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**Author:** Meij, Sancia Esmeralda Theonilla van der

**Title:** Evolutionary diversification of coral-dwelling gall crabs (Cryptochiridae)

**Issue Date:** 2015-06-03



**Reproductive morphology**



# Chapter 15

## Female reproductive morphology of coral-inhabiting gall crabs (Crustacea: Decapoda: Brachyura: Cryptochiridae)

Juliane Vehof, Sancia E.T. van der Meij, Michael Türkay & Carola Becker

### Abstract

Gall crabs are obligate associates of stony corals in which they induce skeletal modifications. In some cryptochirid species, females live in open depressions accessible to males; while in others, females are rather isolated in semi-closed galls, which necessitates elaborate sperm storage capabilities by the female. In this study we investigate the female gross morphology and reproductive systems of *Fungicola syzygia* lodged in semiclosed flattened pits, *Opecarcinus cathyae* with semiopen pits and *Pseudocryptochirus viridis* from shallow open depressions using line drawings and histological methods. The general morphology of the cryptochirids' reproductive systems is uniform and conforms to other thoracotreme brachyurans: paired muscular vaginae of the concave pattern lead from the sternal gonopores into paired seminal receptacles where sperm is stored. The seminal receptacle is internally lined by distinct types of epithelia: a cuticle underlined by a columnar epithelium ventrally, a monolayered secretory epithelium dorsally and a multilayered transfer tissue where the oviducts enter the seminal receptacle. In all studied specimens, the seminal receptacle contained free spermatozoa; however, in specimens of *Pseudocryptochirus viridis* it also contained spermatophores, indicating a recent insemination. In contrast to most other brachyurans ovaries of the investigated cryptochirids extend into the pleon. The specific degree of ovary extension differs between the studied species and is closely related to female body shape.

## Introduction

Gall crabs of the family Cryptochiridae are minute-sized obligate associates of stony corals, in which they induce skeletal modifications. These modifications are used as dwellings (see Fig. 1). Cryptochirids show a certain level of host specificity, insofar that each gall crab species is restricted to a specific group of corals. Distinct types of dwellings are recognized such as shallow depressions (Fize and Serène, 1957; van der Meij, 2012; Wei *et al.*, 2013; Fig. 1I, J), cylindrical pits (Fize and Serène, 1957; Simon-Blecher and Achituv, 1997; Wei *et al.*, 2013) and more or less closed galls (Potts, 1915; Fize and Serène, 1957; Wei *et al.*, 2013). Due to their small size and their hidden lifestyle within the coral skeleton, gall crabs are one of the least studied eubrachyuran groups (Potts, 1915; Kotb and Hartnoll, 2002). The actual impact on the coral host by the gall crab is not determined, and the characterization of cryptochirids as either commensals or parasites is debated. Furthermore, their mode of feeding is still under discussion (see Potts, 1915; Kropp, 1986; Castro, 1988; Abelson *et al.*, 1991; Simon-Blecher and Archituv, 1997); however, the obligate character of the association is undisputed.

Morphologically, Cryptochiridae differ from other brachyurans in several aspects. The carapace, that is the cephalothorax, is elongated in most species and longer than wide in both sexes. Apart from that, a strong sexual dimorphism is recognized, with the sedentary females being considerably larger than the mobile males. Adult females have developed a large brood pouch where embryos mature until hatching (Fig. 1D, G). This so-called marsupium, formed by the abdomen [= pleon] (Potts, 1915), is a synapomorphy of the Cryptochiridae. The breeding female is sedentary and in some species completely isolated by the coral skeleton except for fine pores for water circulation (Potts, 1915). In other species, the female inhabits a pit that remains open so that free-living males can enter, and occasionally, males and females were found in adjoining pits on the same coral (McCain and Coles, 1979; Carricart-Ganivet *et al.*, 2004).

In several aspects, Cryptochiridae resemble Pinnotheridae, another symbiotic brachyuran group. Both families have a similar lifestyle: adult pinnotherid females live sedentary in their hosts (e.g. bivalves, tunicates) and have a strongly widened pleon to carry their large broods (Becker, 2010). Their reproductive investment (Hartnoll, 2006) and output (Hines, 1992) are very high compared to other brachyurans. Analogous to pinnotherids, the reproductive investment of cryptochirids is also considered high (Kotb and Hartnoll, 2002). No data, however, are so far available on their reproductive morphology. Herein, we investigate the female reproductive systems of *Fungicola syzygia* van der Meij, 2015, *Opecarcinus cathyae* van der Meij, 2014 and *Pseudocryptochirus viridis* Hiro, 1938, by histological methods, and present descriptions on their gross morphology. We incorporate these results with data obtained from live observations during fieldwork on species' ecology and cavity types to reveal possible reproductive strategies among Cryptochiridae.

## Material and methods

### *Collection of specimens and fixation*

Gall crabs were hand-collected by scuba diving on coral reefs in the Semporna district (eastern Sabah, Malaysia) in December 2010. The research area is described in detail in Waheed and Hoeksema (2013) and Van der Meij and Hoeksema (2013).

Corals were searched for galls and pits, photographed and subsequently split with hammer and chisel. The following gall crab species were included in this study: one male and four females (three ovigerous) of *Fungicola syzygia* collected from Pom Pom Isl. (04°35'29.8"N 118°51'43.1"E)

in *Pleuractis paumotensis* (Stutchbury, 1833) from 0 to 10 m depth; five ovigerous females of *Opearcinus cathyae* collected at Creach Reef (04°18'58.8"N 118°36'17.3"E) in *Pavona clavus* (Dana, 1846) from 10 to 14 m depth; one male and four females (two ovigerous) of *Pseudocryptochirus viridis* collected from Bakungan Isl. (04°45'11.1"N 118°29'16.0"E) in *Turbinaria* sp. from ca. 15 m depth. Whole specimens were fixed in a mixture of formalin, acetic acid, mercuric chloride and trichloroacetic acid ('Susa Heidenhain' after Romeis, 1989), washed with 100%, 90% and 80% ethanol for two hours each and subsequently stored in 70% ethanol. Samples and slides are stored at Senckenberg Research Institute in Frankfurt. Further gall crab specimens belonging to the same series were fixed in 80% ethanol and are stored in the collections of the Naturalis Biodiversity Center in Leiden, the Netherlands (collection coded as RMNH.Crus.D).

### *Gross morphology and histology*

Line drawings were prepared with the help of a stereo microscope Leica MZ8 equipped with a camera lucida and subsequently digitized using Adobe Illustrator and Photoshop (after Coleman, 2003).

For decalcification, samples were incubated in 20% EDTA (ethylenediaminetetraacetic acid) for 72 h. In a graded ascending series of ethanol with steps of 80%, 90%, 96% and 100% (p. A.) ethanol (each step for 2 h), samples were dehydrated and then infiltrated with paraffin overnight (Leica TP1020 Histokinette). Subsequently, samples were embedded in paraffin blocks, and histological sections were prepared with a Leica RM2165 microtome at 6–8  $\mu\text{m}$ . A trichromatic Masson–Goldner staining 'light green' (after Romeis, 1989), performed with a Leica Autostainer XL, was used for general tissue differentiation. Covered slides were studied and photographed with a Zeiss microscope equipped with a digital camera (CamScan Prog Res).

## Results

### *Species and gall types*

Field pictures of gall crabs inside their hosts and line drawings of each species with its specific gall type are presented in Fig. 1A–L.

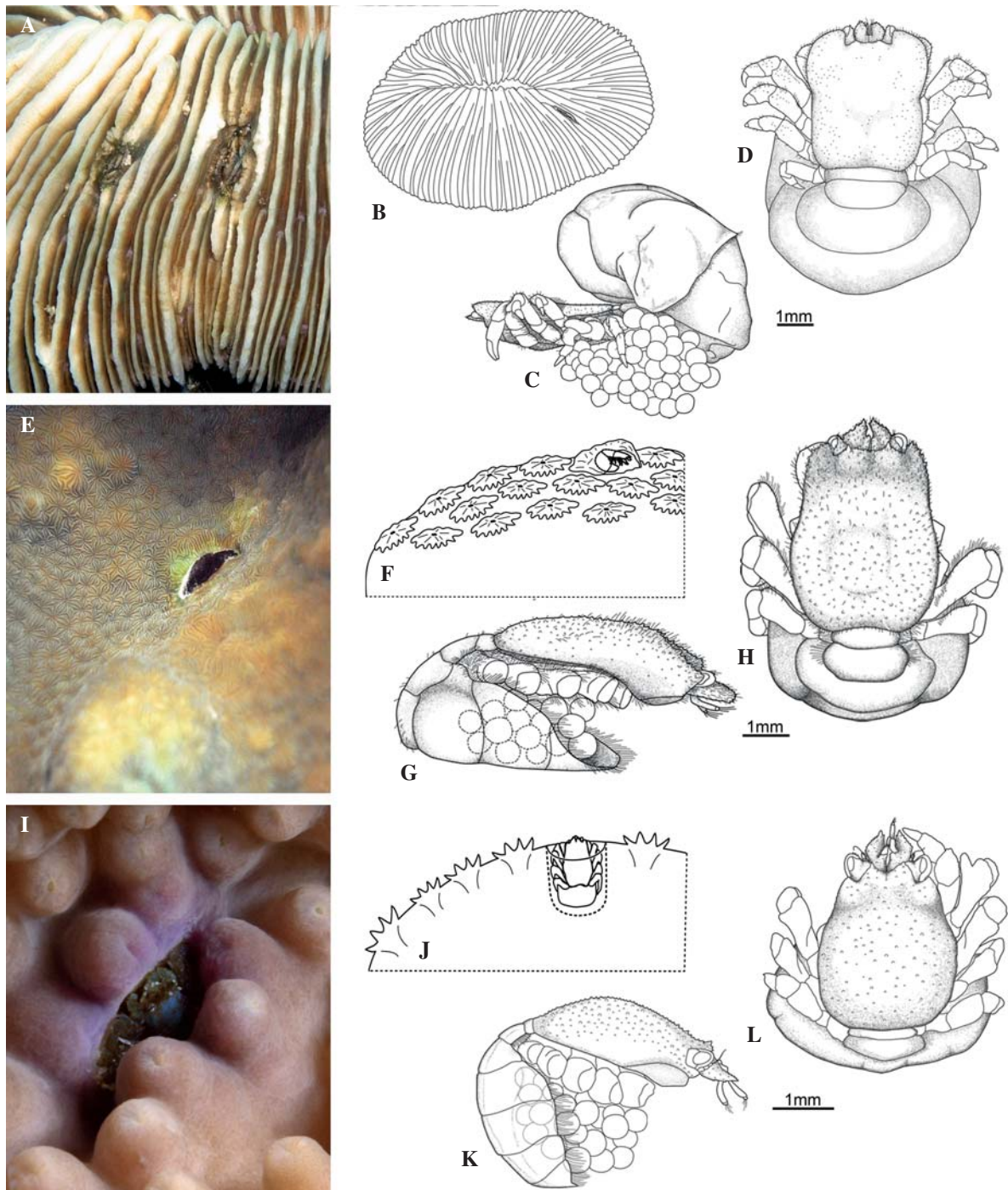
*Fungicola syzygia* is associated with fungiid corals (Fungiidae) and lives in flattened pits similar to those of *F. fagei* (Fize and Serène, 1956). These pits have a very small 'slit-like' opening and are lodged between the septae of the corals (Fig. 1A, B). Mature females cannot leave the galls, while males are sometimes found in separate pits on the same coral or holding onto the septae, without a gall.

The studied specimens of *Opearcinus cathyae* were collected from *P. clavus* (Agariciidae). The pit has a crescent shaped opening, which allows some movement by the crabs (Fig. 1E, F). Crabs were not observed to freely move around on the coral. *Pavona clavus* can occur in large monospecific stands housing many individuals, both males and females, of *O. cathyae* (Hoeksema and van der Meij, 2013).

*Pseudocryptochirus viridis* is associated with *Turbinaria* sp. (Dendrophylliidae), and often a large coral houses many males and females, each in their own pit. The pits shown in Fig. 1I and J are shallow crescent-shaped depressions, which allow full movement for both male and female, nevertheless, moving crabs were only observed when disturbed.

### *Gross morphology*

The studied species have a body form typical for cryptochirids with a cephalothorax that is longer than wide, short walking legs and chelae equal in size (Fig. 1). The cephalothorax is extremely



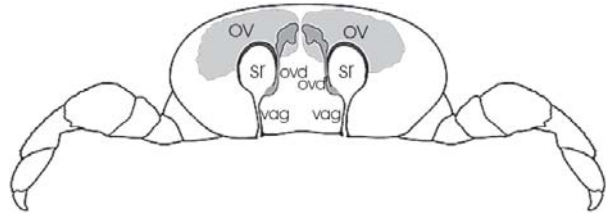
**Fig. 1.** Morphology of the investigated gall crab females and their different gall types **A-D**, *Fungicola syzygia*; **E-H**, *Opecarcinus cathyae*; **I-L**, *Pseudocryptochirus viridis*. **A**, opening of flattened pit including a specimen of *F. syzygia* in *Pleuractis paumotensis*, with only the middle part of the carapace front visible. Photographed by BW Hoeksema; **B**, Schematic drawing of a mushroom coral with a pit lodged between the septae; **C**, Lateral view of *F. syzygia*, the rectangular cephalothorax of this species is extremely flattened, the marsupium of this ovigerous female is turned to the outside by the rough fixation process; **D**, Dorsal view of *F. syzygia* with a well-developed and regular-formed marsupium; **E**, Gall with an overhang and a crescent-shaped opening, induced by *O. cathyae*, on a large colony of *Pavona clavus*. Photographed by SET van der Meij; **F**, Schematic drawing of a gall with overhang; **G**, Lateral view of an ovigerous female of *O. cathyae*; **H**, dorsal view of *O. cathyae*; **I**, Opening of a shallow crescent-shaped depression including a specimen of *P. viridis* in *Turbinaria* sp., the anterior part of carapace and front parts of the chelae are visible. Photographed by BT Reijnen/SET van der Meij; **J**, Schematic longitudinal section of a typical pit; **K**, Lateral view of an ovigerous female of *P. viridis*; **L**, Dorsal view of a female of *P. viridis*.

flattened in *F. syzygia* (Fig. 1C, D), in contrast to the more bulbous form with a anteriorly deflected part in *O. cathyae* (Fig. 1G, H) and *P. viridis* (Fig. 1K, L). The marsupium is built by the strongly widened pleon, which is developed in all studied specimens irrespective of whether females were ovigerous or not. In *F. syzygia* (Fig. 1C, D), the marsupium is more voluminous than in *O. cathyae* (Fig. 1G, H) and *P. viridis* (Fig. 1K, L) so that the walking legs of the female can barely reach ground.

## Morphology of the female reproductive system

### Overview

The general morphology of the female reproductive system in cryptochirids conforms to other eubranchyuran crabs. Paired sternal gonopores (vulvae) lead via cuticular vaginae into the likewise paired sac-shaped, oval to round storage organs: the seminal receptacles (Figs 2, 3A). Both seminal receptacles are connected to the ovaries of each body side – left and right through an oviduct. The receptacle is coated on the outside by connective tissue (Fig. 3A, B); internally, it is lined by three different types of epithelia: a monolayered columnar epithelium with an overlying cuticle ventrally that is continuous with the also cuticular vagina, a monolayered glandular epithelium dorsally (Fig. 3B-D) and a multi-layered tissue where the oviduct runs into the seminal receptacle (Fig. 3E, F). Species differed, however, from each other in histological details of the secretory epithelia, in the seminal receptacle content (secretion and male sperm) and in the location and extension of the ovaries.



**Fig. 2.** Schematic transverse section through the cephalothorax of a female gall crab. Oocytes are produced by the ovary consisting of several lobes whose extension could differ around the light grey area. During spawning, the oviduct leads mature oocytes from the ovary lobes into the seminal receptacles, and subsequently, they leave the body through the vaginae. ov, ovary; ovd, oviduct; sr, seminal receptacle; vag, vagina.

### Vaginae

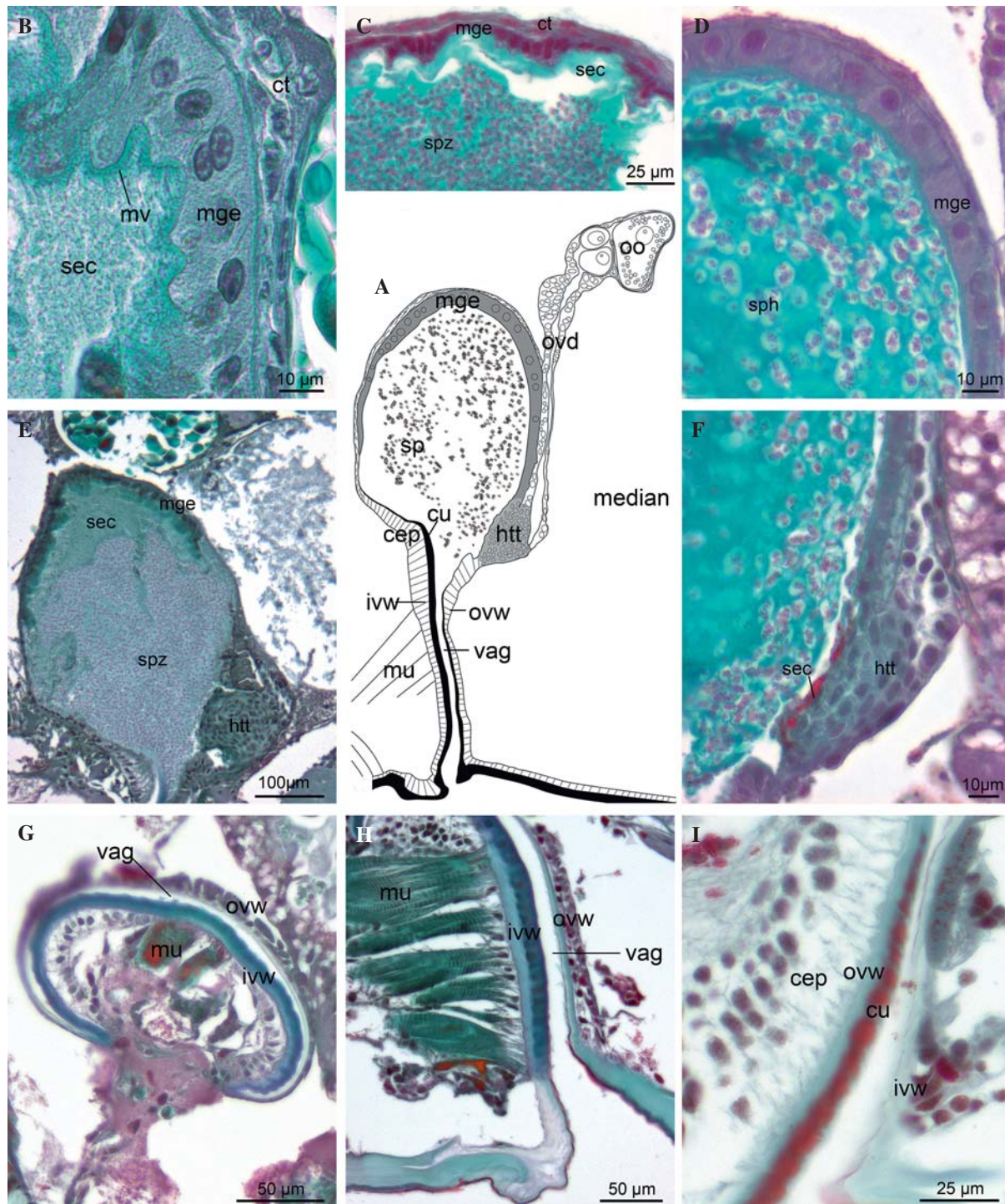
The paired vaginae are cuticle-lined narrowed ducts running perpendicularly from the sternal gonopores into the also paired seminal receptacles (Fig. 2, 3A). The vagina lumen is crescent-shaped in transverse sections ('concave pattern' sensu Hartnoll, 1968) because the lateral half of its wall ('inner vagina wall') is longitudinally folded into the mesial side ('outer vagina wall') (Fig. 3G). The inner vagina wall (Fig. 3H) exhibits strong musculature running to the sternum.

## Seminal receptacle and secretion

### Monolayered glandular epithelium

The epithelium lining the seminal receptacle dorsally is monolayered and produces secretions into the lumen of the seminal receptacle. Slight differences are present in these dorsal epithelia among the investigated species. In *O. cathyae* the glandular epithelium is composed of slim elongated cells that are irregularly shaped and apically lined by microvilli (Fig. 3B). Rounded nuclei are located in the cells basally; some cells have several nuclei (Fig. 3B). Many secretions are present nearby the epithelium (Fig. 3B, E). In *F. syzygia* (Fig. 3C), the cells of the epithelium are comparatively smaller than those in *O. cathyae* so that the nuclei occupy almost the entire cells, with plenty secretions nearby as in *O. cathyae*. The surface of the epithelium of *P. viridis* is regular and





**Fig. 3.** Schematic view of seminal receptacle and vagina of Cryptochiridae with histological details; **A**, schematic drawing of seminal receptacle and vagina, main parts of the seminal receptacle are lined by glandular epithelium; **B**, Glandular epithelium of *Opearcinus cathyae*, apical parts of the cells with microvilli seem; **C**, Glandular epithelium of *Fungicola syzygia*; **D**, Glandular epithelium of *Pseudocryptochirus viridis*; **E**, Ventral localisation of the orifice of oviduct in *O. cathyae*; **F**, Details of the transfer tissue at the orifice of oviduct with orange staining secretion in *P. viridis*; **G**, Vagina of *P. viridis*, crescent-shaped in transverse sections; **H**, Longitudinal section through the vagina of *O. cathyae*, musculature inserts at the inner vagina wall; **I**, The cuticle of the flexible muscular vagina wall differs from the general cuticle and stains orange to red in trichromatic Masson-Goldner staining, well visible here in *F. syzygia* cep, cuticular epithelium; cu, cuticle; ct, connective tissue; htt, holocrine transfer tissue; ivw, inner vagina wall; mge, monolayered glandular epithelium; mu, musculature; mv, microvilli; ovw, outer vagina wall; sec, secretion; sph, spermatophore; spz, spermatozoa; vag, vagina.

rather even, consisting of square-shaped cells, while fluids are homogeneously distributed in the seminal receptacle (Fig. 3D).

#### *Secretory transfer tissue*

At the junction with the seminal receptacles, the oviduct runs into a multilayered tissue that can protrude into the lumen of the receptacle (Fig. 3E, F). Because of its position at the location where oocytes are transferred from the ovary through the oviduct into the seminal receptacle, this tissue is referred to as ‘transfer tissue’. Its cells are irregularly shaped and oriented. Towards the lumen of the seminal receptacle, cells flatten and their plasma becomes increasingly dense; in the periphery, cells are shed as secretion. In the applied Masson-Goldner staining, these secretions stain different from the ones of the dorsal epithelium (Fig. 3F). The transfer tissue is histologically uniform among the investigated species, but differs slightly in the extension towards the lumen of the seminal receptacle.

#### *Seminal receptacle content*

All the studied females (ovigerous and non-ovigerous) had their seminal receptacles filled with spermatozoa, either free or enclosed in spermatophores (Fig. 4A-D). The amount of sperm present within the seminal receptacle varied slightly and, therefore, the size of the seminal receptacle – sometimes even among the right and left seminal receptacle within one specimen. In *F. syzygia* and *O. cathyae* spermatozoa were free (Fig. 4A, B). In *O. cathyae* spermatozoa and fluids were evenly distributed throughout the seminal receptacle and also present close to the vagina. In contrast to this, the sperm mass was concentrated in the dorsal part of the seminal receptacle in *F. syzygia*, while the ventral part around the vagina was free of spermatozoa (Fig. 4B). Only in *P. viridis* were the spermatozoa still enclosed within spermatophores in all studied specimens (Fig. 4C, D).

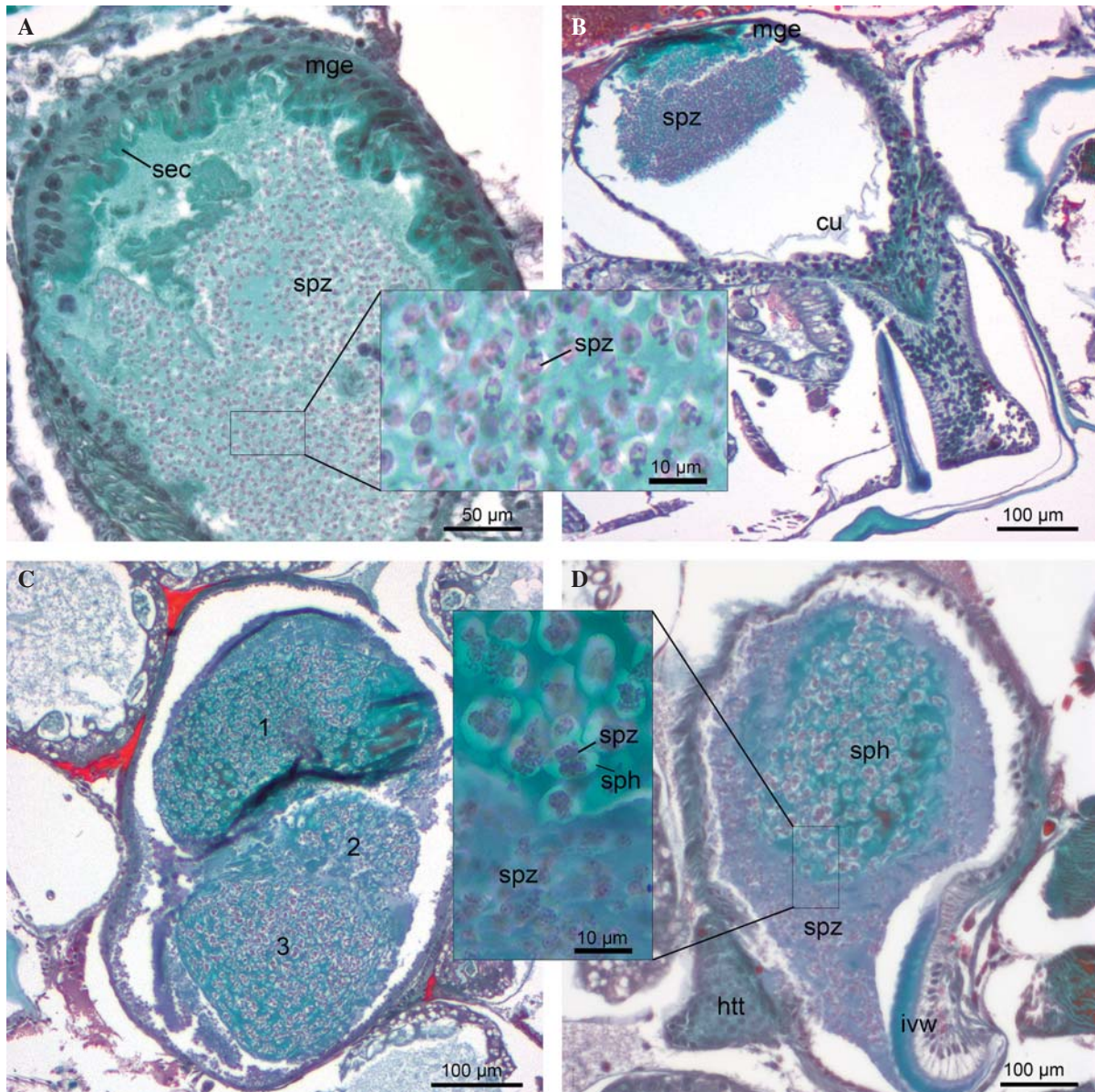
#### *Ovary*

The ovaries of the investigated cryptochirids species, consisted of several lobes, are not restricted to the cephalothorax but extend into the pleon in various degrees. In the ovary, mature oocytes were present next to previtellogenic stages in all three species. In *O. cathyae* and *P. viridis*, ovaries contained mature oocytes centrally in the cephalothorax (Fig. 5A) and in the pleon; while in *F. syzygia*, the main part of the ovary was located in the pleon (Fig. 5B) and reached beyond the sixth pleon segment. In histological sections of the cephalothorax, we identified only a few immature oocytes. The different developmental stages of oocytes were best detectable in the ovary of *F. syzygia* (Fig. 5C). In germinative zones, shown in Fig. 5C, germ cells proliferate by mitosis and develop into oogonia (Fig. 5D). The germinative zones are surrounded by maturation zones in which oogonia undergo the following stages of vitellogenesis. Most of the ovary is filled with mature oocytes in their final stage of vitellogenesis (Fig. 5C).

## **Discussion**

#### *Overview and systematic position*

The main characteristics of the female reproductive systems (a concave vagina, the general construction of the seminal receptacle, its ventral connection with the oviduct, and the types and localisation of epithelia) are uniform among the investigated gall crab species and conform to the reproductive systems of so far investigated representatives of thoracotreme brachyurans from the families Ocypodidae (Sant’Anna *et al.*, 2007; López-Greco *et al.*, 2009; Lautenschlager *et al.*,



**Fig. 4.** Contents of seminal receptacles; **A**, Longitudinal section through seminal receptacle of *Opecarcinus cathyae* filled with free spermatozoa, male seminal plasma and secretions produced by the dorsal glandular epithelium; **B**, Longitudinal section through seminal receptacle of *Fungicola syzygia* with free spermatozoa in its dorsal region; **C**, Transversal section through seminal receptacle of *Pseudocryptochirus viridis* with sperm mass forming distinct portions; **D**, Transversal section through seminal receptacle of *P. viridis* with spermatophores centrally and free spermatozoa close to the periphery. cu, cuticle; htt, holocrine transfer tissue; ivw, inner vagina wall; mge, monolayered glandular epithelium; sph, spermatophore; spz, spermatozoa; vag, vagina.

2010), Varunidae (López-Greco *et al.*, 1999); Pinnotheridae (Becker *et al.*, 2011) and Gecarcinidae (de Souza *et al.*, 2013). Hence, a systematic position of the Cryptochiridae within the Thoracotremata as proposed by Ng *et al.* (2008), Wetzer *et al.* (2009) and Van der Meij and Schubart (2014) is supported by the female reproductive morphology.

#### *Vagina and seminal receptacle*

The cryptochirids' concave vagina which conforms to the 'concave pattern' sensu Hartnoll (1968) is characteristic for thoracotreme crabs and has been found in a number of thoracotreme crab

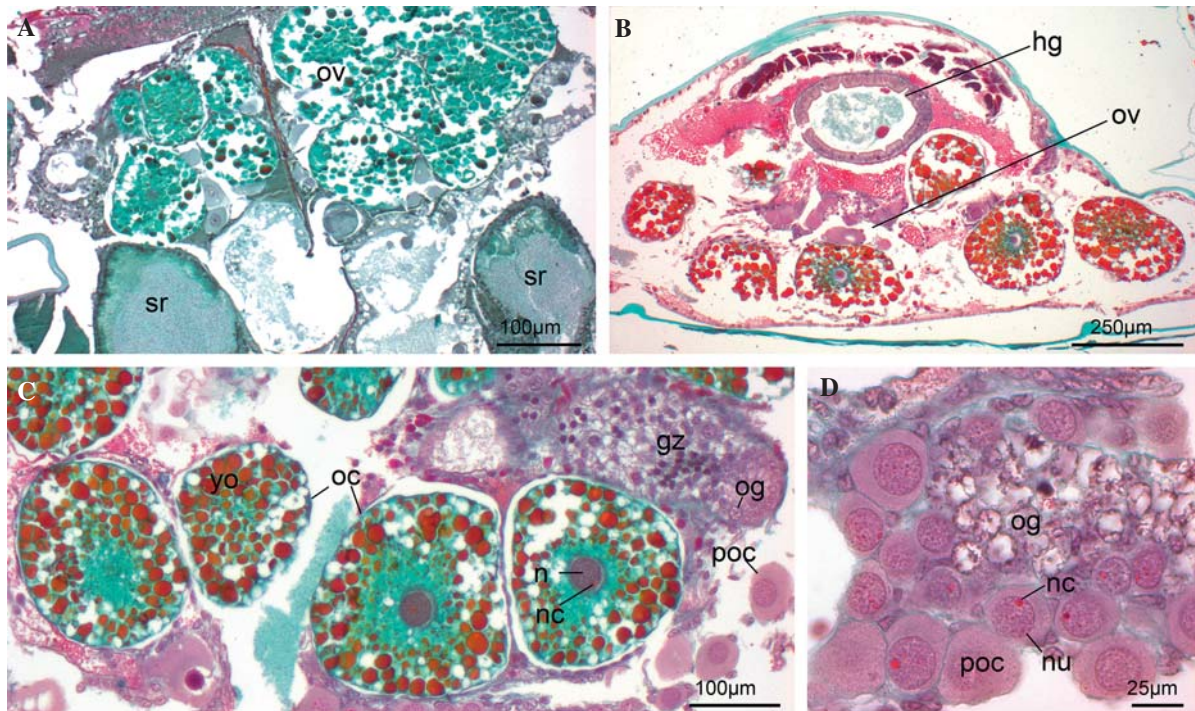
taxa, for example Varunidae (Lee and Yamazaki, 1990), Ocypodidae (Lautenschlager *et al.*, 2010), Pinnotheridae (Becker *et al.*, 2011) and Gecarcinidae (de Souza *et al.*, 2013). However, several ‘advanced’ heterotreme groups also have a vagina of the ‘concave pattern’, while heterotremes with many basal characters often have vaginae of the ‘simple pattern’ sensu Hartnoll (1968). These are simple, round ducts closed by a cuticle, wherein mating is often linked to moult cycles (Hartnoll, 1968). The vagina is closed to the outside by a convex bulge of the inner vagina wall, similar to the operculum described by Hartnoll (1968), and the width of the vagina lumen is actively controlled by the female’s musculature. The histology of the cryptochirid concave vagina corresponds to those of *Uca* (Lautenschlager *et al.*, 2010) and Pinnotheridae (Becker *et al.*, 2011). The width of the vaginal lumen could be actively controlled by the female’s musculature, and no structures were found that necessitate moulting before copulation. This musculature control might play an important role during copulation and oviposition.

Inside the seminal receptacle, the two types of epithelia, the monolayered glandular epithelium and the secretory transfer tissue, produce secretions, which are supposed to be involved in reproductive processes in a way that is not entirely understood (see below).

A specialized transfer tissue at the connection of the seminal receptacle with the oviduct was first described in *Eriochelone sinensis* H. Milne-Edwards, 1853 termed as ‘valve-like tissue’ (Lee and Yamazaki, 1990). The authors assumed that it has a barrier function inhibiting the intrusion of spermatozoa into oviducts and ovaries. Only at the time of ovulation, they observed an open passage in this tissue within the oviduct and seminal receptacle. Beyond a possible barrier function, the transfer tissue produces secretions. Based on its histological characteristics and structural similarities with the tissue in pinnotherids (Becker *et al.*, 2011), we assume a holocrine secretory mechanism in Cryptochiridae, which is characterized by the dissolving of whole cells into secretion (Plattner and Hentschel, 2006). Structurally similar glandular epithelia of the holocrine type are common in eubranchyuran seminal receptacles, for example in portunids (Johnson, 1980), cancrids (Jensen *et al.*, 1996) and majoids (Diesel, 1989; Benninger *et al.*, 1993). In these groups, multilayered holocrine glandular epithelia line the dorsal part of the seminal receptacles; while in cryptochirids and other thoracotremes, for example ocypodids (Lautenschlager *et al.*, 2010), pinnotherids (Becker *et al.*, 2011) and gecarcinids (de Souza *et al.*, 2013), the holocrine secretion is restricted to the transfer tissue at the connection to the oviduct. Whether the holocrine transfer tissue in the Thoracotremata might be homologous to the multilayered holocrine epithelia of the Heterotremata is currently uncertain.

The highly secretory non-holocrine glandular epithelium of the dorsal seminal receptacle is also present in other thoracotreme taxa, for example Ocypodidae (Sant’Anna *et al.*, 2007; López-Greco *et al.*, 2009; Lautenschlager *et al.*, 2010), Pinnotheridae (Becker *et al.*, 2011) and Gecarcinidae (de Souza *et al.*, 2013). Several possible functions, mostly based on studies of heterotreme crabs, have been proposed for these secretions in general: antibacterial activity (Benninger *et al.*, 1993), providing a milieu that allows the growth of useful bacteria (Jensen *et al.*, 1996), dehiscence of spermatophores (Adiyodi and Anilkumar, 1988; Diesel, 1989) or nourishment of spermatozoa inside the seminal receptacle (Anilkumar *et al.*, 1996). To what extent the secretions might contribute to the maintenance of sperm could not be investigated in this study. Involvement in the formation of a sperm plug that closes the female genital ducts after copulation (Bawab and El-Sherief, 1989) or in the dissolution of the sperm plug (Spalding, 1942) is not likely in cryptochirids, as sperm plugs were not present inside the vaginae and/ or seminal receptacles.

The presence of intact spermatophores inside the seminal receptacle of the *Pseudocryptochirus viridis* specimens suggests a recent insemination of the female as the permanence of transmitted spermatophores is usually short (Anilkumar *et al.*, 1999; Jennings *et al.*, 2000). Moreover,



**Fig. 5.** Histology of the ovary; **A**, Transverse section through mature ovary of *Opecarcinus cathyae* within cephalothorax; **B**, Transverse section of ovary inside the pleon of *Fungicola syzygia* with mature oocytes ventrally to the hindgut; **C**, Section through ovary of *F. syzygia* showing germinative zone and mature oocytes (up to 200  $\mu\text{m}$  in diameter) filled with yolk vesicles staining orange to red in Masson–Goldner staining; within mature oocytes the size of the yolk droplets increases from the centre of the cell to its periphery; **D**, Details of oogonia (about 15  $\mu\text{m}$  in diameter) and previtellogenic oocytes (25–30  $\mu\text{m}$ ) which are present at the periphery of germinative zones. gz, germinative zone; hg, hindgut; nc, nucleolus; nu, nucleus; oc, oocyte; og, oogonia; ov, ovary; poc, previtellogenic oocyte; yo, yolk.

the sperm mass agglomeration (containing spermatophores) in several distinct clusters suggests recent multiple mating of the female. In contrast to that, in *Fungicola syzygia* and *Opecarcinus cathyae*, the seminal receptacle content was a homogenous mass of free spermatozoa with no indication of a recent insemination.

#### *Ovaries and reproductive investment*

A postoviposition regeneration of the ovary in ovigerous females or clearly detectable ovary developmental stages (see de Souza and Silva, 2009; Castilho-Westphal *et al.*, 2013) were not observed in the ovaries of cryptochirids. This supports the observation of Kotb and Hartnoll (2002) that brood production lacks seasonality in gall crabs, which is expected considering their tropical distribution.

The extension of the ovaries into the pleon is unusual among brachyuran crabs, in which ovaries are normally restricted to the cephalothorax (Adiyodi and Subramoniam, 1983; Krol *et al.*, 1992). Only few exceptions are known; for example, mature ovaries of *Cardisoma guanhumi* Latreille, 1828 and *Goniopsis cruentata* (Latreille, 1803) reach from the cephalothorax into the second or third pleomer, respectively (de Souza and Silva, 2009; de Souza *et al.*, 2013). Only in pinnotherids were ovaries found to extend into the last segments of the strongly broadened pleon (Becker *et al.*, 2011), as it is the case in the cryptochirid species investigated here. The extreme expansion of ovaries in pinnotherids in cephalothorax and pleon enables brood weights from 66% up to 97% of the female body weight (Hines, 1992). No data on brood weights are available for

the species investigated in the present study, but in the gall crab *Hapalocarcinus marsupialis* Stimpson, 1859 a similarly high reproductive investment has been shown: the brood weight is 59% of the female body weight (Kotb and Hartnoll, 2002). In comparison with free-living crabs with mean brood weights of 11-20% of the female body weight (Hines, 1992; Hartnoll, 2006), the reproductive investment of gall crabs is much higher than average. Just like pinnotherids, gall crabs benefit from the host-related lifestyle, for example in being protected against predators. However, this delimits body size and hence influences the potential space for yolk accumulation in the cephalothorax, which in turn influences the production of embryonic mass (Hines, 1992). At the same time, associated living animals (especially small ones) need a larger number of offspring, because the free-living larval life phase, including the search for a suitable host, is such a critical phase in their life cycle (Bush *et al.*, 2001). In cryptochirids, reproduction can be maximized at cost of mobility (marsupium), as females do not have to leave their dwelling.

#### *Gall type and possible mating strategies*

From the observations of the gall type in the field and the histological results, we conclude that the entrance of the crescent-shaped gall in *P. viridis* is wide enough to allow males to enter for repetitive mating, even with already ovigerous females. In this species, several females and males live together on a coral host, and therefore, repetitive mating and promiscuity are likely to occur (see 'visiting type' in Asakura, 2009). On the contrary, in *F. syzygia*, the entrance to the gall wherein the female lives is a narrow slit that is unlikely to allow the female to leave or a male to enter while an ovigerous female is inside. In this case, we suggest that copulation only takes place in a specific (early) life stage of the female when the pit is still accessible. Therefore, sperm storage over a longer period of time is crucial, and probably, several broods are fertilised by spermatozoa received in an earlier life stage. The gall crab *H. marsupialis* lives in semiclosed galls in corals of the family Pocilloporidae and may have a similar mating strategy: females of this species presumably copulate when the gall opening is still wide enough for males to enter. After the gall closes the female produces eight or more egg batches within the following 10 months (Kotb and Hartnoll, 2002).

## Conclusions

The investigated species have a generally uniform reproductive system of the thoracotreme type and a high investment in reproduction apparent from the enlargement of ovaries extending into the pleon. In *Fungicola syzygia*, inhabiting narrow slit-like pits, the marsupium and the displacement of the ovaries was most developed with the broadened pleon seriously hampering locomotion of ovigerous females. According to our study, this is the species most adjusted to the symbiotic lifestyle and possibly isolated from mating as an adult, similar to *Hapalocarcinus marsupialis* (Kotb and Hartnoll, 2002). *Pseudocryptochirus viridis* and *Opecarcinus cathyae* living in open to (semi)open dwellings are seemingly less hampered in locomotion by the broadened pleon, and the ovaries in the pleon are less developed.

Only in *P. viridis*, with dwellings most accessible to males was a recent copulation confirmed. According to our results, cryptochirids might have different mating strategies from each other, and knowledge of the specific gall/pit type and the coral host is crucial for understanding reproduction in gall crabs.

## **Acknowledgements**

The gall crabs used in this study were collected during the 2010 Semporna Marine Ecological Expedition (SMEE2010), jointly organized by WWF-Malaysia, Universiti Malaysia Sabah's Borneo Marine Research Institute, Universiti Malaya's Institute of Biological Sciences and the Naturalis Biodiversity Center and funded through WWF-Malaysia. Research permits were granted by the Prime Minister's Department, Economic Planning Unit Sabah, Sabah Parks and Department of Fisheries Sabah, Malaysia. For histological work, we were kindly allowed to use the laboratories of Morphisto GmbH in Frankfurt (Main)/Germany, special thanks to Michael Gudo, Martin Thomas, Ines Spöhrer and Claudia Groth. We thank Colin McLay and an anonymous reviewer for their constructive comments.