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Marmorkrebs: Natural crayfish clone as emerging model for various biological disciplines

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1. What is the Marmorkrebs?

In the last decade, the Marmorkrebs or marbled crayfish (figure 1A) has become very popular among hobby aquarists and researchers (Vogt 2008a, 2010; Faulkes 2010). The name comes from its typical coloration that resembles marble. The marbled crayfish belongs to the decapod crustaceans, which include the commercially valuable shrimps, lobsters, crayfish and crabs. It was discovered in the mid-1990s in the German aquarium trade (Scholtz *et al.* 2003; Vogt *et al.* 2004) and has only recently been identified as the parthenogenetic form of the slough crayfish, *Procambarus fallax* (HAGEN, 1870), which is native to Georgia and Florida, USA (Martin *et al.* 2010a). Its scientific name is therefore *Procambarus fallax* (HAGEN, 1870) f. *virginalis* (Martin *et al.* 2010a). The marbled crayfish is the only one of more than 10000 decapods that produces high amounts of genetically identical offspring by obligatory parthenogenesis (Martin *et al.* 2007; Vogt *et al.* 2008). Mainly because of its clonal nature and easy culture, it was introduced as a laboratory animal around the year 2000. At present, it is used by more than a dozen laboratories in Europe, Japan and the United States as an experimental animal for a variety of research purposes. The marbled crayfish grows step-wise by moulting, passing approximately 25 moulting cycles in its lifetime. Adult marbled crayfish usually have body lengths of 4–8 cm (without chelae) and weights of 1.5–15 g. Very large specimens can reach 12 cm and more than 25 g. In the laboratory, the average lifespan of the adults was 720 days, but the record is 1610 days (Vogt 2010). Marbled crayfish can be kept either individually or communally in simple housing systems. They can tolerate broad ranges of environmental conditions for longer periods of time (Vogt *et al.* 2004; Jimenez and Faulkes 2010). The embryonic and first juvenile stages can even be raised in micro-plates (Vogt 2007). All life stages can be fed with the same pellet food (for instance, Tetra WaferMix), which is very exceptional in animal culture. Generation time is approximately 6 months.

2. How do marbled crayfish reproduce and develop?

The marbled crayfish is an all-female species. Males have never been found and could not be induced by exposure of the eggs to male sexual hormones (Vogt 2007). Marbled crayfish reproduce by apomictic parthenogenesis, which is the development of oocytes without fertilization and meiosis (Scholtz *et al.* 2003; Vogt *et al.* 2004; Martin *et al.* 2007). During their lifetime, they can complete up to seven reproduction cycles (Vogt 2010). The number of juveniles produced increases with each cycle in relationship to size increment of the mother. The maximum number of juveniles per batch recorded so far was 427. Like other freshwater crayfish, marbled crayfish develop directly within the eggs that are carried under the mother's pleon. Embryogenesis lasts approximately 17–28 days, depending on the temperature (462 degree days) (Pawlos *et al.* 2010) and is characterized by short-germ development. The first segments, which constitute the cephalic area of the later body, are almost simultaneously formed. All the other segments are then sequentially added by the activity of ectoteloblasts and mesoteloblasts that are located within superimposed rings in the caudal papilla of the embryo (figure 2B) (Seitz *et al.* 2005; Alwes and Scholtz 2006). The first two juvenile stages are incompletely developed and non-feeding, living from their extensive yolk reserves. They remain attached to

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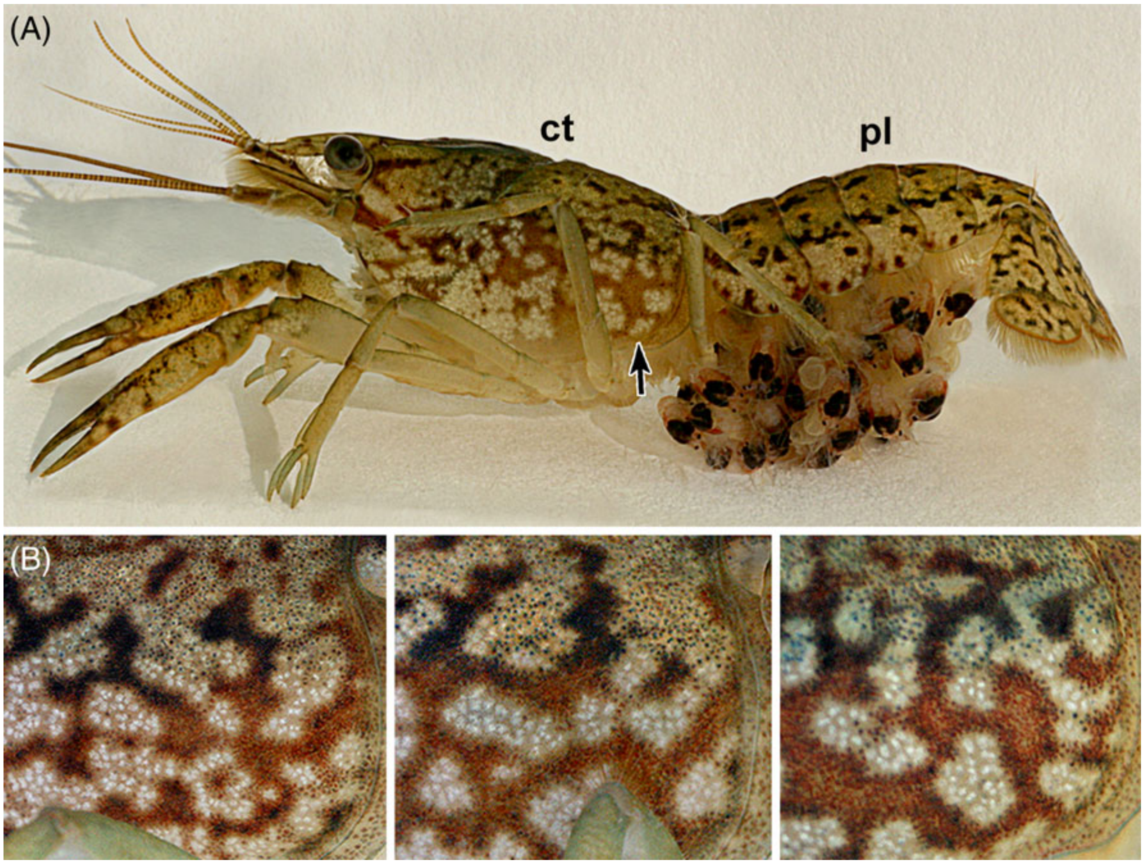

Figure 1. The parthenogenetic marbled crayfish *Procambarus fallax* f. *virginalis* and its phenotypic variation. (**A**) Mother with juveniles under pleon (pl) showing eponymous marmoration (from Vogt and Tolley 2004). (**B**) Variation of marmoration pattern between three genetically identical and communally reared batchmates. Shown is the posterio-lateral part of the cephalothorax (ct) indicated in (**A**) by an arrow (from Vogt *et al.* 2008).

the mother's pleopods (Vogt and Tolley 2004). The third juvenile stage has the fully developed adult habitus. It becomes freelancing and starts feeding but returns to the maternal pleon for resting. These three brooded juvenile stages are then followed by some further 12 juvenile stages and up to 10 adult stages (Vogt 2010). The adult life period is characterized by alternation of growth and reproduction phases.

3. Is it true that the first juvenile stages are secured by a safety line?

Yes, hatchlings (stage-1 juveniles) are safeguarded during hatching and in the hours thereafter by the telson thread (figure 2A) (Vogt and Tolley 2004). This structure originates from secretions and the inner layer of the egg case and keeps the immobile hatchling linked to its mother via the egg case that is firmly attached to the maternal pleopods by an egg stalk (Vogt 2008b). The telson thread acts as a safety line and prevents the hatchling from being dislodged by the water current. Some minutes after hatching, the hatchling becomes active and attaches with its hooked chelae to pleopodal structures of the mother to await the next moulting. Safeguarding of hatching by a safety line is unique in the animal kingdom and an autapomorphy of the freshwater crayfish (Scholtz 2002). Interestingly, there is a second safety line, the anal thread, which secures moulting from juvenile stage-1 to stage-2 (Vogt 2008b). This safety line is different from the telson thread because it originates from delayed shedding of the hindgut.

4. Why are marbled crayfish so attractive to researchers?

In the last decade, marbled crayfish have been used for research on morpho-functional relationships (Vogt and Tolley 2004; Polanska *et al.* 2007; Vogt *et al.* 2008; Gherardi *et al.* 2010; Pawlos *et al.* 2010), development (Seitz *et al.* 2005; Alwes and Scholtz 2006; Jirikowski *et al.* 2010), neurobiology (Vilpoux *et al.* 2006; Rieger and Harzsch 2008;

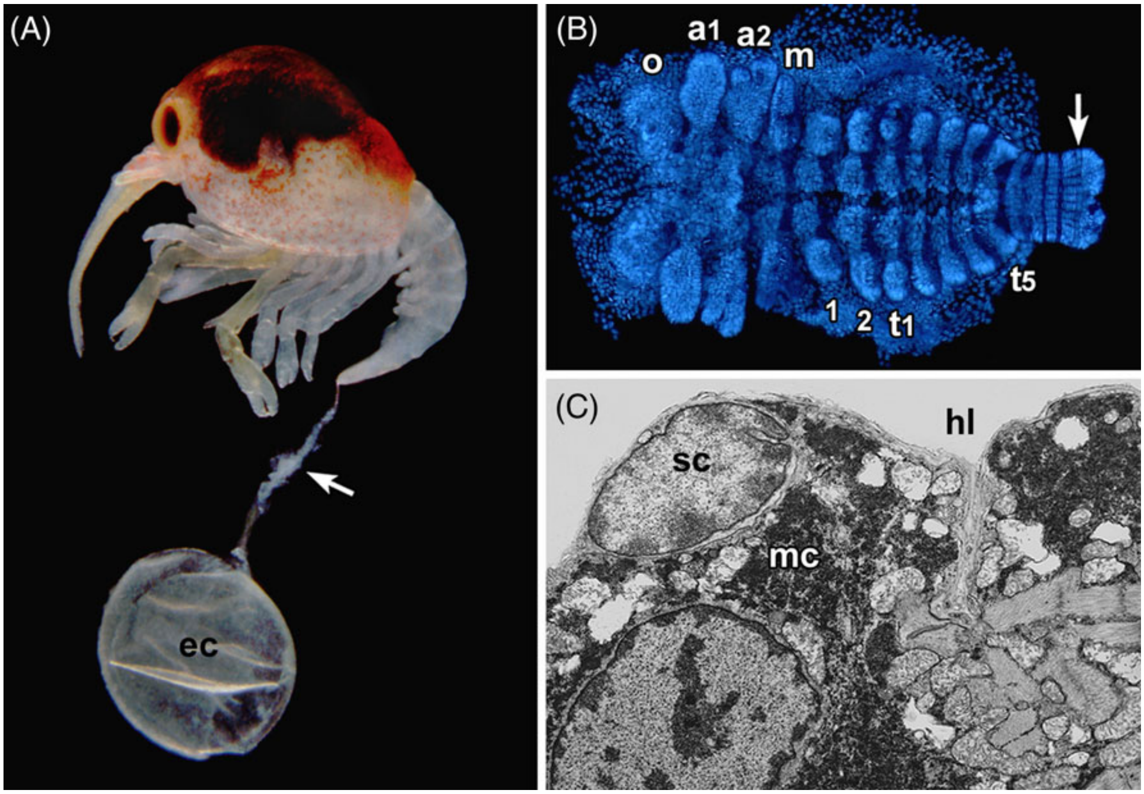

Figure 2. Extraordinary features of the marbled crayfish. (**A**) Hatchling connected to egg case (ec) by telson thread (arrow). *In vivo*, the egg case is firmly attached to the maternal pleopods by an egg stalk (from Vogt and Tolley 2004). (**B**) Ventral view of embryo at about 50% development. The cephalic body part from the ophthalmic anlagen (o) to the mandible anlagen (m) is simultaneously generated. The following maxillae 1 and 2 (1, 2), eight thoracic segments and six pleonal segments are then sequentially added by the ectoteloblasts (arrow) and mesoteloblasts (underneath ectoteloblasts, not visible) of the caudal papilla. In the depicted embryo, the thoracic segments 1 to 5 (t1–t5) are already built. a1 and a2, anlagen of 1st and 2nd antennae (from Alwes and Scholtz 2006). (**C**) Stem cell (sc) of the heart located between myocardium cell (mc) and heart lumen (hl) (from Vogt 2010).

Fabritius-Vilpoux *et al.* 2008), epigenetics (Schiewek *et al.* 2007; Vogt *et al.* 2008, 2009), stem cell biology (Vogt 2010), behaviour (Vogt *et al.* 2008; Farca Luna *et al.* 2009), biogerontology (Vogt 2010), biochronology (Farca Luna *et al.* 2010), toxicology (Vogt 2007; Rubach *et al.* 2011), ecology (Jones *et al.* 2009; Chucholl and Pfeiffer 2010) and evolutionary biology (Sintoni *et al.* 2007). Marbled crayfish fulfil the basic requirements for laboratory culture such as suitable size, robustness against handling stress, high fertility, relatively short generation time and adaptability to a broad range of culture conditions. In addition, they have some special advantages, first of all genetic identity among batchmates and between mother and offspring (Martin *et al.* 2007; Vogt *et al.* 2008). This feature is of particular importance for the investigation of epigenetic aspects of the phenotype. Further advantages are direct development, short-germ development and stereotyped cell lineage in early development (Seitz *et al.* 2005; Alwes and Scholtz 2006), which allow precise experimental interventions in developmental studies. Other attractive features are the possession of numerous morphological characters that are easy to analyse (Vogt *et al.* 2008), indeterminate growth and associated lifelong stem cell activity (Vogt 2010), broad behavioural repertoire, high regeneration capacity and methylation of the DNA in all life-stages (Vogt *et al.* 2008). Moreover, the marbled crayfish is large enough to allow individual physiological and biochemical analyses, which is in contrast to model organisms like the fly *Drosophila melanogaster* and the nematode *Caenorhabditis elegans*. It also allows individual longitudinal studies by taking blood samples or biopsies or by analysing the exuviae of subsequent life stages, which provide an excellent archive of morphological traits. This feature is particularly suitable to study fluctuating asymmetry, the right-to-left difference of a trait.

5. How can marbled crayfish produce different phenotypes from a single genotype?

According to traditional belief, a single genotype maps to only one phenotype in a given environment. This proposition was, among others, the incitement for the development of artificial cloning. However, such a straight genotype-to-phenotype relationship was repeatedly questioned and was recently challenged by experiments with the marbled crayfish. Genetically identical batchmates of the marbled crayfish showed surprisingly broad ranges in variation of coloration, growth, lifespan, reproduction, morphometric parameters, behaviour and fluctuating asymmetry of sense organs even when reared under identical conditions (Vogt *et al.* 2008). Maximal variation was observed for the marmoration pattern, which was unique in each of the several hundred individuals examined (figure 1B). Phenotypic variability among clonemates is apparently caused by stochastic cellular events including stochastic gene expression, reaction-diffusion-like patterning in early development and nonlinear, self-reinforcing circuitries involving behaviour and metabolism in later life stages (Vogt *et al.* 2008). These stochastic and nonlinear phenomena are best summarized in the term stochastic developmental variation (SDV). SDV does affect many biological issues, among them individuation, ageing, susceptibility to diseases and ecological and evolutionary adaptation, and is probably related to alterations of the epigenetic code.

6. Is negligible senescence in the marbled crayfish related to lifelong stem cell activity?

Marbled crayfish show no marked reproductive senescence and structural-functional decay of the tissues and organs when they grow old (Vogt 2010). For instance, spawners younger than 6 months and older than 3 years produced similarly large clutches (~7% of body weight). This feature and the permanent renewal of the tissues are related to the indeterminate growth format of crayfish and the associated lifelong activity of the somatic and germline stem cells. Marbled crayfish possess numerous adult stem cells, among them the satellite cells of the heart (figure 2C) and skeletal musculature, the neurogenic stem cells in various parts of the brain, the E-cells of the hepatopancreas, the haematopoietic stem cells and the oogonia (Vogt 2008a). These adult stem cells and several extraordinary embryonic stem cells like the ectoteloblasts and mesoteloblasts (Alwes and Scholtz 2006) provide very promising targets for stem cell research.

7. Is it true that marbled crayfish are highly resistant to cancer?

Yes, that is true. The decapod crustaceans, which can reach ages of almost 100 years, are generally highly resistant to tumour formation (Vogt 2008c). Although the decapods are well investigated with respect to diseases in the framework of environmental monitoring, aquaculture biosecurity programs and seafood quality controls, there are only a few cases of neoplasias known. In the marbled crayfish, tumours have not been found yet. This is interesting because marbled crayfish have approximately the same lifespan as mice, which have high prevalence of cancer, particularly in their final period of life.

8. Are marbled crayfish good guys only?

Unfortunately, no. In contrast to sexually reproducing animals, escape or deliberate release of a single individual can result in establishment of a wild population. This has happened already in Europe (Marzano *et al.* 2009; Chucholl and Pfeiffer 2010; Martin *et al.* 2010b), Japan (Kawai *et al.* 2009) and Madagascar. In Madagascar, somebody released one or a few individuals near the capital Antananarivo in around 2003. These specimen(s) multiplied then at an enormous speed and invaded habitats such diverse as rice paddies, rivers, lakes and swamps in 8 of the country's 22 regions (Jones *et al.* 2009; Kawai *et al.* 2009). The problems related with the invasion of marbled crayfish into new environments include potential displacement or even eradication of native crayfish species by competition or transfer of diseases (Jones *et al.* 2009; Martin *et al.* 2010b; Feria and Faulkes 2011). Marbled crayfish kept indoors as research animals or as pets pose no threat when a few simple security measures are taken to prevent their escape (Vogt 2008a).

9. How can I be kept informed about ongoing research with the marbled crayfish?

Zen Faulkes from the University of Texas–Pan American (USA) has set up a homepage, which is updated weekly. Here you can find all past and future scientific and popular scientific papers on the marbled crayfish either at *marmorkrebs.org* or *http://www.utpa.edu/faculty/zfaulkes/marmorkrebs/*.

References

Alwes F and Scholtz G 2006 Stages and other aspects of the embryology of the parthenogenetic Marmorkrebs (Decapoda, Reptantia, Astacida). *Dev. Genes Evol.* **216** 169–184

Chucholl C and Pfeiffer M 2010 First evidence for an established Marmorkrebs (Decapoda, Astacida, Cambaridae) population in Southwestern Germany, in syntopic occurrence with *Orconectes limosus* (Rafinesque, 1817). *Aquatic Invasions* **5** 405–412

Fabritius-Vilpoux K, Bisch-Knaden S and Harzsch S 2008 Engrailed-like immunoreactivity in the embryonic ventral nerve cord of the Marbled Crayfish (Marmorkrebs). *Invertebr. Neurosci.* **8** 177–197

Farca Luna AJ, Hurtado-Zavala JI, Reischig T and Heinrich R 2009 Circadian regulation of agonistic behavior in groups of parthenogenetic marbled crayfish, *Procambarus sp. J. Biol. Rhythms* **24** 64–72

Farca Luna AJ, Heinrich R and Reischig T 2010 The circadian biology of the marbled crayfish. *Front. Biosci.* **E2** 1414–1431

Faulkes Z 2010 The spread of the parthenogenetic marbled crayfish, Marmorkrebs (*Procambarus* sp.), in the North American pet trade. *Aquatic Invasions* **5** 447–450

Feria TP and Faulkes Z 2011 Forecasting the distribution of Marmorkrebs, a parthenogenetic crayfish with high invasive potential, in Madagascar, Europe, and North America. *Aquatic Invasions* **6** 55–67

Gherardi F, Souty-Grosset C, Vogt G, Diéguez-Uribeondo J and Crandall KA 2010 Infraorder Astacidea Latreille, 1802 pp: The freshwater crayfish; in *Treatise on zoology - anatomy, taxonomy, biology – the Crustacea. Volume 9, part A: Eucarida: Euphausiacea, Amphionidacea, and Decapoda (partim)* (eds) FR Schram, JC von Vaupel Klein, J Forest and M Charmantier-Daures (Leiden: Brill) pp. 269–423

Jimenez SA and Faulkes Z 2010 Establishment and care of a colony of parthenogenetic marbled crayfish, Marmorkrebs. *Invertebr. Rearing* **1** 10–18

Jirikowski G, Kreissl S, Richter S and Wolff C 2010 Muscle development in the marbled crayfish - insights from an emerging model organism (Crustacea, Malacostraca, Decapoda). *Dev. Genes Evol.* **220** 89–105

Jones JPG, Rasamy JR, Harvey A, Toon A, Oidtmann B, Randrianarison MH, Raminosoa N and Ravoahangimalala OR 2009 The perfect invader: a parthenogenic crayfish poses a new threat to Madagascar's freshwater biodiversity. *Biol. Invasions* **11** 1475–1482

Kawai T, Scholtz G, Morioka S, Ramanamandimby F, Lukhaup C and Hanamura Y 2009 Parthenogenetic alien crayfish (Decapoda: Cambaridae) spreading in Madagascar. *J. Crust. Biol.* **29** 562–567

Martin P, Kohlmann K and Scholtz G 2007 The parthenogenetic Marmorkrebs (marbled crayfish) produces genetically uniform offspring. *Naturwiss.* **94** 843–846

Martin P, Dorn NJ, Kawai T, van der Heiden C and Scholtz G 2010a The enigmatic Marmorkrebs (marbled crayfish) is the parthenogenetic form of *Procambarus fallax* (Hagen, 1870). *Contrib. Zool.* **79** 107–118

Martin P, Shen H, Füller G and Scholtz G 2010b The first record of the parthenogenetic Marmorkrebs (Decapoda, Astacida, Cambaridae) in the wild in Saxony (Germany) raises the question of its actual threat to European freshwater ecosystems. *Aquatic Invasions* **5** 397–403

Marzano FN, Scalici M, Chiesa S, Gherardi F, Piccinini A and Gibertini G 2009 The first record of the marbled crayfish adds further threats to fresh waters in Italy. *Aquatic Invasions* **4** 401–404

Pawlos D, Korzelecka-Orkisz A, Formicki K, Durkowski T and Winnicki A 2010 Egg volume and membrane resistance during embryogenesis of the marbled crayfish (*Procambarus* sp.). *Freshwater Crayfish* **17** 239–243

Polanska MA, Yasuda A and Harzsch S 2007 Immunolocalisation of crustacean-SIFamide in the median brain and eyestalk neuropils of the marbled crayfish. *Cell Tissue Res.* **330** 331–344

Rieger V and Harzsch S 2008 Embryonic development of the histaminergic system in the ventral nerve cord of the Marbled Crayfish (Marmorkrebs). *Tissue Cell* **40**, 113–126

Rubach M, Crum S and Van den Brink P 2011 Variability in the dynamics of mortality and immobility responses of freshwater arthropods exposed to chlorpyrifos. *Arch. Environ. Contam. Toxicol.* **60** 708–721

Schiewek R, Wirtz M, Thiemann M, Plitt K, Vogt G and Schmitz OJ 2007 Determination of the DNA methylation level of the marbled crayfish: an increase in sample throughput by an optimised sample preparation. *J. Chromatogr. B.* **850** 548–552

Scholtz G 2002 Phylogeny and evolution; in *Biology of freshwater crayfish* (ed) DM Holdich (Oxford: Blackwell) pp. 30–52

Scholtz G, Braband A, Tolley L, Reimann A, Mittmann B, Lukhaup C, Steuerwald F and Vogt G 2003 Parthenogenesis in an outsider crayfish. *Nature (London)* **421** 806

Seitz R, Vilpoux K, Hopp U, Harzsch S and Maier G 2005 Ontogeny of the Marmorkrebs (marbled crayfish): a parthenogenetic crayfish with unknown origin and phylogenetic position. *J. Exp. Zool. A* **303** 393–405

Sintoni S, Fabritius-Vilpoux K and Harzsch S 2007 The Engrailed-expressing secondary head spots in the embryonic crayfish brain: examples for a group of homologous neurons in Crustacea and Hexapoda? *Dev. Genes Evol.* **217** 791–799

Vilpoux K, Sandeman R and Harzsch S 2006 Early embryonic development of the central nervous system in the Australian crayfish and the Marbled crayfish (Marmorkrebs). *Dev. Genes Evol.* **216** 209–223

Vogt G 2007 Exposure of the eggs to 17α-methyl testosterone reduced hatching success and growth and elicited teratogenic effects in postembryonic life stages of crayfish. *Aquat. Toxicol.* **85** 291–296

Vogt G 2008a The marbled crayfish: a new model organism for research on development, epigenetics and evolutionary biology. *J. Zool.* *276* 1–13

Vogt G 2008b Investigation of hatching and early post-embryonic life of freshwater crayfish by *in vitro* culture, behavioral analysis, and light and electron microscopy. *J. Morphol.* **269** 790–811

Vogt G 2008c How to minimize formation and growth of tumours: potential benefits of decapod crustaceans for cancer research. *Int. J. Cancer* **123** 2727–2734

Vogt G 2010 Suitability of the clonal marbled crayfish for biogerontological research: a review and perspective, with remarks on some further crustaceans. *Biogerontol.* **11** 643–669

Vogt G and Tolley L 2004 Brood care in freshwater crayfish and relationship with the offspring's sensory deficiencies. *J. Morphol.* **262** 566–582

Vogt G, Tolley L and Scholtz G 2004 Life stages and reproductive components of the Marmorkrebs (marbled crayfish), the first parthenogenetic decapod crustacean. *J. Morphol.* **261** 286–311

Vogt G, Huber M, Thiemann M, van den Boogaart G, Schmitz OJ and Schubart CD 2008 Production of different phenotypes from the same genotype in the same environment by developmental variation. *J. Exp. Biol.* **211** 510–523

Vogt G, Wirkner CS and Richter S 2009 Symmetry variation in the heart-descending artery system of the parthenogenetic marbled crayfish. *J. Morphol.* **270** 221–226

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