophila bifurca*, which produces sperm of 58.2 mm, which is 20 times longer than the

142   specimens that manufacture them (Pitnick *et al.*, 1995). However, in contrast to insect giant

143   sperm, in which most of the length is accounted for by exceptionally long flagella, ostracod

144   sperm are aflagellate and represent an extremely elongated sperm-head. The nucleus runs

145   from one end to the other and is partially encased by two enormous, spiralling mitochondria

146   (Fig. 2C, D). Although tailless, these spermatozoa are motile because contractile elements can

147   produce longitudinal rotation of the entire sperm (Matzke-Karazs *et al.*, 2014). During

148   fertilization the entire spermatozoon enters the egg although even the shortest giant sperm is

149   five times as long as the diameter of the corresponding egg (Matzke-Karazs, 2005).

150   The spermatozoa of the Decapoda Reptantia, which include freshwater crayfish, lobsters

151   and crabs, are called "Explosionsspermien" (explosion sperm) in the German literature. Like

152   the sperm of other decapods they are aflagellate and nonmotile (Jamieson, 1991; Tudge &

153   Koenemann, 2009) but can achieve an abrupt short-term motility by a special acrosomal

154   reaction. Reptantian sperm are composed of a compact main body consisting of the nucleus

155   and acrosome and radial arms (Fig. 2E) (Brown, 1966; Talbot & Chanmanon, 1980a; López-

156   Camps *et al.*, 1981; Vogt, 2002). The radial arms are rich in microtubules and become free

157   shortly before fertilization when the spermatozoa are released from the spermatophore

158   (Niksirat *et al.*, 2014).

159   The acrosomal reaction is characterized by an abrupt eversion of the acrosome, which

160   causes a leap-like forward movement (Fig. 2F, G) (Talbot & Chanmanon, 1980b). In vitro

161   induction of sperm reaction in the lobster *Homarus americanus* and video microscopy

162   revealed a reaction time from 0.66 to 5.16 seconds (Tsai & Talbot, 1993). The acrosomal

163   reaction occurred in two phases, eversion of the acrosome and forward tearing of the nucleus.

164   It propelled the entire sperm forward about 18 µm (Fig. 2F) (Tsai & Talbot, 1993). The force

165   generated by the acrosome reaction is sufficient to push the sperm nucleus through the egg

166   coat as shown for lobster (Fig. 2H) and crabs (Brown, 1966; Goudeau, 1982; Talbot *et al.*,

167   1991).

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IV. SPERM STORAGE AND PATERNITY

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171 Multiple paternity seems to be widespread in Crustacea. It is mainly known for Decapoda but

172 there are also examples for Isopoda, Cirripedia and Copepoda (Avise *et al.*, 2011;

173 Dennenmoser & Thiel, 2015). Avise *et al.* (2011) have compiled data on paternity in 11

174 decapod species including shrimps, crayfish, lobsters and crabs. In nine species, multiple

175 paternity was proven with the microsatellite technique for between 20-100% of clutches. The

176 mean number of fathers per clutch and species ranged from 1 to 5.3. The maximum number of

177 fathers per clutch was 11 in *Caridina ensifera* (Yue & Chang, 2010). Multiple paternity

178 requires promiscuous behaviour of the females and sperm storage until egg-laying and

179 fertilization, which can occur weeks, months and even years after the last mating (Jensen &

180 Bentzen, 2012).

181 Usually, the multiple sires contribute differently to the broods (Dennenmoser & Thiel,

182 2015). For example, in crayfish clutches fertilized by more than three males, one sire always

183 dominated the brood by sharing his genes with 50-80% of the offspring (Walker *et al.*, 2002;

184 Yue *et al.*, 2010). The skewed offspring numbers are the result of cryptic female choice and

185 sperm competition, which is, among others, dependent on the mode of sperm storage and

186 sperm mobilization in the females (Birkhead & Pizzari, 2002; Wigby & Chapman, 2004;

187 Dennenmoser & Thiel, 2015). In decapods, sperm can be stored either externally by the

188 attachment of spermatophores to the ventral side of the female or internally in specialized

189 spermathecas and seminal receptacles of the female. In the following, I will present examples

190 for both types and discuss consequences for paternity.

191 In freshwater crayfish, the spermatophores are either externally attached as in Astacidae

192 and Parastacidae or stored in an unpaired cuticular spermatheca without luminal connection to

193 the oviducts as in Cambaridae. Fertilization is generally external (Holdich, 2002). In astacids,

194   the spermatophores are glued to the sternal plate between the genital openings (Fig. 3A) and

195   to the underside of the tail fan. In multiply mated females, late males may leave the

196   spermatophores of earlier males untouched or may remove them partly (Fig. 3B, C) or

197   completely, which significantly influences paternity in the clutch. For example, in

198   *Austropotamobius italicus*, 33% of the males completely removed the spermatophores of

199   previous rivals, 63% left intact some of the rivals' spermatophores and 3% simply added their

200   own sperm (Galeotti *et al.*, 2007). Paternity in species with external sperm storage may also

201   be incidentally biased by mechanical abrasion of spermatophores during locomotion (Galeotti

202   *et al.*, 2007). In cambarid crayfish, males transfer the sperm into the annulus ventralis and

203   then close the opening with a sperm plug (Galeotti *et al*., 2012). Such plugs seem to secure

204   the sperm in the spermatheca rather than block insemination by later males as may be deduced

205   from the high frequency of multipaternity in clutches of cambarids (Walker *et al.*, 2002; Yue

206   *et al.*, 2010; Kahrl *et al.*, 2014).

207   In brachyuran crabs, some species are primarily unipaternal and others are primarily

208   multipaternal (Avise *et al.*, 2011; Dennenmoser & Thiel, 2015). This feature seems to depend

209   mainly on the species-specific mode of sperm storage and sperm competition. In

210   eubrachyurans, the sperm is deposited in paired ventrally located seminal receptacles that are

211   continuous with the oviducts and the vaginas (Fig. 3D) (Becker *et al.*, 2011; McLay & López

212   Greco, 2011; Pardo *et al.*, 2013). The oocytes are fertilized in a fertilization area close to the

213   opening of the oviduct (Fig. 3D) (Becker *et al.*, 2011; McLay & López Greco, 2011). Since

214   the spermatozoa are non-motile the sperm stored closest to the fertilization area have the

215   highest chance to fertilize the eggs. Principally, males have two possibilities aside of female

216   guarding to ensure dominance over the next clutch. They could either remove the sperm of

217   preceding rivals from the seminal receptacle by the intromittent organ or displace it to an

218   unfavourable position and seal it off. Beninger *et al.* (1991) have advocated for the first

219   alternative because the male gonopods of some crabs have recurved spoon-like tips with

220   swap-like bundles of setae (Fig. 3G) resembling the male genitalia of insects for which sperm

221   removal is proven (Waage, 1979; Tsuchiya & Hayashi, 2014). However, until now there is no

222   experimental proof for active sperm removal of predecessors in brachyurans.

223   In contrast, the alternative possibility to gain last-male precedence by ejaculate

224   stratification and sealing (Fig. 3E, F) is proven for several brachyuran species (Diesel, 1990;

225   Urbani *et al.*, 1998; Sainte-Marie *et al.*, 2000; Pardo *et al.*, 2013). For example, in spider crab

226   *Inachus phalangium* the last male to mate displaces the ejaculate of his predecessor dorsally

227   towards the apex of the receptacle and seals it off with a hardening sperm gel. He then places

228   his own sperm closest to the oviduct opening. Similar observations were made in the

229   laboratory in multiply mated snow crab *Chionoecetes opilio*, in which the last male was

230   mostly the only father of the clutch (Urbani *et al.*, 1998). Stratification and sealing of the

231   sperm packages of rivals seems to be more effective in some species than in others (Sainte-

232   Marie *et al.*, 2000; Pardo *et al.*, 2013; Rojas-Hernandez *et al.*, 2014) but even in the most

233   effective species stratification occasionally fails, particularly if the fresh ejaculate is large in

234   volume, resulting in multiple paternity in otherwise unipaternal species (Sainte-Marie *et al.*,

235   2000; Jensen & Bentzen, 2012).

236   The mode of sperm storage and related sperm competition has considerable consequences

237   for population biology, ecology and fisheries. In long-lived species like clawed lobsters and

238   some crabs, sperm can be stored across moults and be utilized for years (Factor, 1995; Jensen

239   & Bentzen, 2012; Pardo *et al.*, 2013). This is made possible by sperm storage in mesodermal

240   sectors of the seminal receptacles that are not shed during ecdysis (Becker *et al.*, 2011; Pardo

241   *et al.*, 2013). For example, in the Tanner crab *Chionoecetes bairdi* females isolated from

242   males after copulation produced viable eggs by 100% in the mating year and 97% and 71% in

243   the following two years (Paul, 1984). In such species, males that die or are captured soon after

244   copulation can contribute to the gene pool of the population even years later (posthumous

245   paternity). In exploited species, in which males are selectively fished, long-term storage of

246   sperm from multiple mates is thought to contribute to the viability and genetic diversity of

247   populations and may even be essential for a sustainable fishery (Sainte-Marie *et al.*, 2008;

248   Pardo *et al.*, 2013).

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250   **V.   GELATINOUS FERTILIZATION TENT**

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252   Freshwater crayfish produce a gelatinous tent-like structure for fertilization of the eggs and

253   their attachment to the pleopods. This tent is formed by the females immediately before egg

254   laying by a secretion from the glair glands and disrupted and shed several hours later when

255   the fertilized eggs are firmly glued to the pleopods. Glair glands and fertilization tent are

256   shared by all crayfish families, the Astacidae, Cambaridae and Parastacidae.

257   Glair glands develop in the weeks before spawning (Thomas & Crawley, 1975) and are

258   good indicators of forthcoming egg-laying. They appear as creamy-white patches on the last

259   thoracic sternal plates, the sterna of the abdomen, the inner sides of the pleura, the pleopods

260   and the uropods of the female. In the final stage of development they are so prominent that

261   they can even be seen from the dorsal side (Fig. 4A). The glair glands are structurally

262   different from other integumental glands and terminate with many pores on the underside of

263   the thorax and pleon (Andrews, 1904; Thomas & Crawley, 1975).

264   Prior to egg laying, crayfish form a pouch by bending the pleon towards the underside of

265   the cephalothorax. This pouch is then filled with a gelatinous secretion from the glair glands

266   (Fig. 4B), which lasts about 30 minutes in *Orconectes limosus* (Andrews, 1906). Lying on her

267   back, the female then releases the eggs into the glair and fertilizes them by sperm that is

268   mobilized from the externally attached spermatophores or the *annulus ventralis* (Mason,

269   1970; Gherardi, 2002). Egg laying takes about 10-30 min in *Orconectes limosus* but may last

270   for hours in other species (Andrews, 1904). The inner mass of the glair is rather fluid but at

271   the contact zones with the water it forms a more rigid parchment-like structure resembling the

272   flysheet of a tent. Within this tent-like structure the eggs are transferred backwards by gravity

273   and paddling movements of the pleopods to be fastened to the oosetae of the pleopods. The

274   attachment process then requires repeated sideward turnings, which usually lasts several hours

275   (Andrews, 1904, 1906; Mason, 1970).

276   The function of the fertilization tent is probably manifold. It may protect the freshly laid

277   eggs that are soft and highly labile from the osmotic stress of fresh water and may provide an

278   optimal milieu for fertilization. The eggs achieve their typical round shape and rigid

279   consistency only after formation of the outermost shell layer during attachment to the

280   pleopods (Andrews 1904, 1906). Another possible function of the fertilization tent is the

281   mechanical confinement of the eggs to the brood pouch during the complicated and time-

282   consuming attachment procedure. This idea is corroborated by the generally very low loss of

283   eggs at this time and the observation that the eggs are not properly attached and decay rapidly

284   if the fertilization tent is damaged.

285   Glair glands and fertilization tents occur in all crayfish species independent of whether

286   they store sperm externally or internally. They are absent in the marine lobsters, the closest

287   relatives of freshwater crayfish (Factor, 1995) and may therefore represent a special

288   adaptation to reproduction in fresh water. However, the other freshwater decapods lack this

289   fertilization tent too, even the freshwater shrimps and aeglids that have external fertilization

290   like the crayfish (Chow *et al.*, 1982; Almerão *et al.*, 2010). Therefore, the fertilization tent has

291   to be regarded as a special characteristic of freshwater crayfish, which has evolved in the stem

292   group that invaded fresh water from the marine environment.

293

294   **VI.   BROODING COMPARTMENTS FOR POSTHATCHING STAGES**

295

296   The simplest pattern of reproduction in Crustacea is the release of eggs and sperm into the

297   water (broadcasting strategy) and larval development in the plankton. However, most

298   crustacean groups have evolved mechanisms to brood their embryos, larvae or even the first

299   juvenile stages on or in the body of the female (Gruner, 1993; Thiel, 2000, 2003). In some

300   clades, for instance in the Peracarida, parental care is obligatory and morphologically so

301   specific that it can be used as a character for phylogenetic analysis (Richter & Scholtz, 2001).

302   In other lineages like the Euphausiacea, brood care is limited to a fraction of the species only

303   (Gómez-Gutiérrez, 2003). Here, I focus on brooding of the offspring in or on the body of the

304   mother requiring specific structural adaptations. Information on brood care in dwellings that is

305   based more on behavioural interactions of mother and offspring is found in reviews by Thiel

306   (2003, 2007) and Vogt (2013).

307   The simplest type of brood care is carrying of the eggs on the mother until hatching of the

308   nauplius larva. Examples are the Euphausiacea among the higher crustaceans (Malacostraca)

309   and the Copepoda among the lower crustaceans ("Entomostraca"). Both groups include

310   broadcasting and sac-spawning species. Species of the krill genera *Nyctiphanes* and

311   *Nematoscelis* brood their eggs in a membranous sac on the thoracopods (Fig. 5A) (Gómez-

312   Gutiérrez, 2003) whereas cyclopoid and some calanoid copepods carry the eggs in

313   membranous sacs on the urosome (Fig. 10I) (Kiørboe & Sabatini, 1994).

314   Brooding of embryos and posthatching stages in dorsal brood chambers is typical of some

315   entomostracan groups like the Cladocera (Fig. 5B) and Ostracoda (Fig. 11G). In cladocerans,

316   the brood chamber is the space between the dorsal side of the trunk and the carapace. The

317   eggs are laid in this pouch (Fig. 10A) and the embryos develop until a miniature adult is

318   released (Fig. 5B) (Mittmann *et al.*, 2014). Some cladocerans with small eggs secrete a fluid

319   rich in nutrients into this chamber, which may be absorbed by the eggs and developing

320   embryos (Schminke, 2007). Interestingly, a dorsal brood pouch is also found in the

321   Thermosbaenacea, which raise the embryos and four posthatching stages in this chamber

322   (Olesen *et al.*, 2015). This type of brood care is exceptional among the Malacostraca, which

323   normally brood their offspring on the ventral side of the body (Fig. 5C, E).

324   Like all Peracarida, the isopods have a ventral brood chamber that is delimited by the

325   oostegites, which are specialized parts of the thoracopods (Fig. 5C) (Csonka *et al.*, 2015).

326   This marsupium probably evolved for mechanical protection of eggs and developing embryos

327   in the sea but has become particularly important for colonization of the land (Hoese &

328   Janssen, 1989). In terrestrial Isopoda, this chamber serves as a micro-aquarium to raise the

329   developmental stages. It contains cotyledons (Fig. 5C) that secrete the marsupial fluid and

330   probably supply the young with nutrients. The latter possibility was inferred from the

331   presence of lipid globules in the cotyledons (Fig. 5C) and the continuity of these organs with

332   the lipid storing fat body of the mother (Hoese & Janssen, 1989).

333   In the Decapoda, the simplest type of brood care is carriage of the eggs until hatching. It

334   is rare in Dendrobranchiata, the basal subclass of the Decapoda, but obligatory in the derived

335   Pleocyemata. Dendrobranchiate shrimps usually broadcast eggs and sperm and only the

336   pelagic *Lucifer* species carry their eggs on the 3rd thoracopods until the nauplii emerge (Lee

337   *et al.*, 1992). The pleocyemates brood their eggs on the pleopods at least until hatching of the

338   zoea larva. Egg carriage can be as long as 16 months as shown for the American lobster

339   *Homarus americanus* (Goudeau *et al.*, 1987), and therefore, egg attachment must be very

340   firm. This requirement is fulfilled by a special egg attachment system that consists of a rigid

341   egg envelope, a firm but elastic egg stalk and the oosetae that arise laterally from the

342   pleopods. This highly effective egg attachment system presumably originated in the common

343   ancestor of the Pleocyemata some 430 million years ago (Porter *et al.*, 2005).

344   In many Pleocyemata, carriage of the young is extended to the larval stages or even to the

345   juveniles. For example, in freshwater crayfish the juveniles are brooded on the pleopods (Fig.

346   5E) at least until the first feeding stage, which is juvenile stage 3 in cambarids. However, if

347   shelters are scarce the brooding period is prolonged (Vogt, 2013). Brood care in freshwater

348   crayfish includes special structural adaptations that secure the young to the mother during

349   hatching and the first moult as discussed in detail in the next chapter. Other decapod examples

350 of brood care extension until the juvenile stage are the freshwater crabs and aeglid anomurans.

351 In these freshwater inhabitants, the brooded stages are carried on the pleopods and are

352 protected in a ventral pouch that is formed by forward folding of the pleon under the

353 cephalothorax. An exceptional case of brood care in the Decapoda is the terrestrial crab

354 *Geosesarma notophorum*, which carries the juveniles on top of the carapace covered by a film

355 of water (Fig. 5D) (Ng & Tan, 1995).

356   Brooding decapods provide protection and grooming but usually do not feed the young,

357 although the offspring may sometimes participate in the maternal meal. The main advantage

358 of brood care is the increased survival and growth of the young (Clutton-Brock, 1991).

359 Disadvantages are the higher energy expenditure of the caring mothers and the pronounced

360 reduction of egg number in brooding species (Anger, 2001; Vogt, 2013). Therefore, this life

361 history strategy should be favoured only under specific circumstances. The Decapoda are

362 particularly suitable to examine this issue in depth because they include non-brooders and

363 brooders of different degrees and have evolved extended brood care independently in several

364 lineages. Interestingly, brooding until the juvenile stage was convincingly demonstrated for

365 less than 30 species of the ~12,000 marine decapods (mainly from polar regions and harsh

366 coastal environments) but is supposed to occurs in about 70% of the ~3.000 species of

367 freshwater decapods (Vogt, 2013). This imbalance suggests that brood care was mainly

368 selected for in environments with heavily fluctuating nutritional and abiotic conditions

369 (Anger, 2001; Vogt, 2013).

370

371 **VII. SAFETY LINES FOR SAFEGUARDING OF HATCHING AND FIRST MOULT**

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373 Freshwater crayfish have evolved effective safety lines, the telson thread and anal thread, to

374 secure the brooded offspring during the immobile and helpless phases of hatching and first

375 moult, respectively. At other times the juveniles can actively hold on to their mother with

376   terminal hooks on the chelipeds (Vogt & Tolley, 2004; Vogt, 2008). Such safety lines are

377   unique in the animal kingdom and have probably evolved during colonization of fresh water

378   by the stem group of crayfish (Scholtz & Kawai, 2002; Vogt, 2013).

379   The telson thread occurs in all crayfish families (Andrews, 1907; Scholtz, 1995; Vogt &

380   Tolley, 2004). It emerges during hatching and probably originates from two sources, a

381   secretion and the detaching inner layer of the egg case. The telson thread extends from the

382   posterior end of the telson of the hatchling to its egg case, which persists on the pleopods after

383   hatching (Fig. 6A, B). During eclosion it keeps the helpless hatchling passively secured to the

384   mother (Fig. 6A) preventing it from being dislodged by the water current. Thereafter, it

385   secures the hatchling during its attempts to hook into pleopodal structures of the mother

386   (Vogt, 2008).

387   The anal thread occurs only in the Cambaridae and Parastacidae. It is composed of

388   cuticular material and originates from delayed moulting of the hindgut (Scholtz, 1995;

389   Rudolph & Rojas, 2003; Vogt, 2008). During moulting of the hatchling it keeps the emerging

390   stage-2 juvenile passively linked to its exuvia (Fig. 6C), which itself remains hooked into

391   pleopodal structures of the mother. In vitro tests revealed that this curious anal thread-exuvia-

392   pleopod connection is firm enough to protect the freshly moulted juveniles from being washed

393   away (Vogt, 2008). After some hours, the anal thread is disconnected by flapping movements

394   of the juveniles, which are now attached to the maternal pleopods by their peraeopodal hooks

395   (Vogt, 2008). A special situation is found in the parastacid species *Astacopsis gouldi* and

396   *Astacopsis franklinii* which secure three juvenile stages by anal threads (Hamr, 1992).

397

398   **VIII. DWARF MALES**

399

400   Dimorphism between females and males is common in crustaceans and results from

401   secondary sex characteristics related to sperm transfer and brood care. The larger sex is

402   sometimes the female and sometimes the male. An extreme form of sexual dimorphism is the

403   reduction of males to dwarf males, which has independently evolved in sessile cirripeds and

404   parasitic isopods, copepods and cirripeds. Dwarf males are much smaller than females of the

405   same species and are usually equipped with special structures to hold on the female. Their

406   internal organs except of the gonads are reduced to different degrees, depending on taxon.

407   The advantage of dwarf males is the permanent availability of males at a relatively low

408   physiological cost under conditions of limited mating opportunities (Vollrath, 1998).

409   In isopods, dwarf males are typical of the ten families of the Bopyroidea and

410   Cryptoniscoidea, which parasitize on other crustaceans from shallow waters to the deep sea.

411   These families comprise 795 species or 7.7% of all isopods and have a shared evolutionary

412   history with their hosts dating back to the Jurassic (Williams & Boyko, 2012). The males

413   reside on the females but their feeding biology is largely unknown. An example is the bopyrid

414   *Pseudione overstreeti* (Fig. 7A) that infests the branchial chamber of the Mexican ghost

415   shrimp *Callichirus islagrande* (Adkison & Heard, 1995). The greatest total length of the

416   females is 19.1 mm and the greatest width is 14.4 mm. In males, the corresponding values are

417   4.9 mm and 2.3 mm, respectively. The male usually attaches to the ventral side of the female's

418   pleon. In the dajid *Zonophryxus quinquedens*, which parasitizes on the carapace of the deep

419   sea Antarctic shrimp *Nematocarcinus longirostris,* the females and males have maximum

420   lengths of 20 mm and 4.5 mm, respectively (Brandt & Janssen, 1994). The dwarf males live

421   on the underside of the attached female (Fig. 7B).

422   In sessile Cirripedia, which are basically hermaphrodites, dwarf males have evolved

423   several times probably because of the limited opportunity to find sexual partners and to reach

424   them with the penis for insemination (Yusa *et al.*, 2012). An example is the pedunculate

425   gooseneck barnacles, which show a broad diversity of sexual systems including simultaneous

426   hermaphroditism, the coexistence of hermaphrodites and dwarf males (androdioecy) and the

427   combination of pure females and dwarf males (dioecy) (Yusa *et al.*, 2012). Androdioecy

428 occurs in shallow-water species that live in large groups and dioecy occurs in the deep sea and

429 in symbiotic species. In the androdioecious *Scalpellum scalpellum* two to five dwarf males are

430 usually attached to a large hermaphrodite. In the deep sea species *Trianguloscalpellum regium*

431 that depends entirely on the presence of dwarf males for fertilization the dwarf males are very

432 small and are attached to the female in groups located in a specific receptacle inside the scutal

433 edge (Fig. 7C).

434   The rhizocephalan cirripeds are endoparasites in marine Decapoda. The body of the

435 female is composed of two parts, a root-like interna that penetrates the host organs and serves

436 for the absorption of nutrients and a sac-like externa that is located on the host's pleon and

437 serves for reproduction. The eggs in the externa are fertilized by the sperm of dwarf males.

438 These can invade the mantel cavity of the female as a very small trichogon of only 200 µm in

439 length that develops from the cypris larva. The trichogons migrate to specific receptacles

440 close to the ovaries, moult at the entrance of the receptacle and seal it with the shed cuticle.

441 Therefore, each of the two receptacles of a female is inhabited by a single trichogon only,

442 which produces sperm and remains together with the female until the end of life (Hoeg, 1991;

443 Schminke, 2007). In species with closed mantles, dwarf males attach to the outside of the

444 mantle and inject spermatogonia through their antennae into the mantle cavity. Depending on

445 species, these spermatogonia settle then in the receptacles or form balls of spermatogonia that

446 float in the mantle cavity to produce sperm (Hoeg, 1991; Schminke, 2007).

447   Several families of parasitic copepods have evolved dwarf males, which are many times

448 smaller than their corresponding females (Østergaard & Boxshall, 2004). In the

449 Chondracanthidae, a family parasitic on marine fishes, the males attach to immature females

450 at the second copepodite stage, complete their development on the female and remain there

451 until they die. A maximum of eight males on a single female has been recorded but in most

452 members of the family adult females rarely have more than one male attached. An example is

453 *Chondracanthus lophii*, in which tiny males adhere near the female genital apertures to so-

454   called nuptial organs (Fig. 7D), which are special structures for holdfast and probably

455   nourishment. The males use the claws on the transformed antennae (Fig. 7E) for attachment.

456   The pinecone-like nuptial organs (Fig. 7F) contain glandular tissue and are assumed to

457   produce a secretion to nourish the males. Adult males have well developed mouthparts (Fig.

458   7E) and a functional oesophagus and midgut but no anus, suggesting that they feed on easily

459   digestible stuff like mucous produced by the female (Østergaard, 2004; Østergaard &

460   Boxshall, 2004).

461

462   **IX. FEMINIZATION, CASTRATION AND GIGANTISM**

463

464   Parasitic crustaceans and microorganisms can significantly change the reproductive biology

465   of crustacean hosts by manipulating sex or causing infertility. The latter is sometimes

466   paralleled by gigantism promoting multiplication of the parasite.

467   Parasitic isopods and cirripeds are well known for their ability to cause feminization or

468   infertility of their crustacean hosts (Schminke, 2007; Williams & Boyko, 2012). For example,

469   in the ghost shrimp *Callichirus islagrande* the reproductive activity is obviously suppressed

470   when infected with the bopyrid *Pseudione overstreeti.* In all of the more than 100 host-

471   parasite associations investigated, the gonads of the hosts were greatly reduced (Adkison &

472   Heard, 1995). Moreover, since only female shrimp were found to be infested some female

473   morphotypes may represent primary males that have changed sex after parasitic manipulation

474   (Adkison & Heard, 1995). Among the parasitic isopods some bopyrids and dajids and all

475   entoniscids and Cryptoniscoidea are assumed to be parasitic castrators of their hosts

476   (Williams & Boyko, 2012).

477   A well-known bacterial manipulator of crustacean reproduction is the intracellular alpha-

478   proteobacterium *Wolbachia pipientis*. Bouchon *et al.* (1998) detected this bacterium in 19

479   species from eight terrestrial isopod families and in one limnic and two estuarine isopod

480   species. Meanwhile, it was also found in a limnic amphipod and a marine gooseneck barnacle

481   (Cordaux *et al.*, 2012). *Wolbachia* was shown to be able to induce feminization in isopods by

482   converting genetic males into functional females (Juchault *et al.*, 1992; Rigaud *et al.*, 1997,

483   2001). However, not all infected males are transformed into females. Transinfection

484   experiments established that the susceptibility or resistance to sex conversion depends much

485   on the combination of host species and *Wolbachia* strain (Cordaux *et al.*, 2004). *Wolbachia* is

486   usually vertically transmitted via the egg cytoplasm but some horizontal transmission has also

487   occurred as demonstrated by genetic analysis. This mode of transmission may explain the

488   occurrence of *Wolbachia* in highly diverse crustacean groups and "infectious feminization"

489   may threaten the genetic diversity of wild populations.

490   A good example of castration and the induction of gigantism in a crustacean host is the

491   *Daphnia magna – Pasteuria ramosa* system (Ebert *et al.*, 1996, 2004; Cressler *et al.*, 2014).

492   *Daphnia* females continue to produce eggs within the first 12 days after infection by the

493   bacterial manipulator (Ebert *et al.*, 1996) but thereafter, most of the infected individuals are

494   castrated (Fig. 8A) to direct nutrients and energy towards reproduction of the parasite. By

495   manipulating food levels during the infection Ebert *et al.* (2004) showed that both antagonists

496   are resource-limited and that there is a negative correlation between host and parasite

497   reproduction, indeed. Curiously, a certain proportion of the saved energy is channelled into

498   growth of the host resulting in gigantism (Fig. 8A). Although illogical at first glance, the

499   parasite benefits from this resource allocation because it can produce more spores in a bigger

500   host (Ebert *et al.*, 2004). *Pasteuria ramosa* has a polymorphic life cycle beginning with

501   cauliflower-like rosettes and ending with individual spores (Fig. 8B). It is horizontally

502   transmitted through spores that are released from dead host bodies into the water.

503

504   **X.   RECORD-BREAKING CLUTCH SIZE AND CHROMOSOME NUMBER**

505

506 Crustaceans are among the animals with the highest clutch sizes. Particularly high numbers of

507 eggs per clutch are produced by brachyuran decapods. Examples are *Metacarcinus anthonyi*

508 and *Scylla tranquebarica*, which can have 3.8 and 5 million eggs per clutch, respectively

509 (Hines, 1991; Srinivasagam *et al.*, 2000). In the blue crab *Callinectes sapidus*, the eggs are

510 brooded until hatching of the zoea larvae in a so-called sponge under the pleon (Fig. 9A, B).

511 The average sponge contains about three million eggs but in large females it was shown to

512 include up to 8 million eggs (Prager *et al.*, 1990). In some *Cancer* species, the number of eggs

513 produced per female and lifetime was estimated to more than 20 million (Hines, 1991) but in

514 *Callinectes sapidus*, which has up to 18 broods (Graham *et al.*, 2012), this value may be

515 exceeded.

516 The record in chromosome number in animals is hold by the freshwater crayfish

517 *Pacifastacus leniusculus trowbridgii* with a diploid set of 376 chromosomes (Niiyama, 1962).

518 This corresponds to a chromosome number of 188 in the gametes. The second highest

519 chromosome number (3n=276) was recently found in the triploid crayfish *Procambarus*

520 *virginalis* (Fig. 9C) (Martin *et al.*, 2015). *Procambarus virginalis* is the only obligatory

521 parthenogenetic decapod known (Martin *et al.*, 2015; Vogt *et al.*, 2015). It reproduces by

522 apomictic parthenogenesis, i. e. without meiosis, and therefore, the eggs should include 276

523 chromosomes as well, making *Procambarus virginalis* the new animal world record holder

524 with respect to chromosome number of gametes.

525 The high chromosome numbers in freshwater crayfish are explained by whole genome

526 duplication events in their early evolution. The arguments for this assumption come from the

527 observation that some crayfish species have the double and four-fold chromosome numbers of

528 others (Lécher *et al.*, 1995; Martin *et al.*, 2015) and the generally exceptionally high polyploid

529 index in Decapoda (Otto & Whitton, 2000). The decapods have haploid chromosome numbers

530 ranging from 27 to 188 and a polyploid index of 41.7%. Ancient polyploidization is

531 particularly supposed for the Astacidea, Palinuridae and Paguroidea (Otto & Whitton, 2000).

532 However, the increase in chromosome number within a lineage is not paralleled by an

533 increase in genome size, which would contradict the polyploidization hypothesis. For

534 example, *Astacus astacus* has fewer chromosomes (2n=176) than *Orconectes virilis* (2n=200)

535 (Martin *et al.*, 2015) but has a much larger genome size (19.64 pg versus 4.69 pg) (Jeffery,

536 2015), suggesting that high chromosome numbers may at least partly result from evolutionary

537 chromosome fragmentation.

538

539 **XI.   RECORD-BREAKING VIABILITY OF DIAPAUSING EGGS**

540

541 Short-lived members of the limnic and coastal marine plankton and ephemeral water bodies

542 often produce resting eggs that can survive adverse environmental conditions for years

543 (Radzikowski, 2013). In the Crustacea, such diapausing eggs and cysts are produced by the

544 Branchiopoda, Copepoda and Ostracoda. Under laboratory conditions resting eggs of the

545 anostracan *Branchinecta packardi*, the notostracan *Triops longicaudatus* and the

546 conchostracan *Caenestheriella gynecia* remained viable for a minimum of 16, 14 and 8 years,

547 respectively (Radzikowski, 2013). Eggs of the cladoceran *Daphnia pulicaria* (Fig. 10A) and

548 the copepods *Boeckella poppei* and *Onychodiaptomus sanguineus* (Fig. 10I) isolated from

549 accurately dated lake sediments even hatched after 125, 196 and 332 years, respectively

550 (Hairston *et al.*, 1995; Cáceres, 1998; Jiang *et al.*, 2012). The adaptation of short-lived

551 animals to adverse and changing environments by resting eggs has apparently evolved in the

552 earliest metazoans already (Cohen *et al.*, 2009).

553   Crustacean resting eggs can be the result of parthenogenetic or bisexual reproduction,

554 depending on taxon (Radzikowski, 2013). Cladocerans like *Daphnia* usually produce

555 subitaneous summer eggs (Fig. 10A) by parthenogenesis and resting winter eggs (Fig. 10B-D)

556 by sexual reproduction. Resting eggs are usually highly tolerant to environmental stressors

557 like drying, freezing, UV radiation and mechanical damage. For example, the cysts of the

558 anostracan *Artemia franciscana*, a common model of dormancy, can survive prolonged

559 exposure to temperatures of -271°C and +100°C (Radzikowski, 2013). This stress tolerance is

560 achieved by protecting coverings that are often shaped and ornamented in a group or species-

561 specific manner (Fig. 10E-H) (Thiery & Gasc, 1991; Fryer, 1996). These coverings also

562 permit passage through the guts of birds facilitating dispersal and colonization of isolated

563 water bodies (Radzikowski, 2013). In some cladocerans, the resting eggs are additionally

564 enveloped by a cuticular ephippium, which is derived from the carapace (Fig. 10C). Stress

565 resistance is further provided by cryoprotectants like trehalose and glycerol and molecular

566 chaperones like heat shock proteins (MacRae, 2010; Radzikowski, 2013).

567 The production of long-lived diapausing eggs in crustaceans is a bet-hedging strategy that

568 constitutes an ecological and evolutionary reservoir. Mobilization of this reservoir can help

569 the actual population to respond to environmental changes by enhancing the genetic variation

570 and species richness (Hairston, 1996; Cáceres, 1998). In Oneida Lake, New York, diapausing

571 eggs accumulate in the sediments to densities of 2.5 x 10^4 eggs/m^2 for *Daphnia galeata* and

572 8.0 x 10^4 eggs/m^2 *for Daphnia pulicaria* (Cáceres, 1998). ^{210}Pb dating of sediments suggest

573 that these eggs can remain viable for >125 years and that the two *Daphnia* populations have

574 persisted in the lake for >200 years. Annual emergence rates back to the water column range

575 between 0 and 25 *Daphnia*/m^2. Because annual variation in the size of the overwintering

576 water-column population ranges between 0 and 2.5 individuals/L, the contribution of

577 emergence to the development of the spring population is considerable in some years and

578 negligible in others (Cáceres, 1998). In some copepod species, the density of diapausing eggs

579 is >10^6/m^2 and their annual mortality rate is approximately 1% (Hairston *et al.*, 1995;

580 Hairston, 1996).

581 Egg banks of crustaceans are in many ways analogous to the seed banks of terrestrial

582 plant species (Cáceres, 1998) and are valuable tools for ecological, biogeographical and

583 evolutionary research. For example, they are useful to reconstruct ancient plankton

584   communities and to study their change over time (Ohtsuki *et al.*, 2015). They also reflect

585   invasion histories as shown for the introduction and spreading of a parthenogenetic American

586   *Daphnia pulex* clone in Africa and the subsequent displacement of native *Daphnia pulex*

587   populations by this clone (Mergeay *et al.*, 2006). Crustacean egg banks were also used to

588   investigate the influence of man-made eutrophication on plankton communities (Brede *et al.*,

589   2008). Short-term evolutionary changes of the genotype were studied on the example of the

590   increasing resistance of *Daphnia galeata* in Lake Constance, Central Europe, to nutritionally

591   poor and toxic cyanobacteria that drastically proliferated after eutrophication (Hairston *et al.*,

592   1999).

593

594   **XII.   RECORD-BREAKING FOSSIL AGES OF REPRODUCTIVE STRUCTURES**

595

596   Ostracod crustaceans provide one of the most complete and consistent fossil records of any

597   animal group including tens of thousands fossil species dating back to the Ordovician

598   (Rodriguez-Lazaro & Ruiz-Muñoz, 2012). Usually, only their shells are preserved but in the

599   last decade some exceptional fossils with soft body parts have been detected. They revealed,

600   among other things, record-breaking ages of copulatory organs, sperm and brooded embryos.

601   Most ostracods are sexually reproducing and transfer sperm with complex paired

602   copulatory organs called hemipenes (Fig. 11A) (Mc Gregor & Kesling, 1969; Karanovic,

603   2012). In an exceptionally well-preserved specimen of the myodocopid *Colymbosathon*

604   *ecplecticos* from the Lower Silurian of Herefordshire, England, 3-D reconstruction revealed

605   amazing details of the soft body including a copulatory organ (Siveter *et al.*, 2003). This

606   ostracod was preserved as a three-dimensional calcite infill in nodules hosted within volcanic

607   ash. The copulatory organ detected is relatively large and stout (Fig. 11B), projects anteriorly

608   and has lobe-like distal flanks. With an age of 425 million years it is the oldest penis

609   documented for any animal.

610   Ostracods of the suborder Cypridocopina are famed for having some of the longest sperm

611   in the animal kingdom as discussed above. Matzke-Karasz *et al.* (2014) have recently

612   discovered fossil giant sperm by X-ray synchrotron microtomography in five ostracods (one

613   male and four females) from the early Miocene of Queensland, Australia. The 16 million

614   years old specimens belong to the species *Heterocypris collaris* and *Newnhamia mckenziana*.

615   Giant sperm bundles were found in the seminal vesicles and vasa deferentia of the male (Fig.

616   11C) and the sperm receptacles of the females. These bundles included spermatozoa of

617   excellent three-dimensional preservation showing subcellular features such as longitudinal

618   coiling (Fig. 11D) and spiraling of the sperm nucleus. Other well-preserved reproductive

619   structures in the male were the paired Zenker organs (Fig. 11E, F), which are chitinous and

620   muscular pumps that help to transfer the sperm into the females (Yamada & Matzke-Karasz,

621   2012).

622   The sperm of *Heterocypris collaris* and *Newnhamia mckenziana* are the oldest giant

623   sperm on record and the third oldest sperm of any animal. Older fossil sperm were found in a

624   50 million years old annelid cocoon from Antarctica (Bomfleur *et al.*, 2015) and a spring tail

625   trapped 40 million years ago in Baltic amber (Poinar, 2000). The giant sperm of ostracods are

626   exceptional in as far as they are considered to have originated only once some 100 million

627   years ago and have been retained since then, which stands in contrast to the rapid sperm

628   evolution in other taxa (Smith *et al.*, 2015). Indirect evidence of their occurrence already in

629   the Cretaceous comes from the detection of Zenker organs (Fig. 11F), which are restricted to

630   taxa with giant sperm (Matzke *et al.*, 2009). The long history and persistence of giant sperm

631   in ostracods makes them a unique model to study the evolutionary significance and function

632   of this unusual sperm type in animals (Matzke-Karasz *et al.*, 2014; Smith *et al.*, 2015).

633   Structures indicative of brood care are only rarely found in invertebrate fossils (Wang et

634   *al.*, 2015). In 2007, Siveter and colleagues detected a 425 million years old myodocopid

635   ostracod, *Nymphatelina gravida*, in Herefordshire, England, which included 20 ovoid and two

636   valve-shaped structures of a mean length of 558 mm in the posterior domiciliar area. These

637   globules were interpreted as eggs and juveniles in a marsupium-like brood chamber, which is

638   a unique combination in fossil invertebrates (Siveter *et al.*, 2007). Later, Siveter *et al.* (2014)

639   found a pyritized ostracod, *Luprisca incuba*, with well preserved embryos in the Upper

640   Ordovician of central New York State, USA (Fig. 11H). This discovery provides conclusive

641   evidence of a conserved brood-care strategy within the myodocopid Ostracoda for at least 450

642   million years (Fig. 11G, H).

643   The oldest crustacean fossil showing structural features of brood care is *Kunmingella*

644   *maotianshanensis* from the Lower Cambrian Chengjiang Lagerstätte in China aged to 525-

645   530 million years ago. This specimen belongs to the Bradoriida, an early derivative of the

646   stem line Crustacea. *Kunmingella* was dorsoventrally flattened and crawling on the surface of

647   the sediment (Shu *et al.*, 1999). Clusters of rounded bodies of 150 µm in diameter were found

648   in a ventral chamber of the fossil delimited by the post-antennular appendages and their distal

649   setae (Fig. 11I). These globular bodies are interpreted as brooded embryos, resembling brood

650   care in the recent phyllocarid *Nebalia bipes*, which has a similar basket-like brood pouch

651   created by intersecting setae of the thoracic appendages (Shu *et al.*, 1999). Brood care in

652   *Kunmingella maotianshanensis* suggests that this reproductive strategy dates back to the early

653   radiation of the Metazoa.

654

655   **XIII. CONCLUSIONS**

656

657   (1) Most crustaceans transfer sperm with copulatory organs. These can be either

658   extensible as in cirripeds or form-invariant as in decapods. The construction of the former

659   type resembles vertebrate penises whereas permanent stiffness in the latter type is achieved by

660   thick cuticles. In sessile cirripeds, penis length and width can be adapted to population density

661   and habitat. Their penises are among the record-holders with respect to relative size.

662   (2) Sperm structure is very variable in crustaceans. Particularly interesting are the

663   aflagellate giant sperms of ostracods and the explosion sperms of decapods. The ostracods are

664   among the record holders with respect to sperm size. The immobile explosion sperm of

665   lobsters generate short-term motility by abrupt eversion of the acrosome, which is essential

666   for penetration of the ovarian envelope.

667   (3) Multiple mating is widespread in decapods. Paternity in such species is much

668   dependent on the mode of female sperm storage and male strategies to remove or seals the

669   ejaculates of predecessors (sperm competition). Long-term storage of sperm in female

670   receptacles for months and years is of particular relevance in commercially exploited

671   populations, in which gender proportions fluctuate heavily due to selective fishery of males.

672   (4) Prior to egg-laying, the females of freshwater crayfish produce a unique gelatinous

673   tent on the underside of their body, which facilitates external fertilization of the eggs and their

674   attachment to the pleopods. Interestingly, such a fertilization tent is lacking in clawed

675   lobsters, the closest marine relatives of crayfish, but also in other freshwater decapods.

676   (5) Brood care has independently evolved in many groups of the Crustacea. Brooded

677   embryos, larvae and juveniles are carried either in internal brood pouches or on the

678   thoracopods and pleopods of the mothers. Unique brooding structures are the cotyledons of

679   terrestrial isopods that secrete a nutrient-rich fluid and the telson thread and anal thread of

680   freshwater crayfish that safeguard hatching and the first moult.

681   (6) A most curious phenomenon related to crustacean reproduction is the feminization,

682   castration and induction of gigantism by parasitic crustaceans and bacterial manipulators.

683   Bacteria can significantly change the dynamics of populations by infectious feminization. A

684   further curiosity is the occurrence of dwarf males in ecto-parasitic isopods, copepods and

685   cirripeds and sessile cirripeds from extreme environments. In some copepods the males are

686   obviously nourished by a secretion from specialized female organs.

687   (7) The highest chromosome numbers in diploid and polyploid animals and animal

688   gametes were found in freshwater crayfish with 376, 276 and 276 chromosomes, respectively.

689   This phenomenon is probably the result of polyploidisation and chromosome fragmentation

690   events during crayfish evolution. The clutch sizes of up to eight million eggs in marine crabs

691   are record-breaking as well.

692   (8) Some cladocerans and copepods produce resting eggs with record-breaking viabilities

693   of up to 332 years, providing excellent examples of posthumous parenthood. Egg banks with

694   dormant eggs enable the investigation of plankton fluctuation over time, the reconstruction of

695   pristine aquatic ecosystems before man-made pollution, invasion histories, and short-term

696   genotype changes within species.

697   (9) Crustaceans also hold some records with respect to fossil ages of reproductive

698   structures. A 425 million years old ostracod copulatory organ is the oldest penis ever found,

699   16 million years old ostracod sperm is the oldest giant sperm on record and the third oldest

700   sperm of any animal, and eggs in a marsupium-like chamber of a 525 million years old

701   Crustacean stem line derivative is the oldest evidence of brood care in animals.

702

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723

724   **XV. REFERENCES**

725

726   ADKISON, D. L. & HEARD, R. W. (1995). *Pseudione overstreetii*, new species (Isopoda:

727   Epicaridea: Bopyridae), a parasite of *Callichirus islagrande* (Decapoda: Anomura:

728   Callianassidae) from the Gulf of Mexico. *Gulf Research Reports* **9**, 105–111.

729   ALMERÃO, M., BOND-BUCKUP, G. & MENDONÇA, M. S. JR. (2010). Mating behavior of *Aegla*

730   *platensis* (Crustacea, Anomura, Aeglidae) under laboratory conditions. *Journal of*

731   *Ethology* **28**, 87–94.

732   ANDREWS, E. A. (1904). Breeding habits of crayfish. *American Naturalist* **38**, 165–206.

733   ANDREWS, E. A. (1906). Egg-laying of crayfish. *American Naturalist* **40**, 343–356.

734   ANDREWS, E. A. (1907). The young of the crayfishes *Astacus* and *Cambarus*. *Smithsonian*

735   *Contributions to Knowledge* **35**, 1–79.

736   ANGER, K. (2001). *The Biology of Decapod Crustacean Larvae.* Crustacean Issues 14.

737   Balkema, Lisse.

738 AVISE, J. C., TATARENKOV, A. & LIU, J.-X. (2011). Multiple mating and clutch size in

739   invertebrate brooders versus pregnant vertebrates. *Proceedings of the National Academy*

740   *of Sciences of the United States of America* **108**, 11512–11517.

741 BARNES, M. (1992). The reproductive periods and condition of the penis in several species of

742   common cirripedes. *Oceanography and Marine Biology Annual Reviews* **30**, 483–525.

743 BECKER, C., BRANDIS, D. & STORCH, V. (2011). Morphology of the female reproductive

744   system of European pea crabs (Crustacea, Decapoda, Brachyura, Pinnotheridae). *Journal*

745   *of Morphology* **272**, 12–26.

746 BECKER, C., TÜRKAY, M. & BRANDIS, D. (2012). The male copulatory system of European pea

747   crabs (Crustacea, Brachyura, Pinnotheridae). *Journal of Morphology* **273**, 1306–1318.

748 BENINGER, P. G., ELNER, R. W. & POUSSART, Y. (1991). Gonopods of the majid crab

749   *Chionoecetes opilio* (O. Fabricius). *Journal of Crustacean Biology* **11**, 217–228.

750 BIRKHEAD, T. R. & PIZZARI, T. (2002). Postcopulatory sexual selection. *Nature Reviews*

751   *Genetics* **3**, 262–273.

752 BOMFLEUR, B., MÖRS, T., FERRAGUTI, M., REGUERO, M. A. & McLOUGHLIN, S. (2015).

753   Fossilized spermatozoa preserved in a 50-Myr-old annelid cocoon from Antarctica.

754   *Biology Letters* **11**, 20150431.

755 BOUCHON, D., RIGAUD, T. & JUCHAULT, P. (1998). Evidence for widespread *Wolbachia*

756   infection in isopod crustaceans: molecular identification and host feminization.

757   *Proceedings of the Royal Society B* **265**, 1081–1090.

758 BRANDIS, D., STORCH, V. & TÜRKAY, M. (1999). Morphology and function of the copulatory

759   system in freshwater crabs of the Genus *Potamon*. *Journal of Morphology* **239**, 157–166.

760 BRANDT, A. & JANSSEN, H. H. (1994). Redescription of *Zonophryxus quinquedens* Barnard,

761   1913 (Crustacea, Isopoda, Dajidae) from the Weddell Sea, Antarctica, with notes on its

762   biology and zoogeography. *Polar Biology* **14**, 343–350.

763 BREDE, N., SANDROCK, C., STRAILE, D., SPAAK, P., JANKOWSKI, T., STREIT, B. & SCHWENK, K.

764   (2008). The impact of human-made ecological changes on the genetic architecture of

765   *Daphnia* species. *Proceedings of the National Academy of Sciences of the United States*

766   *of America* **106**, 4758–4763.

767 BROWN, G. G. (1966). Ultrastructural studies of sperm morphology and sperm-egg interaction

768   in the decapod *Callinectes sapidus*. *Journal of Ultrastructure Research* **14**, 425–440.

769 CÁCERES, C. E. (1998). Interspecific variation in the abundance, production, and emergence of

770   *Daphnia* diapausing eggs. *Ecology* **79**, 1699–1710.

771 CHASE, R. & BLANCHARD, K. C. (2006). The snail's love-dart delivers mucus to increase

772   paternity. *Proceedings of the Royal Society B* **273**, 1471–1475.

773 CHOW, S., OGASAWARA, Y. & TAKI, Y. (1982). Male reproductive system and fertilization of

774   the palaemonid shrimp *Macrobrachium rosenbergii*. *Bulletin of the Japanese Society of*

775   *Scientific Fisheries* **48**, 177–183.

776 CLUTTON-BROCK, T. H. (1991). *The Evolution of Parental Care.* Princeton University Press,

777   Princeton.

778 COHEN, P. A., KNOLL, A. H. & KODNER, R. B. (2009). Large spinose microfossils in Ediacaran

779   rocks as resting stages of early animals. *Proceedings of the National Academy of Sciences*

780   *of the United States of America* **116**, 6519–6524.

781 CORDAUX, R., MICHEL-SALZAT, A., FRELON-RAIMOND, M., RIGAUD, T. & BOUCHON, D.

782   (2004). Evidence for a new feminizing *Wolbachia* strain in the isopod *Armadillidium*

783   *vulgare*: evolutionary implications. *Heredity* **93**, 78–84.

784 CORDAUX, R., PICHON, S., BEN AFIA HATIRA, H., DOUBLET, V., GRÈVE, P., MARCADÉ, I.,

785   BRAQUART-VARNIER, C., SOUTY-GROSSET, C., CHARFI-CHEIKHROUHA, F. & BOUCHON, D.

786   (2012). Widespread *Wolbachia* infection in terrestrial isopods and other crustaceans.

787   *ZooKeys* **176**, 123–131.

788 CRESSLER, C. E., NELSON, W. A., DAY, T. & MCCAULEY, E. (2014). Starvation reveals the

789 cause of infection-induced castration and gigantism. *Proceedings of the Royal Society B*

790 **281**, 20141087.

791 CSONKA, D., HALASY, K. & HORNUNG, E. (2015). Histological studies on the marsupium of

792 two terrestrial isopods (Crustacea, Isopoda, Oniscidea). *ZooKeys* **515**, 81–92.

793 DAWSON, T. J. (1995). *Kangaroos: Biology of the Largest Marsupials*. Cornell University

794 Press, Ithaca.

795 DECKER, R. W. R. J. & BROM, T. G. (1992). Megapode phylogeny and the interpretation of

796 incubation strategies. *Zoologische Verhandelingen* **278**, 19–31.

797 DENNENMOSER, S. & THIEL, M. (2015). Cryptic female choice in crustaceans. In *Cryptic*

798 *Female Choice in Arthropods* (eds A. V. Peretti and A. Aisenberg), pp. 203–237.

799 Springer, Heidelberg.

800 DIESEL, R. (1990). Sperm competition and reproductive success in the decapod *Inachus*

801 *phalangium* (Majidae): a male ghost spider crab that seals off rivals' sperm. *Journal of*

802 *Zoology* **220**, 213–223.

803 DIODATO, S. L., BERASATEGUI, A. A. & HOFFMEYER, M. S. (2006). Morphological types and

804 seasonal variation in eggs of zooplankton species from bottom sedimants in Bahía Blanca

805 Estuary, Argentina. *Brazilian Journal of Oceanography* **54**, 161–167.

806 EBERT, D., RAINLEY, P., EMBLEY, T. M. & SCHOLZ, D. (1996). Development, life cycle,

807 ultrastructure and phylogenetic position of *Pasteuria ramosa* Metchnikoff 1888:

808 rediscovery of an obligate endoparasite of *Daphnia magna* Straus. *Philosophical*

809 *Transactions of the Royal Society B* **351**, 1689–1701.

810 EBERT, D., CARIUS, H.-J., LITTLE, T. & DECAESTECKER, E. (2004). The evolution of virulence

811 when parasites cause host castration and gigantism. *American Naturalist* **164** *Supplement*,

812 S19–32.

813 EWERS-SAUCEDO, C., HAYER, S. & BRANDIS, D. (2015). Functional morphology of the

814 copulatory system of box crabs with long second gonopods (Calappidae, Eubrachyura,

815 Decapoda, Crustacea). *Journal of Morphology* **276**, 77–89.

816 FACTOR, J. R. (1995). *Biology of the Lobster Homarus americanus*. Academic Press, San

817 Diego.

818 FRYER, G. (1996). Diapause, a potent force in the evolution of freshwater crustaceans.

819 *Hydrobiologia* **320**, 1–14.

820 GALEOTTI, P., PUPIN, F., RUBOLINI, D., SACCHI, R., NARDI, P. A. & FASOLA, M. (2007). Effects

821 of female mating status on copulation behaviour and sperm expenditure in the freshwater

822 crayfish *Austropotamobius italicus*. *Behavioral Ecology and Sociobiology* **61**, 711–718.

823 GALEOTTI, P., BERNINI, G., LOCATELLO, L., SACCHI, R., FASOLA, M. & RUBOLINI, D. (2012).

824 Sperm traits negatively covary with size and asymmetry of a secondary sexual trait in a

825 freshwater crayfish. *PLoS ONE* **7**, e43771.

826 GHERARDI, F. (2002). Behaviour. In *Biology of Freshwater Crayfish* (ed. D. M. Holdich), pp.

827 258–290. Blackwell, Oxford.

828 GÓMEZ-GUTIÉRREZ, J. (2003). Hatching mechanism and accelerated hatching of the eggs of a

829 sac-spawning euphausiid *Nematoscelis difficilis*. *Journal of Plankton Research* **25**, 1397–

830 1411.

831 GOUDEAU, M. (1982). Ferilization in a crab: I. Early events in the ovary, and cytological

832 aspects of the acrosome reaction and gamete contacts. *Tissue and Cell* **14**, 97–111.

833 GOUDEAU, M., TALBOT, P. & HARPER, R. (1987). Mechanism of egg attachment stalk

834 formation in the lobster, *Homarus*. *Gamete Research* **18**, 279–289.

835 GRAHAM, D. J., PERRY, H., BIESIO, P. & FULFORD, R. (2012). Fecundity and egg diameter of

836 primiparous and multiparous blue crab *Callinectes sapidus* (Brachyura: Portunidae) in

837 Mississippi waters. *Journal of Crustacean Biology* **32**, 49–56.

838   GRUNER, H.-E. (1993). Crustacea. In *Arthropoda (ohne Insecta). Lehrbuch der Speziellen*

839   *Zoologie, Band I, 4. Teil* (ed. H.-E. Gruner), pp. 448–1030. Gustav Fischer Verlag, Jena.

840   HAIRSTON, N. G. JR. (1996). Zooplankton egg banks as biotic reservoirs in changing

841   environments. *Limnology and Oceanography* **41**, 1087–1092.

842   HAIRSTON, N. G. JR., VAN BRUNT, R. A., KEARNS, C. M. & ENGSTROM, D. R. (1995). Age and

843   survivorship of diapausing eggs in a sediment egg bank. *Ecology* **76**, 1706–1711.

844   HAIRSTON, N. G. JR., LAMPERT, W., CÁCERES, C. E., HOLTMEIER, C. L., WEIDER, L. J.,

845   GAEDKE, U., FISCHER, J. M., FOX, J. A. & POST, D. M. (1999). Rapid evolution revealed

846   by dormant eggs. *Nature* **401**, 446.

847   HAMR, P. (1992). Embryonic and postembryonic development in the Tasmanian freshwater

848   crayfishes *Astacopsis gouldi, Astacopsis franklinii* and *Parastacoides tasmanicus*

849   *tasmanicus* (Decapoda: Parastacidae). *Australian Journal of Marine and Freshwater*

850   *Research* **43**, 861–878.

851   HARVEY, T. H. P., VÉLEZ, M. I., BUTTERFIELD, N. J. (2012). Exceptionally preserved

852   crustaceans from western Canada reveal a cryptic Cambrian radiation. *Proceedings of the*

853   *National Academy of Sciences of the United States of America* **109**, 1589–1594.

854   HINES, A. H. (1991). Fecundity and reproductive output in nine species of *Cancer* crabs

855   (Crustacea, Brachyura, Cancridae). *Canadian Journal of Fisheries and Aquatic Sciences*

856   **48**, 267–275.

857   HOBBS, H. H. JR. (1989). An illustrated checklist of the American crayfishes (Decapoda:

858   Astacidae, Cambaridae, and Parastacidae). *Smithsonian Contributions to Zoology* **480**, 1–

859   236.

860   HOEG, J. T. (1991). Functional and evolutionary aspects of the sexual system in the

861   Rhizocephala (Thecostraca: Cirripedia). In *Crustacean Sexual Biology* (eds R. T. Bauer

862   and J. M. Martin), pp. 208–227. Columbia University Press, New York.

863 HOESE, B. & JANSSEN, H. H. (1989). Morphological and physiological studies on the

864 marsupium in terrestrial isopods. *Monitore Zoologico Italiano, Nuova Serie, Monografia*

865 **4**, 153–173.

866 HOLDICH, D. M. (2002). Background and functional morphology. In *Biology of Freshwater*

867 *Crayfish* (ed. D. M. Holdich), pp. 3–29. Blackwell, Oxford.

868 HORCH, J. M. (2009). Adaptive plasticity of the penis in a simultaneous hermaphrodite.

869 *Evolution* **63**, 1946–1953.

870 JAMIESON, B. G. M. (1991). Ultrastructure and phylogeny of crustacean spermatozoa.

871 *Memoirs of the Queensland Museum* **31**, 109–142.

872 JEFFERY, N. W. (2015). *Genome Size Diversity and Evolution in the Crustacea*. PhD-Thesis.

873 Faculty of Graduate Studies, University of Guelph, Guelph, Canada.

874 JENSEN, P. C. & BENTZEN, P. (2012). A molecular dissection of the mating system of the

875 Dungeness Crab, *Metacarcinus magister* (Brachyura: Cancridae). *Journal of Crustacean*

876 *Biology* **32**, 443–456.

877 JIANG, X., ZHAO, S., XU, Z., WANG, G., HE, J. & CAI, M. (2012). Abundance and age of viable

878 resting eggs of the calanoid copepod *Boeckella poppei* Mrázek in sediments: evidence of

879 egg banks in two Antarctic maritime lakes. *Polar Biology* **35**, 1525–1531.

880 JUCHAULT, P., RIGAUD, T. & MOCQUARD, J. P. (1992). Evolution of sex-determining

881 mechanisms in a wild population of *Armadillidium vulgare* Latr. (Crustacea, Isopoda):

882 competition between two feminizing parasitic factors. *Heredity* **69**, 382–390.

883 KAHRL, A. F., LAUSHMAN, R. H. & ROLES, A. J. (2014). Evidence for multiple paternity in two

884 species of *Orconectes* crayfish. *Canadian Journal of Zoology* **92**, 985–988.

885 KARANOVIC, I. (2012). *Recent Freshwater Ostracods of the World: Crustacea, Ostracoda,*

886 *Podocopida*. Springer, Heidelberg.

887 KIØRBOE, T. & SABATINI, M. (1994). Reproductive and life-cycle strategies in egg-carrying

888 and free-spawning calanoid copepods. *Journal of Plankton Research* **16**, 1353–1366.

889 KLEPAL, W., BARNES, H. & MUNN, E. A. (1972). The morphology and histology of the

890   cirriped penis. *Journal of Experimental Marine Biology and Ecology* **10**, 243–265.

891 KLEPAL, W., RENTENBERGER, C., ZHEDEN, V., ADAM, S. & GRUBER, D. (2010). Structural

892   peculiarities of the penis of *Semibalanus balanoides* (Linnaeus, 1767) and *Chthamalus*

893   *stellatus* (Poli, 1791) (Crustacea: Cirripedia: Thoracica). *Journal of Experimental Marine*

894   *Biology and Ecology* **392**, 228–233.

895 LAWRENCE, S. E. (1992). Sexual cannibalism in the praying mantis, *Mantis religiosa*: a field

896   study. *Animal Behaviour* **43**, 569–583.

897 LÉCHER, P., DEFAYE, D. & NOEL, P. (1995). Chromosomes and nuclear DNA of Crustacea.

898   *Invertebrate Reproduction and Development* **27**, 85–114.

899 LEE, W. Y., OMORI, M. & PECK, R. W. (1992). Growth, reproduction and feeding behavior of

900   the planktonic shrimp, *Lucifer faxoni* Borradaile, off the Texas coast. *Journal of Plankton*

901   *Research* **14**, 61–69.

902 LÓPEZ-CAMPS, J., BARGALLÓ, R., BOZZO, M. G., DURFORT, M. & FONTARNAU, R. (1981). The

903   spermatogenesis of crustaceans. VII. Review of spermatozoon of the crayfish *Astacus*

904   *astacus* (Malacostraca, Decapoda, Macrura, Reptantia). *Gamete Research* **4**, 65–82.

905 MACRAE, T. H. (2010). Gene expression, metabolic regulation and stress tolerance during

906   diapause. *Cellular and Molecular Life Sciences* **67**, 2405–2424.

907 MARTIN, J. W. & DAVIS, G. E. (2001). *An Updated Classification of the Recent Crustacea.*

908   Science Series 39. Natural History Museum of Los Angeles County, Los Angeles.

909 MARTIN, P., THONAGEL, S. & SCHOLTZ, G. (2015). The parthenogenetic Marmorkrebs

910   (Malacostraca: Decapoda: Cambaridae) is a triploid organism. *Journal of Zoological*

911   *Systematics and Evolutionary Research*, in press.

912 MASON, J. C. (1970). Egg-laying in the Western North American crayfish, *Pacifastacus*

913   *trowbridgii* (Stimpson) (Decapoda, Astacidae). *Crustaceana* **19**, 37–44.

914 MATZKE-KARASZ, R. (2005). Giant spermatozoon coiled in small egg: fertilization

915   mechanisms and their implications for evolutionary studies on Ostracoda (Crustacea).

916   *Journal of Experimental Zoology (Molecular Development and Evolution)* **304B**, 129–

917   149.

918 MATZKE-KARASZ, R., SMITH, R. J., SYMONOVÁ, R., MILLER, G. & TAFFOREAU, P. (2009).

919   Sexual intercourse involving giant sperm in Cretaceous ostracode. *Science* **324**, 1535.

920 MATZKE-KARASZ, R., NEIL, J. V., SMITH, R. J., SYMONOVA, R., MOŘKOVSKY, L., ARCHER, M.,

921   HAND, S. J., CLOETENS, P. & TAFFOREAU, P. (2014). Subcellular preservation in giant

922   ostracod sperm from an early Miocene cave deposit in Australia. *Proceedings of the*

923   *Royal Society B* **281**, 20140394.

924 MCGREGOR, D. L. & KESLING, R. V. (1969). Copulatory adaptations in ostracods. Part II.

925   Adaptations in living ostracods. *Contributions from the Museum of Paleontology of the*

926   *University of Michigan* **22**, 221–239.

927 MCLAY, C. L. & LÓPEZ GRECO, L. S. (2011). A hypothesis about the origin of sperm storage

928   in the Eubrachyura, the effects of seminal receptacle structure on mating strategies and

929   the evolution of crab diversity: How did a race to be first become a race to be last?

930   *Zoologischer Anzeiger* **250**, 378–406.

931 MERGEAY, J., VERSCHUREN, D. & DE MEESTER, L. (2006). Invasion of an asexual American

932   water flea clone throughout Africa and rapid displacement of a native sibling species.

933   *Proceedings of the Royal Society B* **273**, 2839–2844.

934 MITTMANN, B., UNGERER, P., KLANN, M., STOLLEWERK, A. & WOLFF, C. (2014).

935   Development and staging of the water flea *Daphnia magna* (Straus, 1820; Cladocera,

936   Daphniidae) based on morphological landmarks. *EvoDevo* **5**, 12.

937 NEUFELD, C. J. & PALMER, A. R. (2008). Precisely proportioned: intertidal barnacles alter

938   penis form to suit coastal wave action. *Proceedings of the Royal Society B* **275**, 1081–

939   1087.

940 NG, P. K. L. & TAN, C. G. S. (1995). *Geosesarma notophorum* sp. nov. (Decapoda, Brachyura,

941   Grapsidae, Sesarminae), a terrestrial crab from Sumatra, with novel brooding behaviour.

942   *Crustaceana* **68**, 390–395.

943 NIIYAMA, H. (1962). On the unprecedentedly large number of chromosomes of the crayfish,

944   *Astacus trowbridgii* Stimpson. *Annotationes Zoologicae Japonenses* **35**, 229–233.

945 NIKSIRAT, H., KOUBA, A. & KOZÁK, P. (2014). Post-mating morphological changes in the

946   spermatozoon and spermatophore wall of the crayfish *Astacus leptodactylus*: insight into

947   a non-motile spermatozoon. *Animal Reproduction Science* **149**, 325–334.

948 OHTSUKI, H., AWANO, T., TSUGEKI, N. K., ISHIDA, S., ODA, H., MAKINO, W. & URABE, J.

949   (2015). Historical changes in the ecosystem condition of a small mountain lake over the

950   past 60 years as revealed by plankton remains and *Daphnia* ephippial carapaces stored in

951   lake sediments. *PLoS ONE* **10**, e0119767.

952 OLESEN, J., BOESGAARD, T. & ILIFFE, T. M. (2015). The unique dorsal brood pouch of

953   Thermosbaenacea (Crustacea, Malacostraca) and description of an advanced

954   developmental stage of *Tulumella unidens* from the Yucatan Peninsula (Mexico), with a

955   discussion of mouth part homologies to other Malacostraca. *PLoS ONE* **10**, e0122463.

956 ØSTERGAARD, P. (2004). Does male *Chondracanthus lophii* (Crustacea: Copepoda) feed?

957   *Journal of the Marine Biological Association of the United Kingdom* **84**, 711–716.

958 ØSTERGAARD, P. & BOXSHALL, G. A. (2004). Giant females and dwarf males: a comparative

959   study of nuptial organs in female Chondracanthidae (Crustacea: Copepoda). *Zoologischer*

960   *Anzeiger* **243**, 65–74.

961 OTTO, S. P. & WHITTON, J. (2000). Polyploid incidence and evolution. *Annual Review of*

962   *Genetics* **34**, 401–437.

963 PARDO, L. M., RIVEROS, M., FUENTES, J. P. & LÓPEZ-GREO, L. (2013). Functional morphology

964   of the seminal receptacle in the crab *Metacarcinus edwardsii*. *Invertebrate Biology* **132**,

965   386–393.

966 PAUL, A. J. (1984). Mating frequency and viability of stored sperm in the Tanner crab

967 *Chionoecetes bairdi* (Decapoda, Majidae). *Journal of Crustacean Biology* **4**, 375–381.

968 PITNICK, S., SPICER, G. S. & MARKOW, T. A. (1995). How long is a giant sperm? *Nature* **375**,

969 109.

970 POINAR, G. (2000). First fossil record of stalked spermatophores with sperm (Collembola:

971 Hexapoda). *Historical Biology* **14**, 229–234.

972 PORTER, M. L., PÉREZ-LOSADA, M. & CRANDALL, K. A. (2005). Model-based multilocus

973 estimation of decapod phylogeny and divergence times. *Molecular Phylogenetics and*

974 *Evolution* **37**, 355–369.

975 PRAGER, M. H., MCCONAUGHA, J. R., JONES, C. M. & GEER, P. J. (1990). Fecundity of blue

976 crab, *Callinectes sapidus*, in Chesapeake Bay: biological, statistical and management

977 considerations. *Bulletin of Marine Science* **46**, 170–179.

978 PRICE, J. S., ALLEN, S., FAUCHEUX, C., ALTHNAIAN, T. & MOUNT, J. G. (2005). Deer antlers: a

979 zoological curiosity or the key to understanding organ regeneration in mammals? *Journal*

980 *of Anatomy* **207**, 603–618.

981 RADZIKOWSKI, J. (2013). Resistance of dormant stages of planktonic invertebrates to adverse

982 environmental conditions. *Journal of Plankton Research* **35**, 707–723.

983 RAUPACH, M. J. & THATJE, S. (2006). New records of the rare shrimp parasite *Zonophryxus*

984 *quinquedens* Barnard, 1913 (Crustacea, Isopoda, Dajidae): ecological and phylogenetic

985 implications. *Polar Biology* **29**, 439–443.

986 RICHTER, S. & SCHOLTZ, G. (2001). Phylogenetic analysis of the Malacostraca (Crustacea).

987 *Journal of Zoological Systematics and Evolutionary Research* **39**, 113–116.

988 RIGAUD, T., JUCHAULT, P. & MOCQUARD, J. P. (1997). The evolution of sex determination in

989 isopod crustaceans. *BioEssays* **19**, 409–416.

990 RIGAUD, T., PENNINGS, P. & JUCHAULT, P. (2001). *Wolbachia* bacteria effects after

991 experimental interspecific transfers in terrestrial isopods. *Journal of Inverebrate Pathology*

992 **77**, 251–257.

993 RODRIGUEZ-LAZARO, J. & RUIZ-MUÑOZ, F. (2012). A general introduction to ostracods:

994 morphology, distribution, fossil record and applications. In *Ostracoda as Proxies for*

995 *Quaternary Climate Change* (eds. D. J. HORNE, J. A. HOLMES, J. RODRIGUEZ-LAZARO and

996 F. A. VIEHBERG). Developments in Quaternary Science 17, pp. 1–14. Elsevier, Amsterdam.

997 ROJAS-HERNANDEZ, N., VÉLIZ, D. & PARDO, L. M. (2014). Use of novel microsatellite

998 markers for population and paternity analysis in the commercially important crab

999 *Metacarcinus edwardsii* (Brachyura: Cancridae). *Marine Biology Research* **10**, 839–844.

1000 RUDOLPH, E. H. & ROJAS, C. S. (2003). Embryonic and early postembryonic development of

1001 the burrowing crayfish, *Virilastacus araucanius* (Faxon, 1914) (Decapoda, Parastacidae)

1002 under laboratory conditions. *Crustaceana* **76**, 835–850.

1003 SAINTE-MARIE, G., SAINTE-MARIE, B. & SÉVIGNY, J.-M. (2000). Ejaculate-storage patterns

1004 and the site of fertilization in female snow crabs (*Chionoecetes opilio*; Brachyura,

1005 Majidae). *Canadian Journal of Zoology* **78**, 1902–1917.

1006 SAINTE-MARIE, B., GOSSELIN, T., SÉVIGNY. J. M. & URBANI, N. (2008). The snow crab mating

1007 system: opportunity for natural and unnatural selection in a changing environment. *Bulletin*

1008 *of Marine Science* **83**, 131–161.

1009 SCHMINKE, H. K. (2007). Crustacea, Krebse. In *Spezielle Zoologie. Teil 1: Einzeller und*

1010 *Wirbellose Tiere*, 2. Auflage (Hrsg. W. WESTHEIDE and R. RIEGER), pp. 555–637.

1011 Spektrum Akademischer Verlag, Heidelberg.

1012 SCHOLTZ, G. (1995). The attachment of the young in the New Zealand freshwater crayfish

1013 *Paranephrops zealandicus* (White, 1847) (Decapoda, Astacida, Parastacidae). *New*

1014 *Zealand Natural Sciences* **22**, 81–89.

1015 SCHOLTZ, G. & KAWAI, T. (2002). Aspects of embryonic and postembryonic development of

1016 the Japanese freshwater crayfish *Cambaroides japonicus* (Crustacea, Decapoda) including

1017 a hypothesis on the evolution of maternal care in the Astacida. *Acta Zoologica* **83**, 203–

1018 212.

1019 SEPKOSKI, J. J. JR. (2000). Crustacean biodiversity through the marine fossil record.

1020 *Contributions to Zoology* **69**, 213–222.

1021 SHU, D., VANNIER, J., LUO, H., CHEN, L., ZHANG, X. & HU, S. (1999). Anatomy and lifestyle

1022 of *Kunmingella* (Arthropoda, Bradoriida) from the Chengjiang fossil Lagerstätte (lower

1023 Cambrian; Southwest China). *Lethaia* **32**, 279–298.

1024 SIVETER, D. J., SUTTON, M. D., BRIGGS, D. E. G. & SIVETER, D. J. (2003). An ostracode

1025 crustacean with soft parts from the Lower Silurian. *Science* **302**, 1749–1751.

1026 SIVETER, D. J., SIVETER, D. J., SUTTON, M. D. & BRIGGS, D. E. G. (2007). Brood care in a

1027 Silurian ostracod. *Proceedings of the Royal Society B* **274**, 465–469.

1028 SIVETER, D. J., TANAKA, G., FARRELL, Ú. C., MARTIN, M. J., SIVETER, D. J. & BRIGGS, D. E. G.

1029 (2014). Exceptionally preserved 450-million-year-old Ordovician ostracods with brood

1030 care. *Current Biology* **24**, 801–806.

1031 SMITH, R. J., MATZKE-KARASZ, R., KAMIYA, T. & DE DECKKER, P. (2015). Sperm lengths of

1032 non-marine cypridoidean ostracods (Crustacea). *Acta Zoologica*, in press.

1033 SRINIVASAGAM, S., KATHIRVEL, M. & KULASEKARAPANDIAN, S. (2000). Captive broodstock

1034 development, induced breeding and larval stages of mud crabs (*Scylla* spp.) *CIBA*

1035 *Bulletin* **12**, 1–26.

1036 TALBOT, P. & CHANMANON, P. (1980a). The structure of sperm from the lobster, *Homarus*

1037 *americanus*. *Journal of Ultrastructure Research* **70**, 275–286.

1038 TALBOT, P. & CHANMANON, P. (1980b). Morphological features of the acrosome reaction of

1039 lobster *(Homarus)* sperm and the role of the reaction in generating forward sperm

1040 movement. *Journal of Ultrastructure Research* **70**, 287–297.

1041 TALBOT, P., POOLSANGUAN, B., AL-HAJJ, H. & POOLSANGUAN, W. (1991). Gamete

1042 interactions during in vitro fertilization of lobster (*Homarus americanus*) oocytes.

1043 *Journal of Structural Biology* **106**, 125–134.

1044 THIEL, M. (2000). Extended parental care behavior in crustaceans - a comparative overview.

1045 In *The Biodiversity Crisis and Crustacea*, Crustacean Issues 12 (eds J. C. VON VAUPEL

1046 KLEIN and F. R. SCHRAM), pp. 211–226. Balkema, Rotterdam.

1047 THIEL, M. (2003). Extended parental care in crustaceans - an update. *Revista Chilena de*

1048 *Historia Natural* **76**, 205–218.

1049 THIEL, M. (2007). Social behavior of parent-offspring groups in crustaceans. In *Evolutionary*

1050 *Ecology of Social and Sexual Systems: Crustaceans as Model Organisms* (eds J. E.

1051 DUFFY and M. THIEL), pp. 294–318. Oxford University Press, Oxford.

1052 THIERY, A. & GASC, C. (1991). Resting eggs of Anostraca, Notostraca and Spinicaudata

1053 (Crustacea, Branchiopoda) occurring in France: identification and taxonomical value.

1054 *Hydrobiologia* **212**, 245–259.

1055 THOMAS, W. J. & CRAWLEY, E. (1975). The glair glands and oosetae of *Austropotamobius*

1056 *pallipes* (Lereboullet). *Experientia* **31**, 534–537.

1057 TSAI, I. & TALBOT, P. (1993). Video microscopic analysis of ionophore induced acrosome

1058 reactions of lobster *(Homarus americanus)* sperm. *Molecular Reproduction and*

1059 *Development* **36**, 454–461.

1060 TSUCHIYA, K. & HAYASHI, F. (2014). Left-handed sperm removal by male *Calopteryx*

1061 damselflies (Odonata). *SpringerPlus* **3**, 144.

1062 TUDGE, C. & KOENEMANN, S. (2009). Spermatozoal morphology and its bearing on decapod

1063 phylogeny. In *Decapod Crustacean Phylogenetics* (eds J. W. MARTIN, K. A. CRANDALL

1064 and D. L. FELDER). Crustacean Issue 18, pp. 101–120. CRC Press, Baton Rouge.

1065 URBANI, N., SAINTE-MARIE, B., SÉVIGNY, J.-M., ZADWORNY, D. & KUHNLEIN, U. (1998).

1066 Sperm competition and paternity assurance during the first breeding period of female

1067   snow crab (*Chionoecetes opilio*) (Brachyura: Majidae). *Canadian Journal of Fisheries*

1068   *and Aquatic Sciences* **55**, 1104–1113.

1069   VOGT, G. (2002). Functional anatomy. In *Biology of Freshwater Crayfish* (ed. D. M.

1070   HOLDICH), pp. 53–151. Blackwell, Oxford.

1071   VOGT, G. (2008). Investigation of hatching and early post-embryonic life of freshwater

1072   crayfish by in vitro culture, behavioral analysis, and light and electron microscopy.

1073   *Journal of Morphology* **269**, 790–811.

1074   VOGT, G. (2013). Abbreviation of larval development and extension of brood care as key

1075   features of the evolution of freshwater Decapoda. *Biological Reviews* **88**, 81–116.

1076   VOGT, G. & TOLLEY, L. (2004). Brood care in freshwater crayfish and relationship with the

1077   offspring's sensory deficiencies. *Journal of Morphology* **262**, 566–582.

1078   VOGT, G., FALCKENHAYN, C., SCHRIMPF, A., SCHMID, K., HANNA, K., PANTELEIT, J., HELM,

1079   M., SCHULZ, R. & LYKO, F. (2015). The marbled crayfish as a paradigm for saltational

1080   speciation by autopolyploidy and parthenogenesis in animals. *Biology Open* **4**, 1583–

1081   1594.

1082   VOLLRATH, F. (1998). Dwarf males. *Trends in Ecology and Evolution* **13**, 159–163.

1083   WAAGE, J. K. (1979). Dual function of the damselfly penis: sperm removal and transfer.

1084   *Science* **203**, 916–918.

1085   WALKER, D., PORTER, B. A. & AVISE, J. C. (2002). Genetic parentage assessment in the

1086   crayfish *Orconectes placidus*, a high-fecundity invertebrate with extended maternal brood

1087   care. *Molecular Ecology* **11**, 2115–2122.

1088   WANG, B., XIA, F., WAPPLER, T., SIMON, E., ZHANG, H., JARZEMBOWSKI, E. A. & SZWEDO, J.

1089   (2015). Brood care in a 100-million-year-old scale insect. *eLife* **4**, e05447.

1090   WIGBY, S. & CHAPMAN, T. (2004). Sperm competition. *Current Biology* **14**, R100–103.

1091   WILLIAMS, J. D. & BOYKO, C. B. (2012). The global diversity of parasitic isopods associated

1092   with crustacean hosts (Isopoda: Bopyroidea and Cryptoniscoidea). *PLoS ONE* **7**,

1093   e35350.2,3.

1094   YAMADA, S. & MATZKE-KARASZ, R. (2012). How is a giant sperm ejaculated? Anatomy and

1095   function of the sperm pump, or "Zenker organ," in *Pseudocandona marchica* (Crustacea,

1096   Ostracoda, Candonidae). *Naturwissenschaften* **99**, 523–535.

1097   YUE, G. H. & CHANG, A. (2010). Molecular evidence for high frequency of multiple paternity

1098   in a freshwater shrimp species *Caridina ensifera*. *PLoS ONE* **5**, e12721.

1099   YUE, G. H., LI, J. L., WANG, C. M., XIA, J. H., WANG, G. L. & FENG, J. B. (2010). High

1100   prevalence of multiple paternity in the invasive crayfish species, *Procambarus clarkii*.

1101   *International Journal of Biological Sciences* **6**, 107–115.

1102   YUSA, Y., YOSHIKAWA, M., KITAURA, J., KAWANE, M., OZAKI, Y., YAMATO, S. & HØEG, J. T.

1103   (2012). Adaptive evolution of sexual systems in pedunculate barnacles. *Proceedings of*

1104   *the Royal Society B* **279**, 959–966.
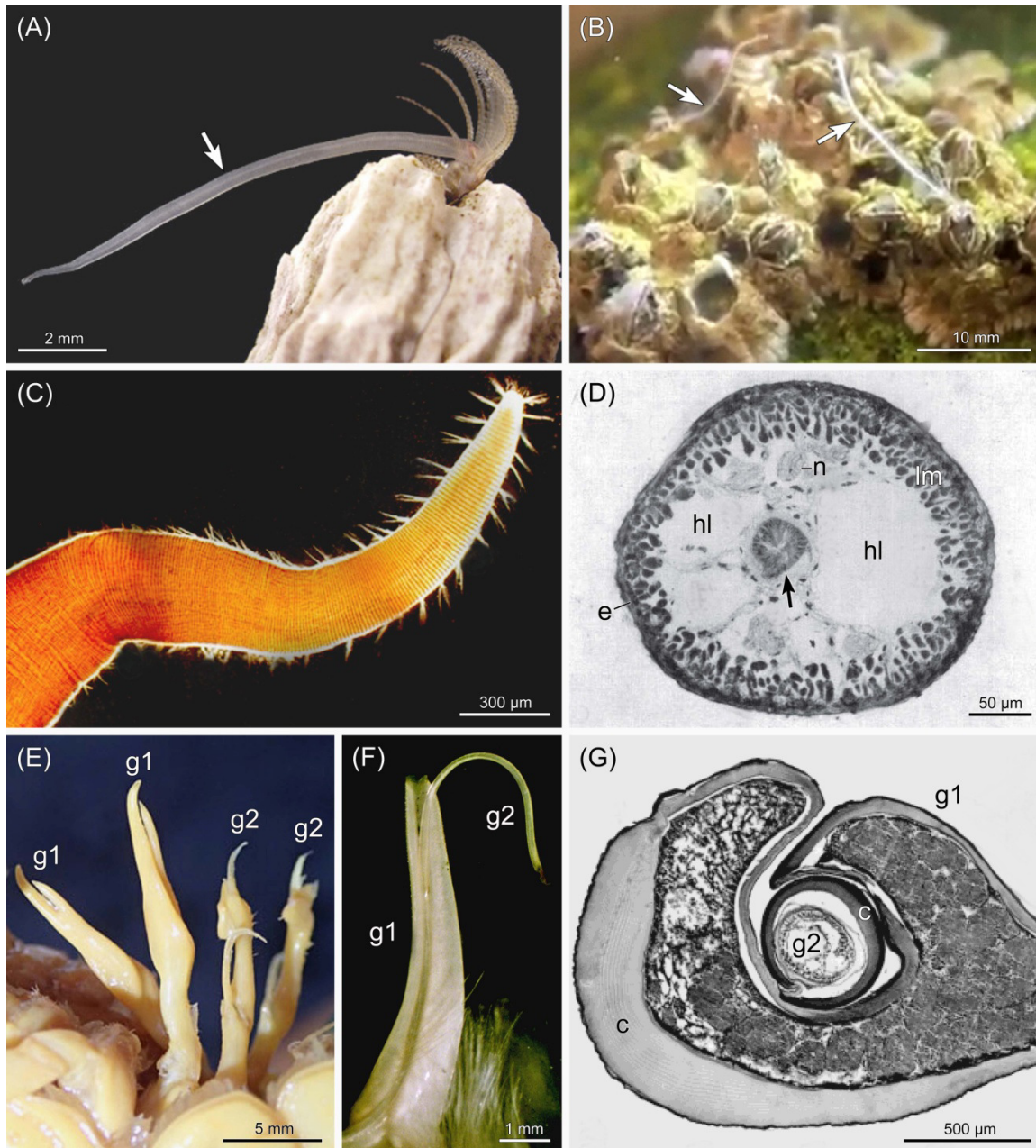
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Fig. 1. Extensible versus form-invariant copulatory organs. (A-D) Hydraulic penis of Cirripedia. (A) Relaxed penis (arrow) of *Balanus glandula*. Photo: Christopher J. Neufeld. (B) Extended penises (arrows) of *Semibalanus balanoides*. From a video 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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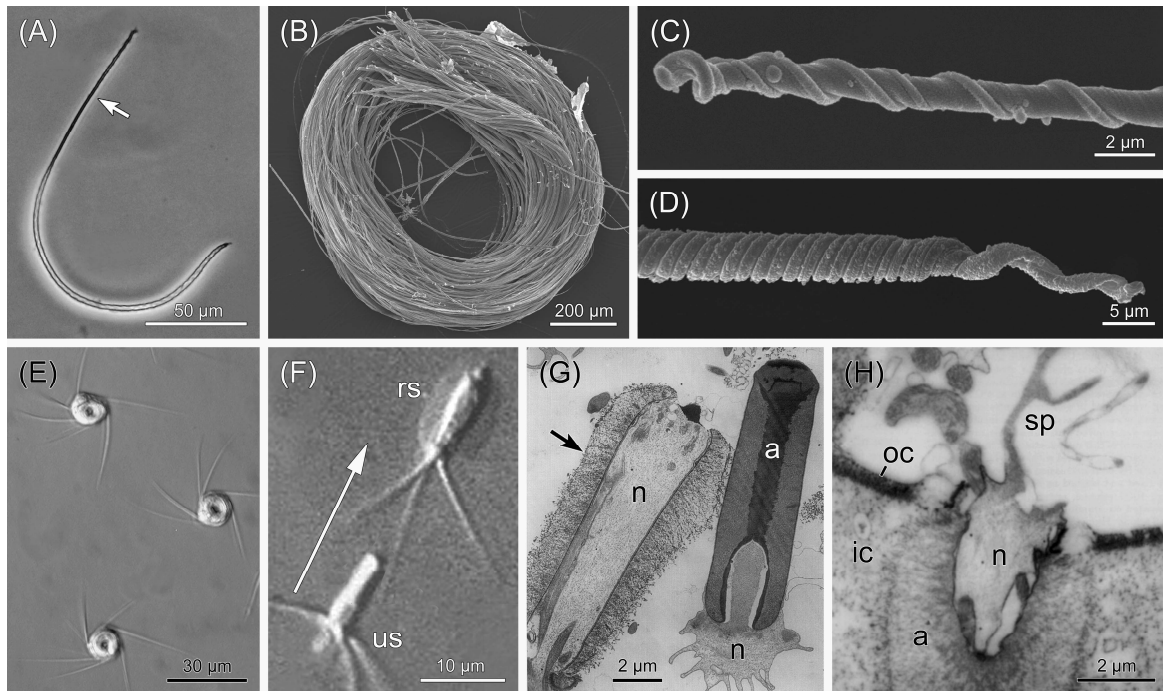
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Fig. 2. Spermatozoa of exceptional form and function. (A-D) Giant sperm of cypridoidean Ostracoda. From Smith *et al.* (2015). (A) Shortest giant sperm from *Fabaeformiscandona velifera*. Arrow denotes anterior region. (B) Sperm bundle from seminal 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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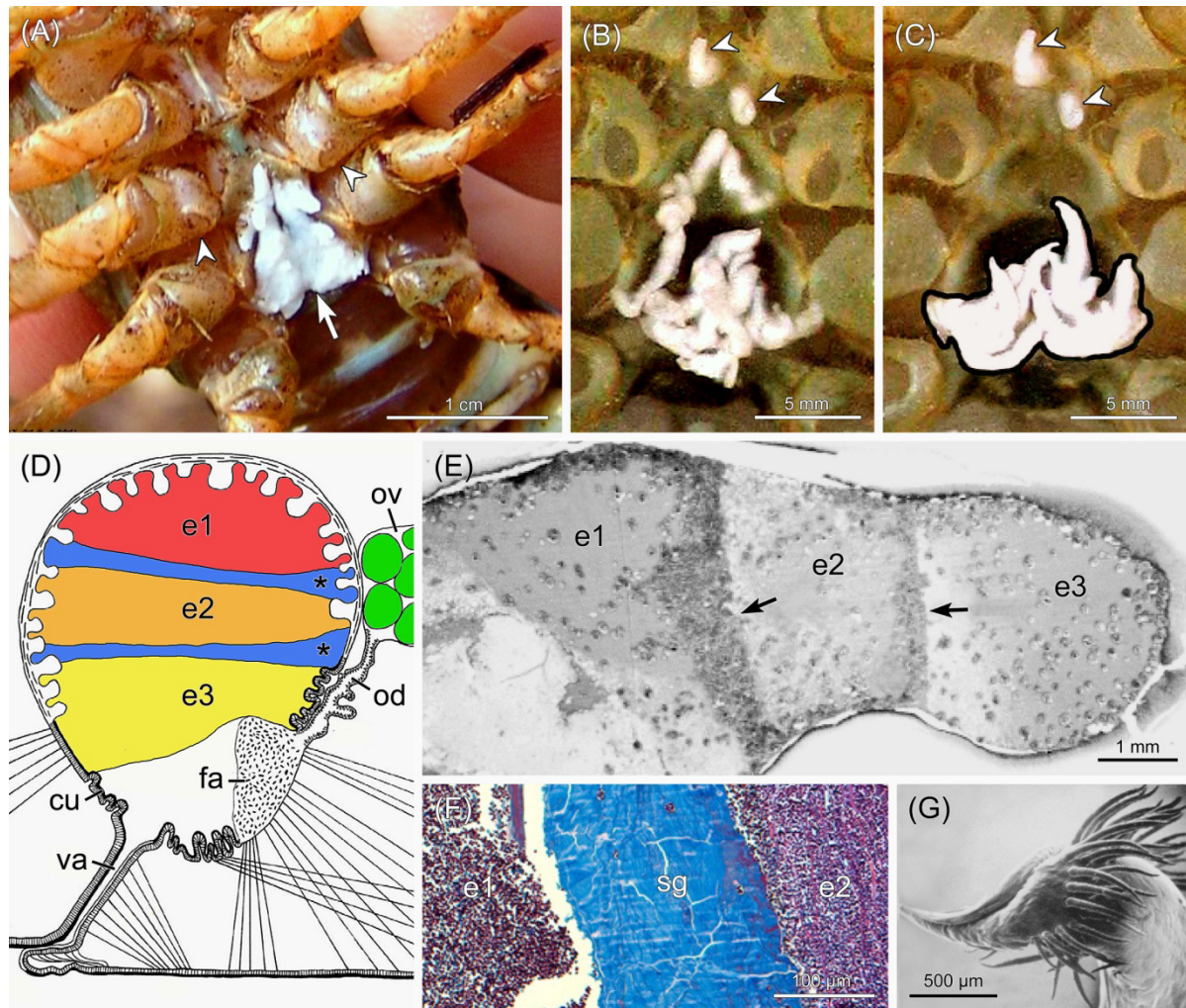
Fig. 3. Storage of sperm from multiple matings in Decapoda. (A-C) External attachment of spermatophores in astacid crayfish. (A) Preferred sperm attachment site (arrow) between female gonopores (arrowheads) in *Astacus astacus*. Photo: Lukáš Konečný. (B, C) Partial removal of spermatophores of earlier 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 sperm stratification from 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 layers of densely packed sperm (arrows). 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* equipped with recurved spoon-like structure and setal brushes. These structures were suspected to serve for the removal of sperm of rivals from the female seminal receptacle. From Beninger *et al.* (1991).
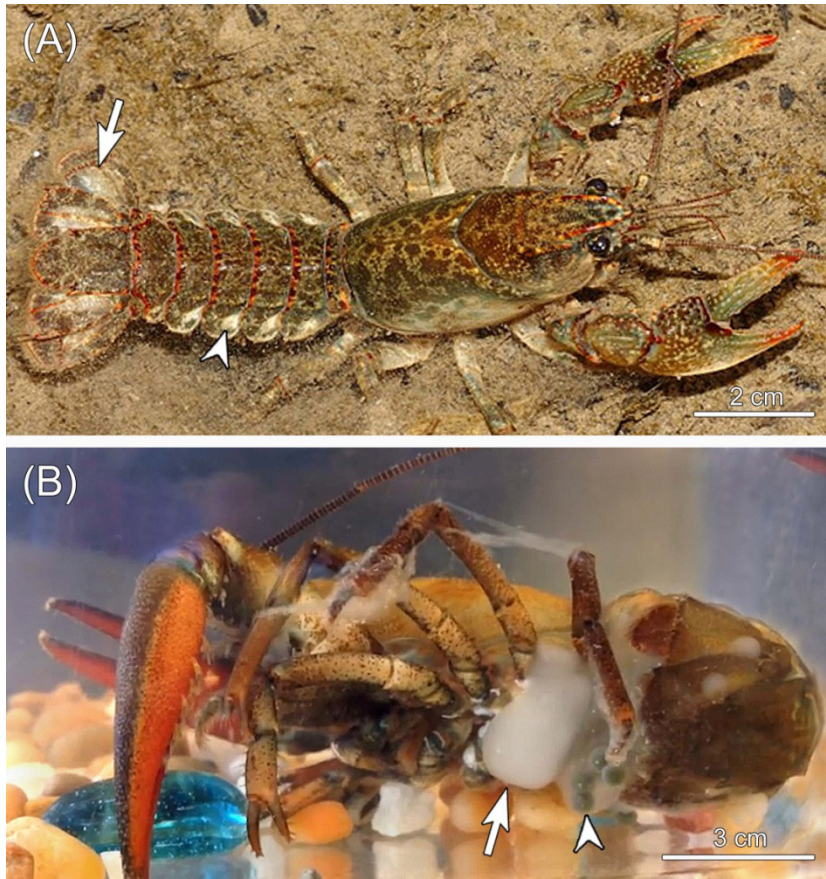
Fig. 4. Glair glands and fertilization tent in crayfish. (A) Whitish glair glands in uropods (arrow) and abdominal pleura (arrowhead) of *Cambarus polychromatus* female. Photo: Andrew Hoffman. (B) Fertilization pouch in egg-laying female of *Pacifastacus leniusculus* filled with gelatinous mass from 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.
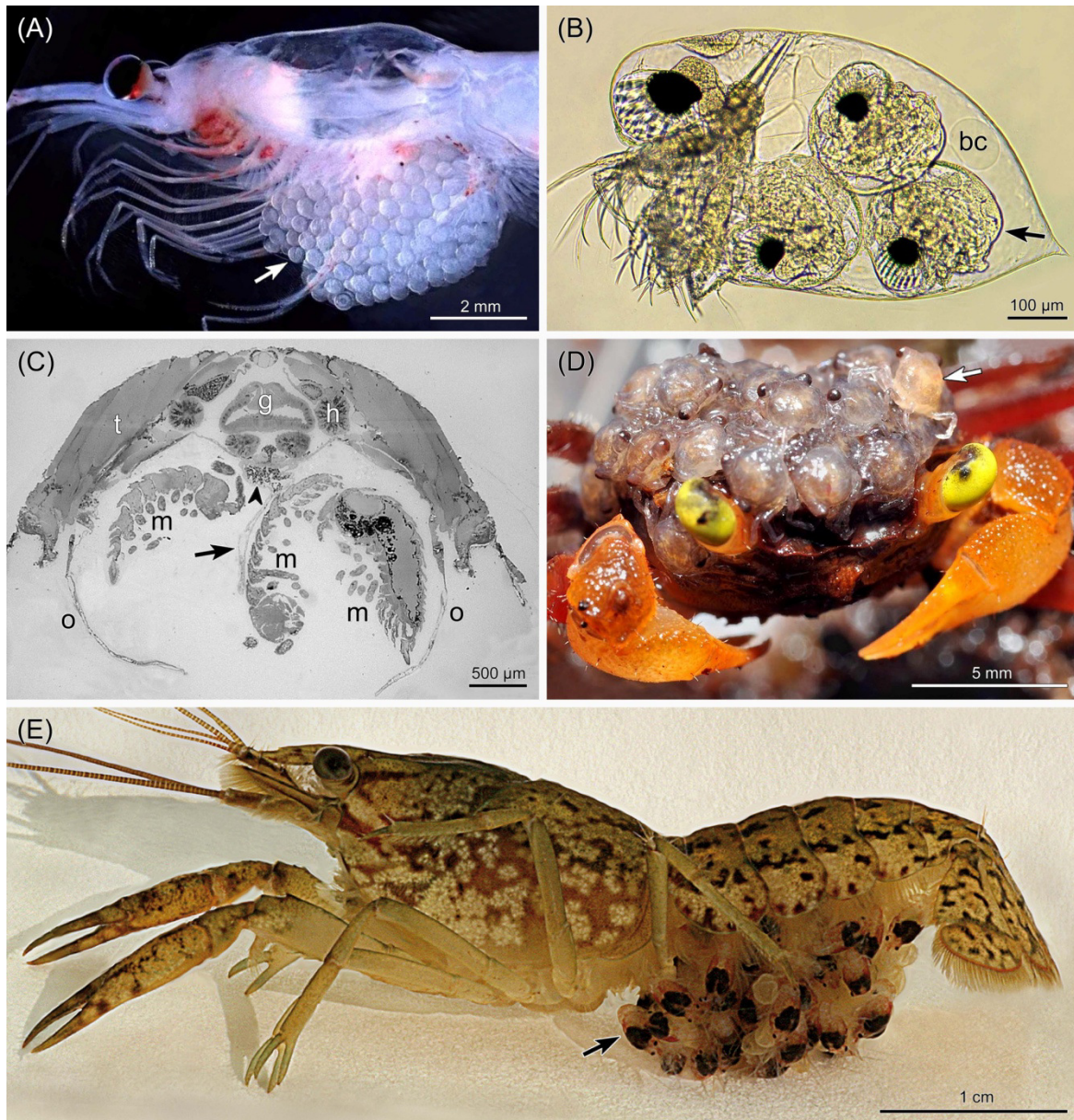
Fig. 5. Brooding of eggs and posthatching stages in specialized body compartments. (A) Krill *Nyctiphanes australis* with egg sac (arrow) between thoracopods. Photo: Anita Slotwinski. (B) Water flea *Evadne nordmanni* with advanced embryos (arrow) in dorsal brood chamber (bc). Photo: Maurice Loir. (C) Cross section through marsupium of isopod *Cylisticus convexus* with brooded mancas (m). The marsupium is delimited by oostegites (o) and includes a liquid and nutrient secreting cotyledon (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).
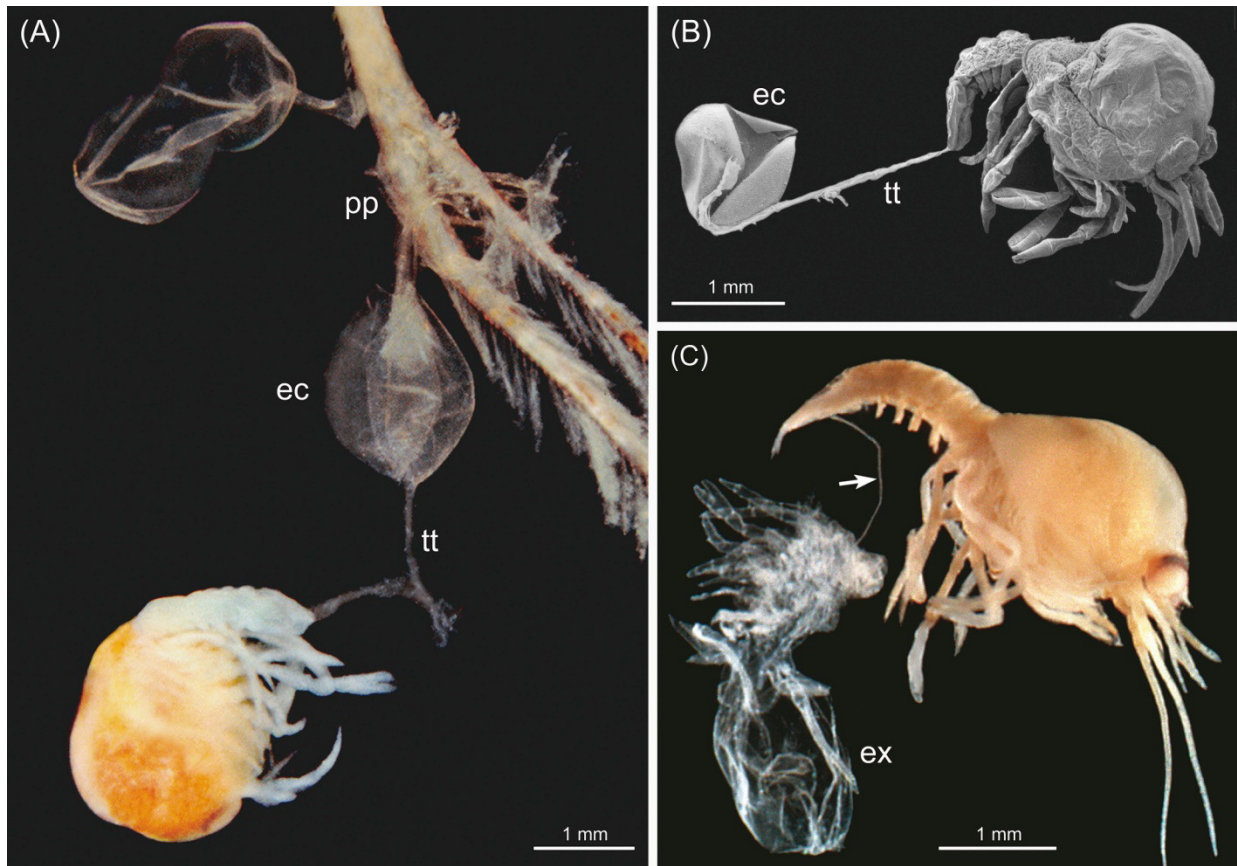
Fig. 6. Safeguarding of brooded crayfish juveniles by safety lines. (A) Safeguarding of hatching by telson thread in *Procambarus virginalis*. The hatchling is secured to the maternal pleopod (pp) via telson thread (tt) and egg case (ec). From Vogt & Tolley (2004). (B) Scanning electron micrograph of 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).
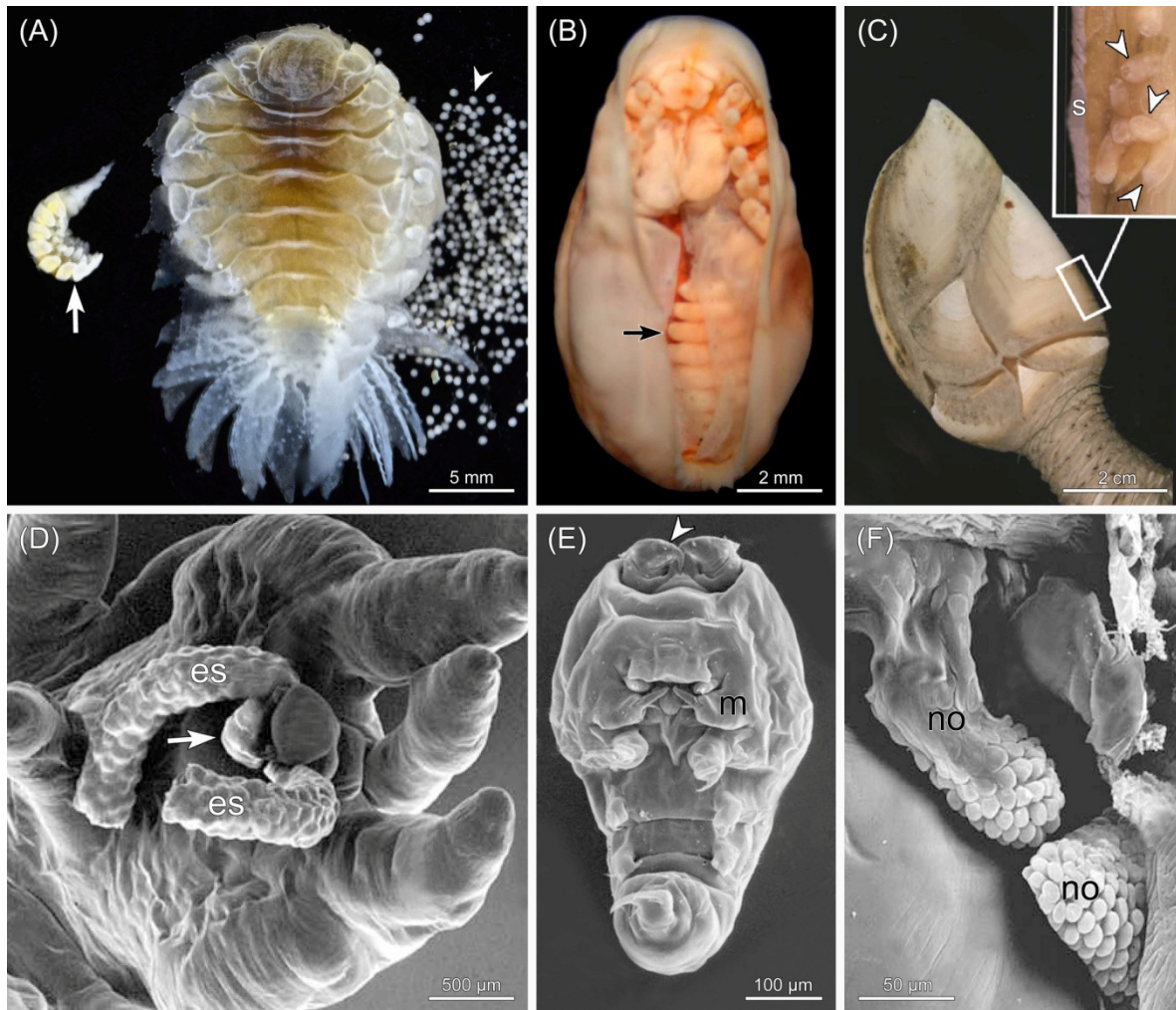
Fig. 7. Dwarf males in isopods (A, B), cirripeds (C) and copepods (D-F). (A) Ovigerous

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).
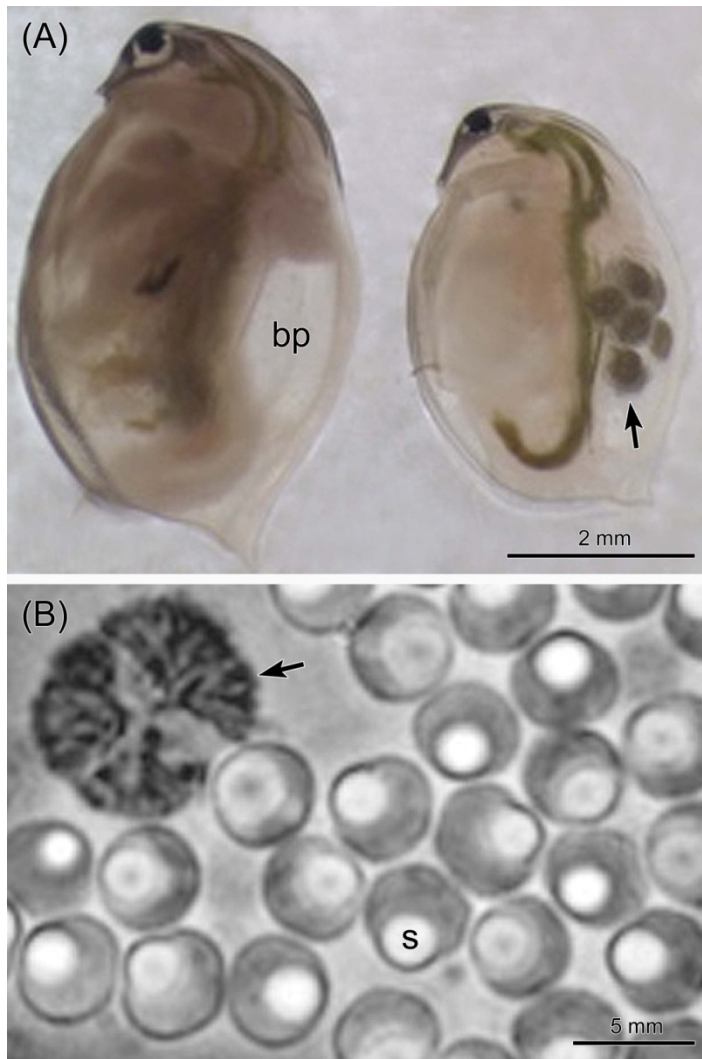
**Fig. 8.** Castration and gigantism in bacteria-infected water flea. (A) Comparison of healthy (right) and *Pasteuria ramosa*-infected (left) clutch-mates of *Daphnia magna*. The healthy specimen carries a clutch of eggs (arrow) in the brood pouch. The infected specimen is much larger and its brood 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.
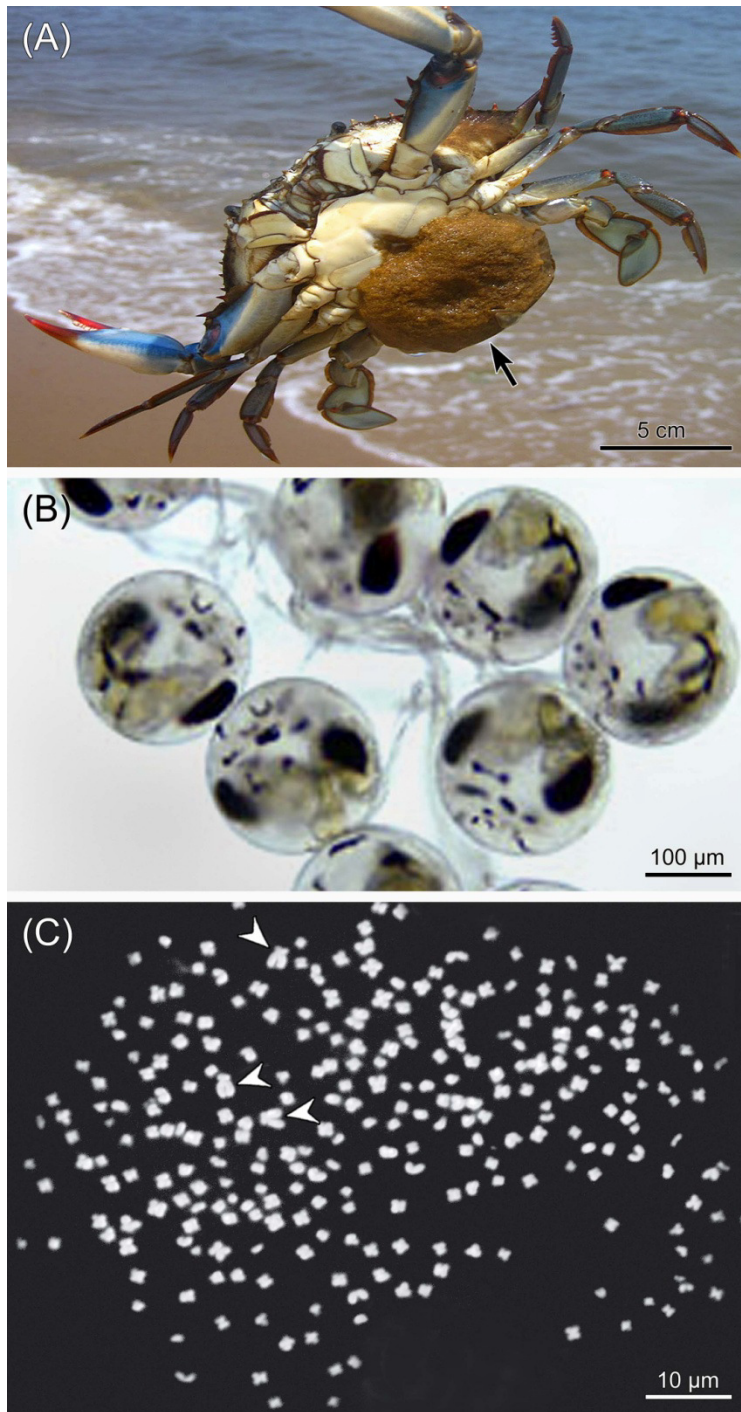
Fig. 9. Record-breaking clutch size and chromosome number in Decapoda. (A) Blue crab

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) Metaphase

chromosome spread of triploid cell of embryo of crayfish *Procambarus virginalis* (3n= 276

chromosomes). Arrowheads indicate three strikingly large subtelocentric chromosomes. From

Martin *et al.* (2015).
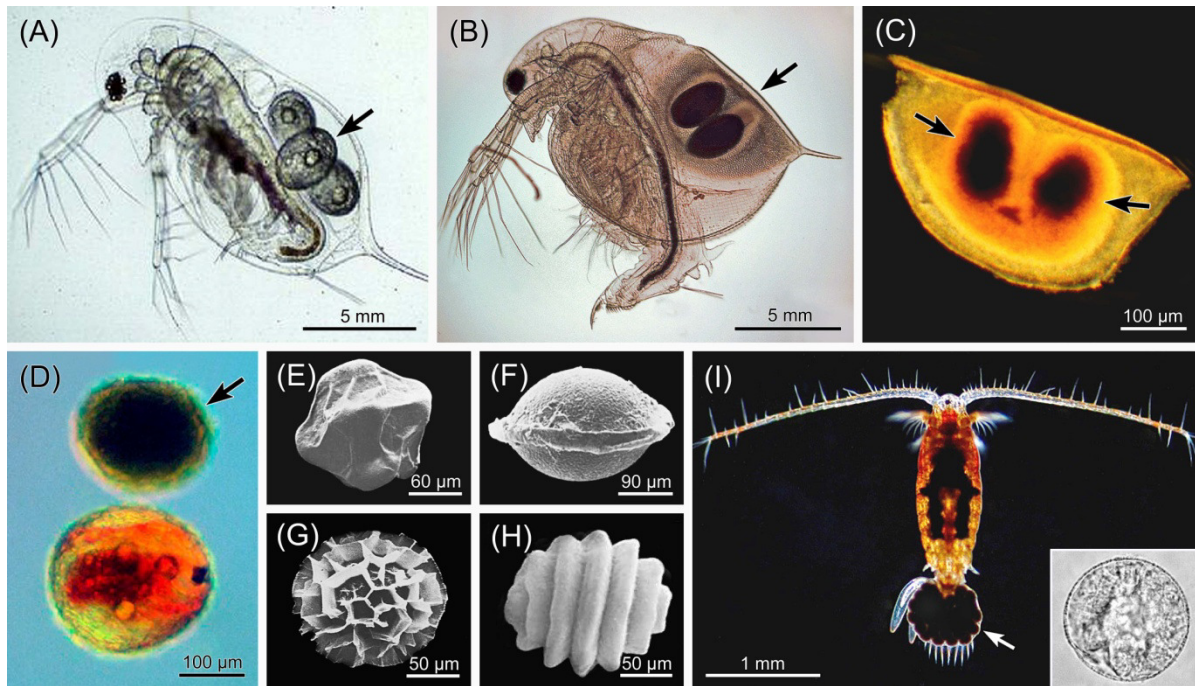
Fig. 10. Resting eggs with record breaking viability in short-lived Crustacea. (A-D) Resting eggs and cysts and their producers in Cladocera. (A) *Daphnia galeata*, the branchiopod record holder with a resting egg viability of 125 years. Arrow denotes brood chamber with subitanous eggs. From novaquatis.eawag.ch. (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 non-cladoceran 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).
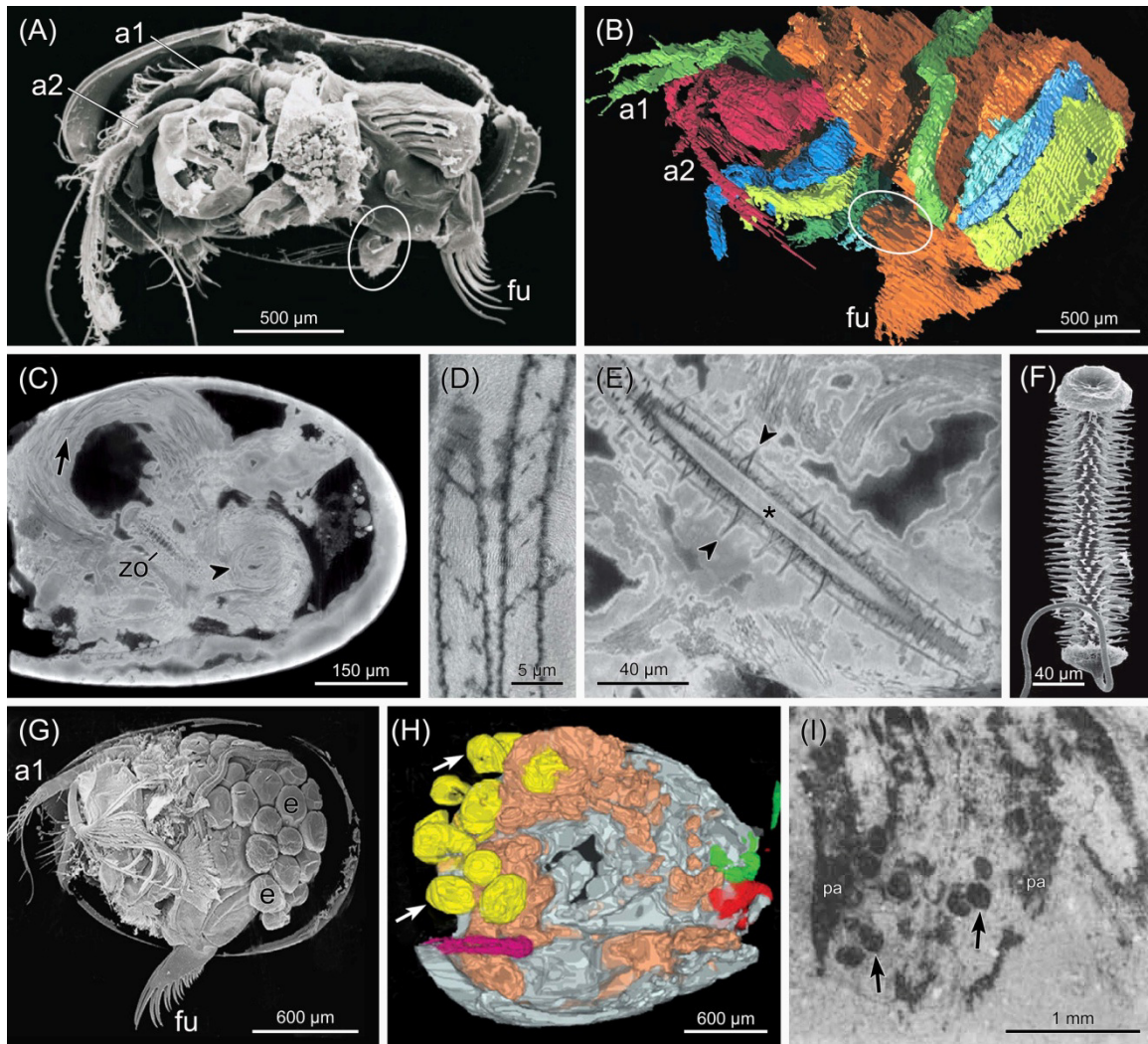
Fig. 11. Record-breaking fossil ages of reproductive structures. (A, B) Copulatory organ of Ostracoda. From Siveter *et al.* (2003). (A) Extant myodocopid *Xenoleberis yamadai* male showing ventrally located copulatory organ (circle). Left lateral view. a1, first antenna; a2, second antenna; fu, furca. (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 530 million years old crustacean stem line derivative *Kunmingella* showing brood chamber with embryos (arrows). The chamber is delimited by the post-antennular appendages (pa). From Shu *et al.* (1999).