European Pea Crabs -
Taxonomy, Morphology, and Host-Ecology
(Crustacea: Brachyura: Pinnotheridae)

Dissertation

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To my Professor

Michael Türkay

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Display cabinet prepared for the exhibition “among parasites” in Senckenberg Natural History Museum.

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European Pea Crabs – Taxonomy, Morphology, and Host-Ecology

Pinnotherids are small crabs symbiotic to a variety of invertebrates. The European species infest bivalves and sea squirts. Their way of life is parasitic and poses a threat to commercially exploited bivalves. While juveniles of both sexes still look very similar - being agile swimmers and partially free living - a metamorphosis takes place in the female after mating and results in a conspicuous sexual dimorphism. Thereafter, the female settles in its host definitely and is morphologically strongly adapted to the parasitic life phase. A very high reproductive output was demonstrated among several pea crab species infesting bivalves. Despite from that, hardly any information is present in the literature on the pinnotherids' reproductive biology and the underlying morphology.

Due to their cryptic way of life, the sexual dimorphism, and the different morphotypes of the female, the taxonomy of the Pinnotheridae is a serious challenge. Two widely accepted species are recognized on European coasts: *Pinnotheres pisum* and *Nepinnotheres pinnotheres*. *Pinnotheres pectunculi* was so far only known from the bivalve *Glycymeris glycymeris* in its type locality Roscoff (France), while *Pinnotheres ascidicola* and *Pinnotheres marioni* were described as living exclusively in ascidians without careful comparison with the previously described species. In order to produce standardized comparative descriptions, pea crabs were collected and studied from different hosts and localities in the Northeast Atlantic and in the Mediterranean. *Nepinnotheres pinnotheres* and *Pinnotheres pisum* were redescribed with consideration to characters of female and male. According to our morphological analysis, *Pinnotheres ascidicola* and *Pinnotheres marioni* are junior synonyms of *Nepinnotheres pinnotheres,* whereas the status of *Pinnotheres pectunculi* as a valid species was ascertained. Important characters are the mouthparts, the male gonopods, and especially chelipeds that showed consistent characteristics among different crab stages of both sexes.

Based on our sampling, we estimated the host-range of the European species. *Nepinnotheres pinnotheres* lives in ascidians and in the pen shell *Pinna nobilis*. *Pinnotheres pisum* infests numerous bivalve species - *Pinna nobilis* included. For *Pinnotheres pectunculi* novel host records are presented, all from the bivalve family Veneridae. Furthermore, feeding of the *Pinnotheres*-species was observed. They use a setae comb ventrally on the claw to brush mucus (and the accumulated food particles) from the bivalve gills. Feeding strategies and host-ecology will be thoroughly discussed in consideration to other Pinnotheridae.

We investigated the reproductive systems of European pinnotherids by histological methods, scanning and transmission electron microscopy, and confocal laser scanning microscopy.

The Eubrachyura have internal fertilization: paired vaginas enlarge into storage structures, the spermathecae, which are connected to the ovaries by oviducts. Sperm is stored until the oocytes are mature and transported into the spermathecae, where fertilization takes place. In the investigated pinnotherids, the vagina is of the 'concave pattern'. Musculature is attached alongside flexible parts of the vagina-wall to control the dimension of its lumen. The genital opening is closed by a muscular mobile operculum.

The spermatheca can be divided into two distinct regions by function and morphology. The ventral part includes the connection with vagina and oviduct and is regarded as the zone where fertilization takes place. It is lined with cuticle except where the oviduct enters the spermatheca by the 'holocrine transfer tissue'. At ovulation, the oocytes have to pass through this multi-layered glandular epithelium, which has a holocrine mode secretion. The dorsal part of the spermatheca is lined by a highly secretory apocrine glandular epithelium, which was to date only found in fiddler crabs of the genus *Uca*.

The male internal reproductive system consists of paired testes and corresponding vasa deferentia. The sperm morphology of pinnotherids conforms to other thoracotremes, with slight differences between *Nepinnotheres pinnotheres* and *Pinnotheres pisum*. Spermatozoa become enveloped into spermatophores in the secretory proximal vas deferens. The medial vas deferens is strongly enlarged and stores spermatophores embedded in seminal plasma. The distal vas deferens holds tubular appendices, which extend into the ventral cephalothorax and slightly into the pleon. These appendices produce and store vast quantities of seminal plasma. The copulatory system of the Brachyura is formed by paired penes and two pairs of gonopods, which function in sperm transfer. In pinnotherids, the long first gonopods transfers the sperm mass to the female. It holds the ejaculatory canal inside, which opens proximally and distally. The second gonopod is solid, short and conical. During copulation, the penis and the second gonopod are inserted into the base of the tubular first gonopod. The second gonopod functions in the transport of the sperm mass inside the ejaculatory canal towards its distal opening. The specific shape of the second gonopod is strongly adapted for a sealing of the tubular first gonopod with longitudinal cuticle foldings that interlock inside the first gonopod. The presented results are discussed concerning their function in reproduction and in respect of the systematic account.

The role of secretion in sperm transfer, storage and fertilization among the Brachyura is still under debate. It is notable that structure and function of secretion are more complex in pinnotherids and probably more efficient than in other brachyuran crabs, which will be discussed, in view of the parasitic way of life and the high fecundity of pinnotherids.

Europäische Muschelwächter – Taxonomie, Morphologie und Wirtsökologie

Krabben der Familie Pinnotheridae leben in Assoziation mit anderen wirbellosen Meerestieren. Die europäischen Vertreter bewohnen Muscheln und Seescheiden. Ihre Lebensweise ist parasitisch und führt zu Schäden bei kommerziell genutzten Muschelarten. Während sich die Juvenilen beider Geschlechter noch gleichen – sie sind gute Schwimmer und fakultativ freilebend – vollzieht sich beim Weibchen nach der Paarung eine Metamorphose, die zu einem ausgeprägten Geschlechtsdimorphismus führt. Anschließend ist es fest an den Wirt gebunden und morphologisch stark an seine parasitische Lebensweise angepasst. Muschelwächter haben eine extrem hohe Reproduktionsleistung im Vergleich zu anderen Krabben. Davon abgesehen sind ihre Sexualbiologie und die zugrunde liegende Morphologie weitgehend unerforscht.

Aufgrund der geringen Größe, ihrer verborgenen Lebensweise, dem Geschlechts-dimorphismus und den unterschiedlichen Morphotypen der Weibchen ist die Taxonomie von Pinnotheriden eine Herausforderung. Zwei allgemein akzeptierte Arten sind an den Küsten Europas vertreten: *Pinnotheres pisum* und *Nepinnotheres pinnotheres*. *Pinnotheres pectunculi* war bislang nur aus der Meermandel *Glycymeris glycymeris* von Roscoff (Bretagne, Frankreich) bekannt. *Pinnotheres ascidicola* und *Pinnotheres marioni* sind als reine Ascidienbewohner beschrieben worden ohne sie vorher eingehend mit den bereits aus Muscheln bekannten Arten zu vergleichen.

Mit dem Ziel, standardisierte, vergleichende Beschreibungen anzufertigen, haben wir Muschelwächter aus zahlreichen Wirten von Fundorten im Nordostatlantik, in der Nordsee und im Mittelmeer gesammelt und untersucht. Entsprechend unserer morphologischen Analyse sind *Pinnotheres ascidicola* und *Pinnotheres marioni* jüngere Synonyme des vorher beschriebenen *Nepinnotheres pinnotheres.* Der Artstatus von *Pinnotheres pectunculi* hat sich hingegen bestätigt. Wichtige Merkmale sind Mundwerkzeuge, männliche Gonopoden und Scheren, welche innerhalb beider Geschlechter konstant sind.

Basierend auf unserer Freilandstudie konnte das Wirtspektrum bestimmt werden. *Nepinnotheres pinnotheres* lebt in Seescheiden und in der Steckmuschel *Pinna nobilis*. *Pinnotheres pisum* infiziert viele verschiedene Muschelarten, darunter die Steckmuschel. Für *Pinnotheres pectunculi* konnten neue Wirtsarten aus der Familie der Venusmuscheln nachgewiesen werden. Außerdem gelang es, das Fressverhalten der beiden *Pinnotheres*-Arten in Muscheln zu beobachten. Sie benutzen einen Borstenkamm an der Unterseite der Schere, um den Kiemenschleim mit den darin angereicherten Nahrungspartikeln zu gewinnen.

Wir diskutieren das Fressverhalten und die zugrundeliegende Morphologie der europäischen Arten im Hinblick auf ihr Wirtsspektrum und den Faktoren, die der Wirtswahl zugrunde liegen könnten.

Der Geschlechtsapparat wurde mit histologischen Methoden, dem Raster- und Transmissions-Elektronenmikroskop und Methoden der konfokalen Lasermikroskopie untersucht. Eubrachyuren haben eine innere Befruchtung: paarige Vaginas erweitern sich zu Spermatheken, welche über Ovidukte mit den Ovarien verbunden sind. Das Sperma wird gespeichert, bis die Eizellen reif sind und in die Spermathek transportiert werden. Bei Muschelwächtern kontrollieren flexible, mit Muskulatur ausgestattete Wandanteile das Lumen der Vagina, die zusätzlich von einem mobilen Operculum bedeckt ist. Die Spermathek ist morphologisch und funktional unterteilt. Ventral ist die Spermathek, einschließlich der Vagina, cuticularisiert. Nur die Mündung des Ovidukts tritt über ein holokrines Drüsengewebe in die Spermathek ein. Dorsal ist die Spermathekenwand ein apokrines Drüsenepithel, welches bislang nur für Winkerkrabben der Gattung *Uca* beschrieben wurde.

Der männliche Geschlechtsapparat besteht aus paarigen Hoden und gewundenen Samenleitern. Die Morphologie der Spermien von Muschelwächtern entspricht anderen Thorakotrematen, differenziert aber *Nepinnotheres pinnotheres* und *Pinnotheres pisum*. Die Spermatozoen werden im sekretorischen proximalen Vas deferens in Spermatophoren verpackt. Der mediale Vas deferens ist stark erweitert, in ihm sind die Spermatophoren in eine Matrix aus seminalem Plasma eingebettet und gespeichert. Der distale Vas deferens besitzt Anhänge, die den Cephalothorax ventral füllen und leicht ins Pleon ziehen. Große Mengen seminales Plasma werden in diesen Anhängen produziert und gespeichert.

Der Kopulationsapparat von Krabben besteht aus paarigen Penes und zwei Paar Abdominalbeinen, die im Dienste der Spermienübertragung zu Gonopoden umgewandelt sind. Bei Pinnotheriden überträgt der lange erste Gonopode die Spermien in die weibliche Geschlechtsöffnung - in ihm verläuft der Spermienkanal mit einer proximalen und distalen Öffnung. Der zweite Gonopode ist kurz und keulenförmig. Während der Paarung sind Penis und zweiter Gonopode in die Basis des röhrenförmigen ersten Gonopoden eingeführt. Der zweite Gonopode ist hydraulisch am Transport des Spermas zur distalen Öffnung des Spermienkanals beteiligt. Seine Form ist spezifisch an die Abdichtung des hydraulischen Systems im ersten Gonopoden angepasst.

Die vorliegenden morphologischen Ergebnisse werden im Vergleich zu anderen Krabben und im Hinblick auf ihre systematische Bedeutung, Funktion und die parasitische Lebensweise von Muschelwächtern diskutiert.

European Pea Crabs - Taxonomy, Morphology, and Host-Ecology (Crustacea: Brachyura: Pinnotheridae)

General Introduction

Pea Crabs - Friend or Foe?

European pinnotherids are small-sized crabs, known as symbionts* of bivalves. Their cryptic way of life and the relationship with the host has sparked interest in their natural history for a long time (see fig. 1.1).

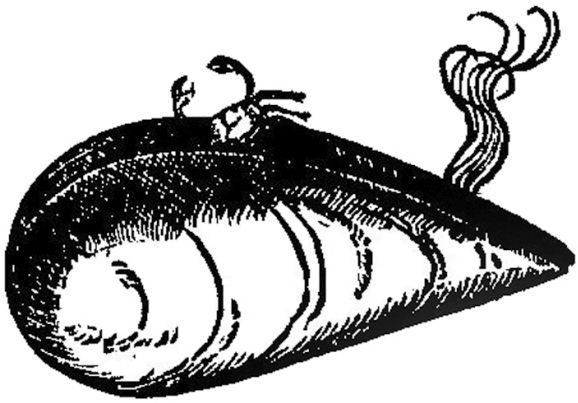

Figure 1.1. Copperplate print from Rondelet (1558) showing *Pinnotheres pisum* (Linné, 1767) crawling out of the blue mussel, *Mytilus edulis*.

In Linnaeus' (1758) fundamental work for zoological nomenclature, Systema Naturae, the name *Cancer pinnotheres* was established for the species named *Nepinnotheres pinnotheres* (Linnaeus, 1758) today. It has frequently been found in the Mediterranean pen shell *Pinna nobilis*, from where its name is deduced. In the hieroglyphs of the ancient Egyptians (ca. 3000 B.C.), the pinnotherids' host *Pinna* has been reckoned as symbol for a man who depends on someone else's help (Herbst 1783). The classical author Aristotle (384-322 B.C.) mentioned pinnotherids in his Historia Animalium, calling them *Pinnophylax*, which is translated as "the

*Symbiosis (Engl.): any relationship of two organisms
Mutualism (Engl.): relationship beneficial for both partners (= "Symbiose" in German)

guard of the pinna" (Thompson 1910). Concerning their ecological relationship, Aristotle stated that "if the Pinna be deprived of this pinna-guard, it soon dies" (Hughes 1981). For a long time it was actually believed that pea crab and host mutually benefit from their relationship. The Roman author Pliny the Elder (23 A.D. – 79 A.D.) gave the following description in his Naturalis Historia:

> *"The pinna and the guard of the pinna assist one another, not being able to remain apart. Now, the pinna is a kind of oyster, but the guard of the pinna is a small crab: and the pinna having opened its shell, remains quiet, watching the fish who are coming towards it; but the guard of the pinna, standing by when anything comes near, bites the pinna, so as to give it a sort of sign; and the pinna being bitten, closes its shell, and in this manner the two share together what is caught inside the pinna's shell."* (Bostock and Riley 1855).

At the present state of knowledge, the feeding strategies of both - bivalve and pinnotherid - clearly differ from the description above. Bivalves are suspension feeders. With their gills, they filter fine organic particles from the seawater. Again, pea crabs feed on the mucus produced by the host gills and the food particles accumulated in that mucus (Coupin 1894, Orton 1920, Kruczynski 1975).

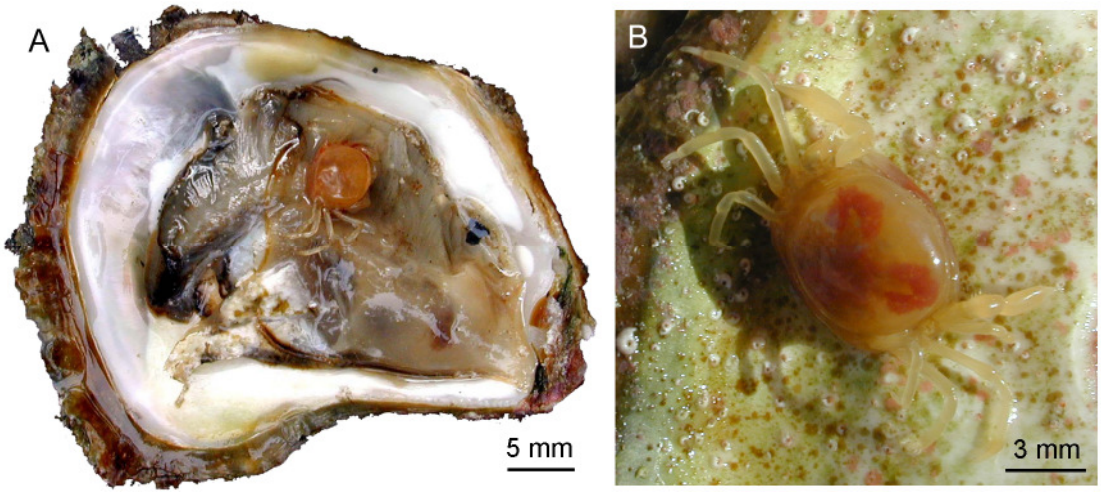

Figure 1.2. *Pinnotheres pisum* (Linnaeus, 1767) in European oyster *Ostrea edulis*. (**A**) Left valve of oyster removed showing adult female. (**B**) Female with red ovaries showing through carapace.

This commensal feeding can damage the bivalve host, e.g. cause mechanical injuries to the gills (Christensen and McDermott 1958, Bierbaum and Ferson 1986), reduce filter efficiency (Sugiura et al. 1960) and oxygen consumption (Bierbaum and Shumway 1988), or decrease

metabolism (Mercado-Silva 2005) and growth (Kruczynski 1972, Navarte and Saiz 2004). The host's gonads may be impacted (O'Beirn and Walker 1999), and, hence, the reproductive potential of the host (Bologna and Heck Jr. 2000). The infection with a pinnotherid does sometimes even result in temporary infertility (Berner 1952). Thus, the pea crabs can be regarded as truly parasitic in their relations to bivalves - at least in the case of the adult female, which is an obligate symbiont (Sun et al. 2005). The male instead is in part free-living and just a facultative commensal.

Owing to the described damages to their hosts, pinnotherids can have a negative commercial impact on aquaculture and fisheries of bivalves (Berner 1952, Bierbaum and Ferson 1986, Bierbaum and Shumway 1988, Navarte and Saiz 2004). *Pinnotheres pisum* (Linnaeus, 1767) (fig. 1.2), one of the species treated in the present study, is a constant pest in edible oysters and blue mussels of European coasts (Atkins 1926, Huard and Demeusy 1968, Haines 1994, pers. obs.). However, the pinnotherid *Zaops ostreum* (Say, 1817), from oysters (*Crassostrea*) on the West-Atlantic coasts, was reported to be collected separately by fishermen in the past and placed on the market as a delicacy (Say 1817; McDermott, per. com.). Moreover, pea crabs have been demonstrated to be a "guard" to their host in one case: the infestation of *Mytilus edulis* with *Tumidotheres maculatus* (Say, 1818) showed an effect on predation of mussels by sea stars *Asterias forbesi*: mussels that did not contain a pea crab were significantly preferred as by the sea stars (Campbell 1993), while infested mussels were often rejected.

Diversity of Pinnotherid-Host Relations

More than 300 exclusively marine species are currently assigned to the family Pinnotheridae De Haan, 1833 (see Ng et al. 2008). They have a worldwide distribution in tropical to temperate waters, from tidal zones to the deep sea (Schmitt et al. 1973). The deepest records are for the genus *Abyssotheres* Manning and Galil, 2000 in ocean depths of over 700 meters (Alcock and Anderson 1899, Komatsu and Ohtsuka 2009).

Members of the subfamily Pinnotherinae De Haan, 1833 live in association with a variety of invertebrates. While the genera *Pinnotheres* Bosc, 1802 and *Arcotheres* Manning, 1993 are endosymbionts in the mantle cavity of bivalves (Campos 2001), *Calyptraeotheres* Campos, 1990 is a symbiont of gastropods, in particular limpets of the family Calyptraeidae (Campos 1990). *Orthotheres haliotidis* Geiger and Martin, 1999 lives inside abalones of the genus *Haliotis* (Geiger and Martin 1999). The scientific names of Pinnotheridae are often deduced from the host. Accordingly, *Tunciotheres* Campos, 1996a is an endosymbiont of sea squirts

(Ascidiacea) (Campos 1996a), whereas *Holotheres* Ng and Manning, 2003 and *Holothuriophilus* Nauck, 1880 inhabit the cloacae or the respiratory trees of sea cucumbers (Holothuroidea) (Ng and Manning 2003). *Dissodactylus* Smith, 1870 is an ectosymbiont on flat irregular sea urchins, the so-called sand dollars (Clyperasteroida, Echinoidea) (Bell 1988, Campos 1990, George and Boone 2003). *Zaops ostreum* (Say, 1817), known from oysters, scallops, and mussels can also be found in the tubes of sessile polychaetes, mainly *Chaetopterus* spp. (Bezerra et al. 2006). However, *Pinnotheres laquei* Sakai, 1961 is the first and only record for a commensal in the mantle cavity of brachiopods (Feldmann et al. 1996).

The genera *Pinnixa* White, 1846 and *Austinixa* Heard and Manning, 1997 of the subfamily Pinnothereliinae Alcock, 1900 (fig. 1.3) live in the burrows of polychaete worms (McDermott 1962b, 2005), ghost shrimps (Callianassidae) (McDermott 2006), mud shrimps (Thalassinidae) (Dos Santos Alves and Pezzuto 1998, Coelho 2005), Echiurida (Anker et al. 2005), and Sipunculida (Campos and Wicksten 1997).

Figure 1.3. *Pinnixa*, *Austinixa* and allied genera of the subfamily Pinnothereliinae collected from burrows of various animals in Panama. Magnification ca. 5 x; source: www.flickr.com (by courtesy of A. Anker, Florida Museum of Natural History, USA).

Calyptraeotheres granti (Glassell, 1933) and *Tumidotheres maculatus* (Say, 1818) (both Pinnotherinae) can also occur as hypersymbionts of pagurids (Williams and McDermott 2004): they live in slipper snails of the genus *Crepidula* (Calyptraeidae, Gastropoda) and *Anomia simplex* (Anomiidae, Bivalvia), which are in turn attached inside the shell of hermit crabs.

Life History and Sexual Dimorphism

As in most marine animals, the dispersal of pinnotherid larvae is planktonic. Males and juvenile females are found inside the host, but also outside. They are capable of swimming in the water column by paddling with their setose walking legs (Hartnoll 1972). After mating, a metamorphosis takes place in the female and it definetly settles a host. The carapace becomes soft through decalcification while cephalothorax and pleon, which accommodate the ovaries, grow disproportionately. The resulting sexual dimorphism (fig. 1.4) reflects the different life histories of the two sexes. The adult female is strongly adapted to its endoparasitic life inside the host while the male stays mobile, which allows switching hosts in the search for females. A detailed review on the life history of European pinnotherid species is given in chapter 3 (Becker and Türkay 2010).

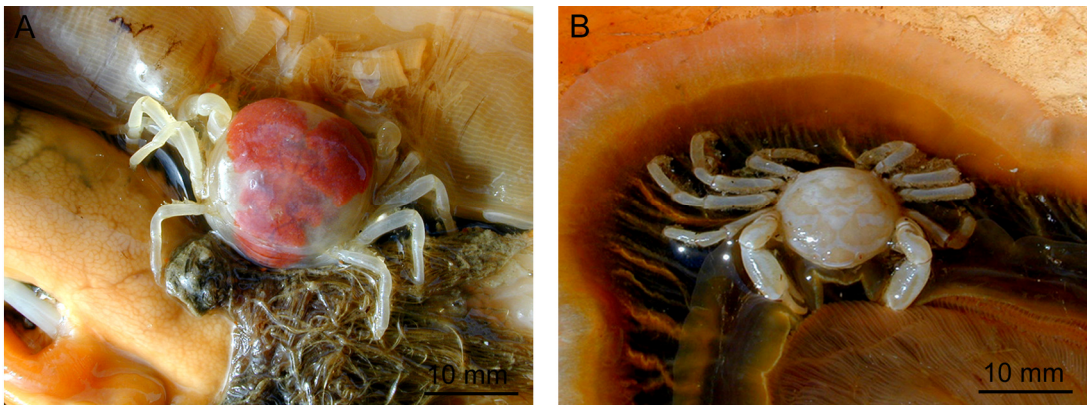

Figure 1.4. Sexual dimorphism of *Pinnotheres pisum*. (**A**) Adult female in *Modiolus modiolus* from the North Sea. (**B**) Male with orange colour pattern on carapace.

Why Study Their Reproductive Morphology?

Brachyuran mating systems are of special interest since they have developed important innovations compared to other crustaceans. Firstly, the males transfer the sperm mass directly into the female gonopores by two pairs of abdominal legs modified for copulation (gonopods). The interaction and specific function of gonopods during copulation show variant patterns among brachyuran sub-groups (see chap. 5, Becker et al., subm.). Furthermore,

internal fertilization has developed within Brachyura by the females' ovaries being internally connected to sperm storage structures, the paired spermathecae. Both, the evolution of specifc copulatory appendages in the male and the development of sperm storage and internal fertilization in the female, have resulted in a fascinating diversity of mating systems (Hartnoll 1969, Bauer 1986, Asakura 2009; chap. 4, Becker et al. 2011), and contributed to opening up new habitats.

Freshwater crabs (Potamoidea) have a direct development with young crab-stages hatching from the eggs. In false spider crabs (Hymenosomatidae), which have also intruded freshwater habitats (Chuang and Ng 1994), some species are viviparous, with females possessing a brood pouch where offsprings develop. Complex parental care and social behaviour is represented in the Jamaican bromeliad crab *Metopaulias depressus* that lives in trees on epiphytic bromeliads. Parents raise their progenies in the pools of water, which are trapped centrally in the leaves. The young crabs are fed by their adult relatives and defended against predators. Brood care also comprises maintaining water quality by removing old leaves and adding gastropod shells for a sufficient calcium supply (Diesel and Schubart 2007).

Reproductive behaviour is not less conspicuous in fiddler crabs of the genus *Uca* (Ocypodidae). Males have one chela prominently enlarged, and use it in performing waving-patterns to communicate with congeners and combat of courtship over females (Crane 1975, Christy 2007). Male-male competition is also known from the spider crab *Inachus phalangium* (Majoidea). Since females mate with several partners subsequently, males try to ensure their fatherhood by sealing off rival sperm from earlier copulations inside the spermatheca (Diesel 1990).

Pinnotherids are exceptional among Brachyura in their great reproductive output (Hines 1996) and a high investment in reproduction (Hartnoll 2006). In other crabs, gonads are restricted to the cephalothorax. Only in pinnotherids, the female ovary extends into the pleon. Furthermore, pinnotherids are particular in mating as juveniles, termed precocious (Hartnoll 1969). The female copulates before metamorphosis and stores the male sperm mass during several moults. Only after metamorphosis is completed, spawning occurs (Atkins 1926).

Many open questions are addressed to the pinnotherids reproductive biology due to their endoparasitic way of life. Whether copulation takes place inside or outside the host, if it happens more than once in a female's life time, and if copulation occurs post-moult or in intermoult, is unknown. In addition, the ability of storing sperm during several moults and the underlying morphology of the spermathecae has not been studied to date.

The male first gonopods are important taxonomic characters at species-level. Second gonopods, their interaction and function in copulation, and the female reproductive systems are relevant on higher systematic ranks.

Systematics of Pinnotherids

The systematics of Pinnotheridae De Haan, 1833 are presently in a state of change. A number of new names and taxa have currently been established, and many species have been removed from the family. The old concept of pinnotherids included very diverse members, rather unified by having symbiotic relationships than reflecting a natural group based on synapomorphic characters. For instance, *Hapalonotus reticulatus* (De Man, 1879), found in the respiratory tree of sea cucumber *Holothuria scabra* (Vandenspiegel et al. 1992), was originally placed in the Pinnotheridae, but later transferred to Eumedoninae Dana, 1852 (Števčić et al. 1988, Chia and Ng 1998).

The former subfamily Tritodynamiinae Števčić, 2005 included species with a remarkable swarming behaviour (Takahashi et al. 1999). They were recently removed from Pinnotheridae and are now assigned to Macrophthalmidae Dana, 1851 (Ahyong and Ng 2009). Furthermore, Astenognathinae Stimpson, 1858, with *Asthenognathus atlanticus* Monod, 1933 distributed on the European Atlantic coast, were transferred into Varunidae H. Milne Edwards, 1853 (Ahyong and Ng 2009). Anomalifrontinae Rathbun, 1931 was excluded from Pinnotheridae too, and the family Xenophthalmidae Stimpson, 1858 was restored for them (Ng et al. 2008). Recently, Aphanodactylidae Ahyong and Ng, 2009 were established to receive the genera *Aphanodactylus* Tesch, 1918, *Gandoa* Kammerer, 2006, and *Uruma* Naruse, Fujita and Ng, 2009, all symbiotic with polychaetes (Ahyong and Ng 2009).

Molecular re-examination has confirmed the recent taxonomic changes within the Pinnotheridae De Haan, 1933 and resulted in only two primary clades: the subfamilies Pinnotherinae De Haan, 1933 and Pinnothereliinae Alcock, 1900 (Palacios-Theil et al. 2009), which is also supported by morphology (e.g. Bürger 1895, Marques and Pohle 1995, Pohle and Marques 1998, Campos 2006, 2009). The species distributed on the coasts of Europe all belong to the subfamiliy Pinnotherinae.

Diagnosis of the Pinnotherinae

Important contributions to the taxonomy of pinnotherids were published by Bürger (1895), Rathbun (1918), Shen (1932), Manning (e.g. 1993b), Ahyong and Ng (2007), and Campos

(e.g. 2009). It is a problem with some of the older publications that definitions published for Pinnotheridae De Haan, 1833, only refer to the subfamily Pinnotherinae De Haan, 1833, but not to Pinnothereliinae Alcock, 1900. Again, diagnoses given for *Pinnotheres* Bosc, 1802 are too broad and apply in present taxonomy for other Pinnotherinae too. In the following, a summary of key characters of pinnotherids, with focus on Pinnotherinae, is given.

Pinnotheridae belong to Thoracotremata Guinot, 1977, thus both sexes have their genital openings located in the sternal cavity of the thorax, covered by the pleon.

Pinnotherinae have zoea larvae with the pleon widening from somite 1 to 5 and a telson that is laterally convex and posteriorly trilobed (Campos 2009). The zoeae of *Pinnotheres* Bosc, 1802 are unusual in lacking a dorsal spine (Lebour 1928a, 1928b, Atkins 1954, Rice 1975).

The carapace is generally smooth and rounded, without sculpturing, and neither lateral thorns nor teeth. Exceptions are represented in the genera *Fabia* Dana, 1851, with two lateral furrows on the anterior carapace (Campos 1996a) and *Durckheimia* DeMan, 1889 that has a peculiar carapace, with a median rigde, and upturned carapace margins (Ahyong and Brown 2003). Normally, the carapace is simply globular or subglobular, sometimes hexagonal in Pinnotherinae (Bürger 1895); in Pinnothereliinae, broad, flattened or transversely cylindric (Campos 2006; see fig. 1.3).

The sexual dimorphism, i.e., the metamorphosis in the female, is characteristic for most of the Pinnotherinae and strongly expressed in *Pinnotheres* (see chap. 3). Free-living males and juvenile females have a convex, rigid carapace and a narrow pleon (Rathbun 1918). The walking legs are slightly flattened in the anterior-posterior axis, bearing setose swimming fringes. The adult female is characterized by its vast subglobular, decalcified and therfore soft carapace (Campos 2009) and a strongly broadened pleon.

The eyes of pinnotherids are small, especially in the adult female, with short peduncles (Stebbing 1893, Rathbun 1918, Shen 1932). *Durckheimia* DeMan, 1889 is said to only have rudimentary eyes (Bürger 1895). Campos (2009) mentions a protuberance on the basal article of the antennae in Pinnotherinae *sensu stricto*.

An important mouthpart-character introduced by Bürger (1895) is the third maxilliped, because it shows apomorphies only known for pinnotherids (fig. 1.5). Merus and ischium are progressively fused within Pinnotherinae (De Haan, 1833), forming one large merus-ischium article (Bürger 1895). In several genera the fusion is complete (Manning 1993a). In others, a suture is still present (Manning 1993b, Ng and Manning 2003). In Pinnothereliinae Alcock, 1900, merus and ischium are separate (Palacios-Theil et al. 2009). In the third maxilliped of other brachyurans, the dactylus of the palpus inserts distally on the propodus (fig. 1.5B). This

is also the case in some pinnotherid genera, such as *Orthotheres* Sakai, 1969 (Campos 1989a). In others, the dactylus is dislocated and inserts subterminally (fig. 1.5C), or it is reduced as in *Ostracotheres* H. Milne Edwards, 1853 (Campos 1996a). Both characters of the third maxilliped show several conditions among pinnotherid subfamilies and genera and, thus, are very useful for taxonomy. Bürger (1895) also applied walking legs to describe genera and species. The relative length of pereiopods as well as shape and length of their distal articles, i.e. the dactyli, are significant characters. Several species have one or two pairs of walking legs, respectively their dactyli, prolonged. Walking legs can also be asymmetrically developed in length (Gordon 1936, Griffin and Campbell 1969). In *Dissodactylus* Smith, 1870, the dactyli of the walking legs are bifurcate (Manning 1993b) and used for climbing on the thorny echinoid hosts (Bell and Stancyk 1983).

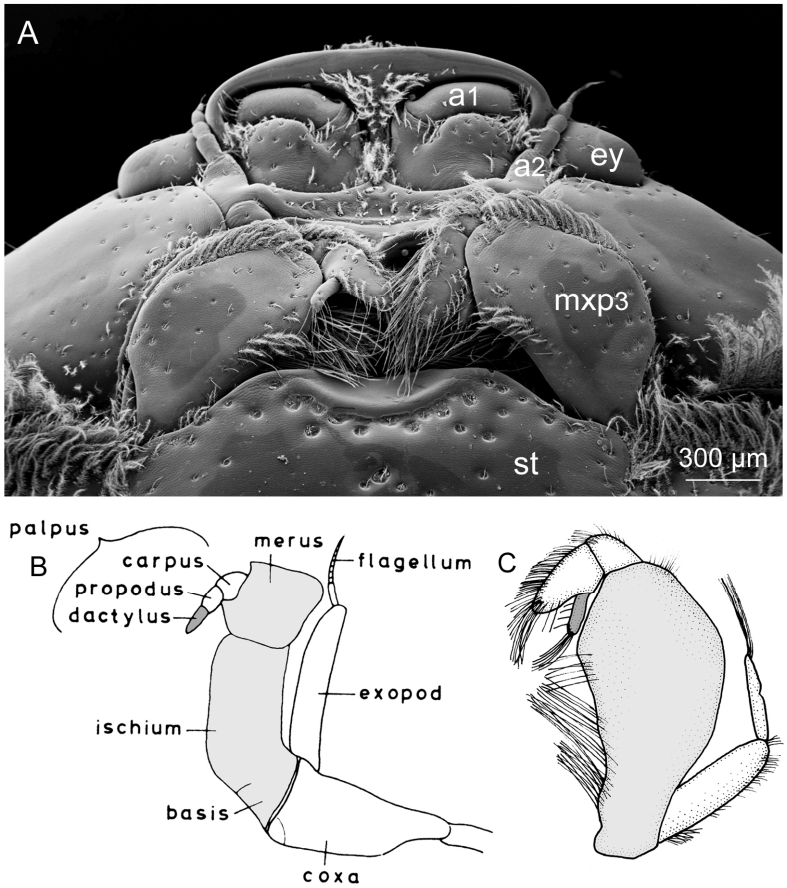

Figure 1.5. Mouthpart characters of Pinnotheridae: third maxilliped. (**A**) SEM-photograph of front in ventral view (*Pinnotheres pisum*). The flattened third maxillipeds cover the inner mouthparts. (**B**) Third maxilliped of Brachyura in general (after Christansen 1969). The palpus is digitiform with the dactylus inserting distally to the propodus. Merus and ischium are separate articles. (**C**) Third maxilliped of *Pinnotheres*. The dactylus inserts subterminally the propodus. Merus and ischium are fused. a1 = antennule; a2 = antenna; ey = eye; mxp3 = third maxilliped; st = sternum.

Problems and Aims

The present study deals with the European species for several reasons. *Pinnotheres pisum* (Linnaeus, 1767) is of particular importance for the taxonomy of pinnotherids, since it is the type species of the genus *Pinnotheres* Bosc, 1802, which is in turn the genus, the family Pinnotheridae De Haan, 1833 refers to. Due to its wide distribution and high abundance, it is relatively available, also from commercially merchandised host. Compared to other species, a basic knowledge is present in literature from earlier studies, which complements the interpretation of subsequent investigations. The negative impact of pea crabs on commercially important bivalves strengthens the significance of studying questions on host ecology, population dynamics, life cycle, and reproductive biology. In this context, the examined European species also serve as model organisms for general issues on pinnotherid biology.

The taxonomy of pinnotherids is difficult due to their small size and the absence of characters in the carapace. Also, sexual dimorphism hampers the association of male and female of one species, if they are not from one host. This, together with the different looking morphotypes of the female before and after metamorphosis, has already led to many synonyms. Although as few as five pinnotherid species have been described for the European coast, their taxonomy is not yet resolved. The most abundant and widely distributed species, *Nepinnotheres pinnotheres* (Linnaeus, 1758) and *Pinnotheres pisum* (Linnaeus, 1767), can be clearly distinguished, for instance in the relative length of dactyli in their last walking legs (Bürger 1895). Nevertheless, misidentifications are found in literature, and confusions in the museum collections, likely due to the fact that both species occur in the same host, the Mediterranean pen shell, *Pinna nobilis*. Three further species were described, but rarely mentioned in literature since then, and never studied in detail. *Pinnotheres pectunculi* Hesse, 1872 from the dog cockle *Glycymeris glycymeris* as well as *Pinnotheres ascidicola* Hesse, 1872 and *Pinnotheres marioni* Gourret, 1888 described from ascidian hosts.

Obscurities in pinnotherid taxonomy lead inevitably to unreliable data on their incidence in particular hosts. So, the first aim of the present study was to collect pinnotherids from a wide range of hosts and localities to determine the pea crabs and their host-range (chap. 2), and to compare and redescribe the European species (chap. 3, Becker and Türkay 2010). For the taxonomic part, drawings were produced.

Furthermore, we investigate the reproductive morphology by histology, scanning and transmission electron microscopy (SEM, TEM) and confocal laser scanning microscopy (CLSM). The morphology of the male and female reproductive systems are described and

conclusions on their functions are presented (chap. 4, Becker et al. 2011; chap. 5, Becker et al., subm.). The results are discussed in comparison with other brachyurans, in regard to their systematic account, and to the particular biology of pea crabs (chap. 6).

Fieldwork and Investigated Hosts

Material - Overview

The Crustacean collection of the Senckenberg Natural History Museum already had a large collection of pinnotherid-material at the start of this project. Long-term collecting series were available for the German Bight (North Sea) and the Northern Atlantic coast of France. Additional material was present from the Mediterranean (Italy, Croatia, Greece, Turkey) and different localities of the Northeast Atlantic coast including the North Sea (Germany, Netherlands, Belgium, France).

However, recent fieldwork was essential to obtain reliable information on host species and infestation rates, which were in part missing for the existing material. Fresh samples were also indispensable for tissue fixations used in histology and electron microscopy.

Sampling in the North Sea was conducted on board of research vessels (RV, fig. 2.1). In the Mediterranean, the material was mainly hand-collected by scuba diving (fig. 2.2). Individual collectors have greatly contributed in providing material as well. Detailed material lists, including information on collectors and sampling sites, are presented in chapter 3.

Part of the older material was undetermined, uncatalogued and/or not yet included in the Senckenberg collections. Thence, it was re-examined and - together with most of the fresh material - integrated into the crustacean collection and digital online database of Senckenberg (access: http://sesam.senckenberg.de/).

North Sea

Helgoland Trench (German Bight). In a long-term study, a population of horse mussels, *Modiolus modiolus*, infested with *Pinnotheres pisum*, was sampled regularly all year round from 1983 to 1992 in the Helgoland Trench of the German Bight. The trench is located south of the Island Helgoland in depths below 50 m, surrounded by shallower waters. Sampling was conducted on board of RV SENCKENBERG with a 2 m – beam trawl (fig. 2.1A, E).

The material was studied to achieve information on seasonal infestation rates, population dynamics, and life cycle. Size ranges of specimens (carapace widths/lengths) and the seasonal occurrence of stages did not give sufficient information for resolving life history.

Figure 2.1. Fieldwork in the North Sea. **A**) RV SENCKENBERG (length: 36 m) on the coast of the Island Helgoland in the German Bight. (**B**) RV HEINCKE (55 m) was used for the Dogger Bank winter cruise (North Sea). (**C**) RV METEOR (97.5 m). (**D**) Ring dredge (90 cm). (**E**) 2 m - Beam trawl. (**F**) Typical benthic sample from the Dogger Bank. Photographs: C. Becker (A, D, E), S. Tränkner (B, F), P. Wintersteller (C).

For example, juvenile female stages before metamorphosis were rare (n = 5) and randomly distributed throughout the year (Becker, unpubl. data). A compilation of the results is represented in table 2.1. Couples have been found all year round, thus no specific mating season could be determined. Spawning occurred from May to August, with a peak in July.

Table 2.1. Long-term study of *Pinnotheres pisum* in the horse mussel *Modiolus modiolus* in the Helgoland Trench of the German Bight from 1983 to 1992. Numbers of investigated hosts and pea crabs, investation rates, and sex-ratios are presented. Proportions of ovigerous females in parenthesis; spawning season shaded in light grey. n = number; ovi = ovigerous.

Month	***Modiolus* (n)**	**infestation (n)**	**infestation rate**	**single ♀ (n)**	**single ♀ (%)**	**pair ♂♀ (n)**	**pair ♂♀ (%)**	**single ♂ (n)**	**single ♂ (%)**
Jan.	27	14	52%	8	57%	5	36%	1	7%
Feb.	30	7	23%	4	57%	3	43%	0	0%
Mar.	50	25	50%	18	72%	5	20%	2	8%
Apr.	33	15	45%	10	67%	3	20%	2	13%
May	85	37	44%	29 (3 ovi)	78%	8 (1 ovi)	22%	0	0%
Jun.	25	6	24%	3 (2 ovi)	50%	3 (1 ovi)	50%	0	0%
Jul.	14	12	86%	7 (7 ovi)	58%	4 (4 ovi)	33%	1	8%
Aug.	54	22	41%	16 (6 ovi)	73%	5	23%	1	5%
Sept.	41	21	51%	18	86%	3	14%	0	0%
Oct.	6	3	50%	1	33%	2	67%	0	0%
Nov.	89	42	47%	33	79%	7	17%	2	5%
Dec.	93	50	54%	29	58%	15	30%	6	12%
Total	**547**	**254**	**47%**	**176**	**69%**	**63**	**25%**	**15**	**6%**

Table 2.2. Sampling in the Helgoland Trench from 2003 to 2010. The annual summer samplings from 2005 to 2010 are summarized. Each year, one or more hauls were conducted.

Date	***Modiolus modiolus***	**infestation with *P. pisum***	**infestation rate (%)**
May 2003	14	5	36%
Aug. 2003	13	-	0%
Aug. 2005 – 2010	2	-	0%

Recent samplings in the Helgoland Trench have shown the decrease of the infested *Modiolus*-population (tab. 2.2). Only few living specimens were found and those were not infested with pea crabs. Actually, the sampled population of horse mussels was already overaged in the 90-ies, mostly consisting of full-grown adult mussels, but no juveniles. By now, the *Modiolus*-population seems to have completely disappeared, which has to be surveyed by ongoing monitoring.

Loreley Bank (German Bight). The Loreley Bank is located east of Helgoland Island, with sandy to gravelly substrates in depths of 12 to 15 m. Samples were taken by a ring dredge of 90 cm diameter with RV SENCKENBERG. The bivalve *Spisula solida* is an abundant component in sandy bottoms of such shallow sand banks. According to our results, the infestation of *Spisula solida* with *Pinnotheres pisum* is a rare exception on the Loreley Bank (tab. 2.3).

Table 2.3. *Pinnotheres pisum* in *Spisula solida* from the Loreley Bank (German Bight). Only one couple was found out of 1869 potential hosts. Numbers of investigated hosts in parenthesis. n/n = number of infestation/number of investigated hosts.

Date	Findings (hosts)
May 1985	1♂♀ (787)
July 1985	- (329)
Aug. 1985	- (448)
Nov. 1985	- (305)
Total n/n	**1/1869**

Dogger Bank (North Sea). The Dogger Bank is a shallow shoal in the central southern North Sea. Annual samplings took place in summer (July/August) in a biweekly cruise with RV SENCKENBERG. The sampling grid consisted of 37 stations covering an area of approximately 17.000 km^2 with depths of 16 to 33 m (Türkay and Kröncke 2004), Every station was sampled by beam trawl and by ring dredge. In January 2010 an additional winter cruise was conducted with RV HEINCKE (fig. 2.1B) (Sonnewald 2010).
Bivalves are not abundant in the sandy to gravely substrates of the Dogger Bank (fig 2.1F) and pinnotherids are very rare. The occasional findings of *Pinnotheres pisum* are presented in table 2.4.

List of non-infested species (numbers of specimens examined in parenthesis):
Bivalves: *Abra alba* (7), *Acanthocardia echinata* (13), *Aequipecten opercularis* (45), *Chamelea* spp. (88), *Clausinella fasciata* (4), *Corbula gibba* (5), *Dosinia* spp. (8), *Ensis* spp. (59), *Nucula* cf. *nitidosa* (15), *Spisula solida* (3), *Spisula subtruncata* (6), *Spisula* sp. (21), *Tapes rhomboides* (17), *Thracia* sp. (11), *Venerupis senegalensis* (23).
Ascidians: *Ascidiella scabra* (> 1000).

Table 2.4. *Pinnotheres pisum* in bivalves from the Dogger Bank. n/n = number of infestation/ numbers of investigated hosts.

Date	*Mactra stultorum*	*Gari fervensis*	*Donax vittatus*	*Spisula elliptica*
2004 summer	1/9	0/3	-	-
2006 summer	3/14	2/8	0/1	-
2008 summer	6/8	1/4	-	0/2
2009 summer	5/15	0/1	0/2	-
2010 winter	1/20	0/10	1/38	1/11
2010 summer	4/34	0/11	1/56	0/99
Total	**21/100**	**3/37**	**2/97**	**1/112**
Infestation rate	**21%**	**8%**	**2%**	**< 1%**

Only *Pinnotheres pisum* was found in the North Sea. Its host-range includes very different bivalve species (tab. 2.1 - 2.4). *P. pisum* does not occur in the Wadden Sea of the German bight (Türkay, pers. com.). Further, it is not regularly present in the Jadebusen (German Bight of the North Sea), just one single specimen was found inside *Mytilus edulis* in the Mellum Balje, a tributy tidal channel of the Jade (Türkay, pers. com.).

Northeast Atlantic

Roscoff, Brittany, France. The coast of Roscoff in Brittany (France) is the type locality of *Pinnotheres pectunculi* Hesse, 1872 and *Pinnotheres ascidicola* Hesse, 1872, therefore, intensive sampling was conducted in cooperation with the ”Station Biologique de Roscoff”. In May 1990 and April 1991, large series of *Pinnotheres pectunculi* from *Glycymeris glycymeris* were collected by J. Klein (formerly Senckenberg). Unfortunately, it was not recorded from how many host specimens pea crabs were obtained. However, also unopened bivalves of different species were preserved, which could be used to find new host records and determine infestation rates for the present study (tab. 2.5).

Further material was obtained from the diving service of the ”Station Biologique de Roscoff” and from individual collectors (chap. 3). The *Glycymeris*-samples from April 2008 contained only female *Pinnotheres pectunculi* of which 59 % were ovigerous (n = 38). The extreme rareness of males in *Glycymeris glycymeris* was remarkable and can only be elucidated by surveys throughout the year.

Table 2.5. *Nepinnotheres pinnotheres* and *Pinnotheres pectunculi* from the Northeast Atlantic coast (Brittany, France). Numbers of investigated hosts in parenthesis.

	N. pinnotheres	***Pinnotheres pectunculi***			
Date	***Ascidia mentula***	***Glycymeris glycymeris***	***Venus verrucosa***	***Venus casina***	***Clausinella fasciata***
May 1990, Apr. 1991	-	> 200♀ 1♂♀ (unknown)	11♀ 3♂ (unknown)	9♀ 2♂♀ 2♂ (28)	2♀ 2♂ (24)
Apr. 2006	1♀ 1♂ (3)	1♀ (12)		-	-
Mar. 2007	3♀ 1♀♂ (49)	-		-	-
Apr. 2008	-	64 ♀ (89)		-	-
Infestation rates	**12%**	**64%**	**?**	**53%**	**17%**

List of non-infested species (numbers of specimens examined in parenthesis):
Bivalves: *Acropagia crassa* (16), *Cerastoderma edule* (41), *Dosinia sp.* (1), *Mactra stultorum* (1). *Venerupis senegalensis* (15).
Ascidians: *Phallusia mammillata* (124).

In addtion to the results presented in table 2.5, *Pinnotheres pisum* was obtained from an unspecified number of blue mussels, *Mytilus* spp., collected on the Atlantic coast of Brittany around Roscoff. However, *Pinnotheres pectunculi* was never found in *Mytilus* (chap. 3)
All three European pea crabs were distributed on the investigated sample site. *Nepinnotheres pinnotheres* was only obtained from *Ascidia mentula*, while new host records could be added to the host range of *Pinnotheres pectunculi*.

Mediterranean

Since the Mediterranean pen shell, *Pinna nobilis,* is an endangered species protected according to the European Council Directive 92/43/EEC (Katsanevakis 2007), it was not targeted in the present study. Specimens were received occasionally as by-catch.

Northern Adriatic Sea. Many different sample sites were investigated around Rovinj (Istria, Croatia) (see chap. 3)**.** Potential hosts were mainly hand-collected by scuba- and skin-diving in depths of 1 to 35 m. Further samplings were carried out with RV BURIN from the Institute Ruđer Bošković with a 1.20 m-beam trawl (fig. 2.2).

Table 2.6. *Nepinnotheres pinnotheres* from sea squirts and *Pinnotheres pisum* from bivalves, Northern Adriatic Sea. Numbers of investigated hosts in parenthesis. n/n = number of findings/number of investigated hosts.

	Nepinnotheres pinnotheres				***Pinnotheres pisum***	
Date	*Ascidia mentula*	*Halocynthia papillosa*	*Phallusia mammilata*	*Microcosmos* spp.	*Ostrea edulis*	*M. gallo-provincialis*
Aug. 2003	3♀, 3♂ 1♂♀ (11)	- (10)	- (31)	2♀, 3♂ (253)	5♀ 2♂♀ (31)	(9)
Mar. 2005	1♂ (6)	-	-	1♀, 2♂ (113)	-	-
Aug. 2005	-	3♀ (21)	- (2)	10♀ (350)	14♀ (75)	1♀ (12)
Aug. 2007	- (2)	-	2♀ (7)	2♀, 1♂ (145)	- (29)	(7)
Aug. 2009	1♀ (1)	-	1♀ (19)	8♀, 4♂ 1♂♀ (228)	-	-
Total n/n	**9/20**	**3/31**	**3/59**	**34/1089**	**21/135**	**1/28**
Infestation rate	**45%**	**7%**	**5%**	**3%**	**16%**	**4%**

Table 2.7. *Nepinnotheres pinnotheres* and *Pinnotheres pisum* from the Mediterranean pen shell, *Pinna nobilis*, Northern Adriatic Sea. n/n = number of findings/number of investigated hosts.

Date	***Pinna nobilis***	***Nepinnotheres pinnotheres***	***Pinnotheres pisum***
Aug. 2003	6/6	2♀ 1♂♀	1♀ 2♂♀
Dec. 2003	2/2	1♀	1♂♀
Mar. 2005	2/2	1♀	1♀
Aug. 2005	0/1	-	-
Aug. 2007	0/1	-	-
Aug. 2009	1/1	-	1♂♀
Total n/n	**11/13**	**5**	**6**
Infestation rate	**total 85%**	**39%**	**46%**

Figure 2.2. Field work in the Mediterranean. (**A**) RV BURIN, small boat from Institute Ruđer Bošković (length: < 10 m) in Rovinj (Croatia), used in the Northern Adriatic Sea. (**B**) Scuba-diving in the Eastern Mediterranean (Lebanon): Carola Becker and Jörg Mehnert. (**C**) *Mactra lilacea* from Southern Lebanon, bought from a fish market. (**D**) Sea squirt *Pyura* sp. in small cave inhabited by muray eel. (**E**) Lessepsian bivalves *Spondylus spinosus* and *Chama pacifica* collected in Lebanon. (**F**) *Spondylus spinosus* with one valve removed. Photographs: C. Becker (A, C, E, F), M. Bariche (B), J. Mehnert (D).

Nepinnotheres pinnotheres and *Pinnotheres pisum* were both regularly found in the Adriatic Sea and infestation rates have been documented (tab. 2.6, 2.7). Both live in the Mediterranean pen shell, *Pinna nobilis*. For *N. pinnotheres*, we recorded new ascidian host species. *P. pisum* was only found in *Pinna nobilis*, *Ostrea edulis,* and *Mytilus galloprovincialis*, although many other potential bivalve hosts – also larger ones – were investigated.

List of non-infested species (numbers of specimens examined in parenthesis):
Bivalves: *Spondylus gaederopus* (15), *Pecten jacobaeus* (7), *Chlamys* spp. (11), *Lima* sp. (5), *Venus verrucosa* (4).
Ascidians: *Phallusia mammilata* 2003 - 2005 (33), *Ascidia virginea* (3).

Sea of Crete (Greece). Samples were hand-collected by scuba-diving around Heraklion at the North coast of the Island of Crete, in depths of 2 – 25 m in January 2007. The pen shells, *Pinna nobilis*, obtained as by-catch from fishermen, were not infested. The only finding was one single female of *Nepinnotheres pinnotheres* in *Halocynthia papillosa* (tab. 2.8).

Table 2.8. Results of field work in the Sea of Crete (coasts of Island Crete). Numbers of investigated hosts in parenthesis.

Pinna nobilis	***Halocynthia papillosa***	***Microcosmos* spp.**
- (4)	1♀ (124)	- (41)

Eastern Mediterranean (Lebanon)

The distribution of pinnotherid species in the Eastern Mediterranean has not yet been fully explored. *Nepinnotheres pinnotheres* and *Pinnotheres pisum* have been recorded on the coasts of Greece and the Western coasts of Turkey (D'Udekem D'Acoz 1999). Both species are distributed in the Ionian Sea, in the Aegean Sea, and in the Sea of Marmara. *Pinnotheres pisum* was also found on the coasts of the Island of Cyprus in *Pinna nobilis* (Lewinsohn and Holthuis (1986) and of Israel in *Mactra* sp. (Holthuis and Gottlieb 1958). For Syria and Lebanon, data are missing in the literature.
The coasts of Lebanon are not only interesting to sample due to the unknown distribution of native Mediterranean pinnotherid species, but also because of the numerous invasive species, which have migrated from the Red Sea through the Suez Canal into the Mediterranean, the so-called lessepsian migrants. Thence, lessepsian pea crab species could appear or novel lessepsian hosts might be recorded, infested by the native Mediterranean pinnotherids.
The expedition to Lebanon was undertaken in the framework of the DAAD-project "Establishment of a Middle Eastern biodiversity network". All samples were hand-collected North of Beirut by scuba-diving with localities ranging from N 33°57.480'N 35°35.807'E to 33°57.490'N 35°35.807'E, in depths of 10 to 35 m. Sampling in Southern Lebanon was not possible at that time (2006) for safety reasons. Anyhow, *Mactra* cf. *lilacea* collected in Southern Lebanon was bought from a fish market.

The investigated bivalve communities in the Sea around Beirut have been dominated by lessepsian species (tab. 2.9). Among ascidians, *Phallusia nigra* and *Pyura* sp. were lessepsian, the others native Mediterranean species (tab. 2.10). Although we investigated numerous potential hosts from different sample sites, no native Mediterranean or Red Sea pinnotherids were found. Pinnotherids have not yet been rediscovered on the coasts of Israel since their first record by Holthuis and Gottlieb (1958) (B. Galil, pers com.; I. Karplus, pers. com). The occurrence of Pinnotheridae in the easternmost Mediterranean certainly needs further investigation and additional data is necessary to explore their distribution in the Levant.

Table 2.9. Non-infested bivalves from field work in Lebanon, with reference to investigated numbers.

Spondylus spinosus	***Pinctada radiata***	***Chama pacifica***	***Malvufundus regula***	***Brachidontes pharaonis***	***Mactra* cf *.lilacea***
101	39	77	112	112	300

Table 2.10. Non-infested ascidians from field work in Lebanon, with reference to investigated numbers.

Phallusia nigra	***Pyura* sp.**	***Ascidia* cf. *virginea***	***Microcosmos* spp.**
48	2	16	112

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Taxonomy and Morphology of European Pea Crabs (Crustacea: Brachyura: Pinnotheridae)

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ABSTRACT

Pea crabs distributed around the coasts of Europe live commensally inside bivalves and sea squirts. Even though just five species are described, their taxonomy is still under development. In order to produce standardized comparative descriptions, pea crabs were collected and studied from different hosts and localities in the Northeast Atlantic and the Mediterranean Sea. Because of the pinnotherids' sexual dimorphism, the male and female are described separately for each species. The common and widely distributed species *Nepinnotheres pinnotheres* (Linnaeus, 1758) and *Pinnotheres pisum* (Linnaeus, 1767) are redescribed, while the status of the questionable species *Pinnotheres ascidicola* Hesse, 1872 and *Pinnotheres marioni* Gourret, 1888 from sea squirts and *Pinnotheres pectunculi* Hesse, 1872 from the dog cockle, *Glycymeris glycymeris*, was finally clarified.

According to the morphological analysis, *Pinnotheres ascidicola* and *Pinnotheres marioni* are junior synonyms of *Nepinnotheres pinnotheres*, whereas the status of *Pinnotheres pectunculi* as a valid species has been demonstrated.

Keywords: *Nepinnotheres, marioni, ascidicola, pisum, pectunculi.*

INTRODUCTION

Crabs of the family Pinnotheridae are associated with a variety of invertebrates (Schmitt et al. 1973). The species of the Northeast Atlantic and the Mediterranean Sea live commensally inside bivalves and sea squirts. Pinnotherids show a conspicuous sexual dimorphism (Orton 1920, Atkins 1926). While the juveniles of both sexes still look very similar, a metamorphosis takes place in the female after the precocious mating. That the copulation occurs precociously in pinnotherids, which means in a juvenile stage of the female, is rather exceptional for true crabs (Hartnoll 1969). However, after copulation, the female's ovary develops moult by moult, the carapace becomes soft and translucent through decalcification and cephalothorax and pleon grow disproportionately compared with chelipeds and walking legs. With the initiation of this metamorphosis, the female never leaves the host again. The adult male is considerably smaller than the adult female. It remains partly free living, and is only occasionally found together with the female inside the host. Males and juvenile females are good pelagic swimmers, paddling with their setose second and third pairs of walking legs (Hartnoll 1972). The male-like morphotype of the juvenile female before metamorphosis is found in the host, but also outside. It is referred to as "hard stage" or "stage I" (Atkins 1926) to distinguish it from the soft-shelled adult. According to this system, the subsequent steps in metamorphosis are characterized as "stage II" to "stage IV", resulting in the sexually mature "stage V" (Atkins 1926). So, pea crabs have a quite complex life history. The most complete information exists for the common and widespread species *Pinnotheres pisum* (Linnaeus, 1767). Important contributions to life cycle and population dynamics have been published by Thompson (1835), Orton (1920), Atkins (1926, 1954, 1958), Stauber (1945), Berner (1952), Christensen (1959), Huard and Demeusy (1966a, 1966b, 1968), Silas and Alagarswami (1967) and Haines et al. (1994). The larval and post-larval development of *Pinnotheres pisum* was described by Lebour (1928a, 1928b), Atkins (1954) and Rice (1975). The larvae of the other abundant species, *Nepinnothereres pinnotheres* (Linnaeus, 1758), were described by Lebour (1928a, 1928b).

Owing to the pinnotherids' cryptic way of life, their small size, the sexual dimorphism, and the metamorphosis of the female resulting in different morphotypes, their taxonomy is quite challenging.

On European coasts, the two aforementioned species, *Nepinnotheres pinnotheres* (Linneaus, 1758) and *Pinnotheres pisum* (Linneaus, 1767), are widely accepted and have been recognized for a long time. A third species, *Pinnotheres pectunculi* Hesse, 1872, was described from the bivalve host *Glycymeris glycymeris* (dog cockle) from the French Atlantic

coasts around its type locality, Roscoff in Brittany. Since it is so similar to *Pinnotheres pisum* and because it has not yet been described in full detail, its status as a valid species is not ascertained beyond doubt. The main distinguishing feature so far is a very small additional tooth on the cutting edge of the fixed finger of the claw (Bourdon 1965, d'Udekem d'Acoz 1988). Even more problematic are two further species described as living exclusively in ascidians: *Pinnotheres ascidicola* Hesse, 1872 (Brittany, France, Northwest Atlantic) and *Pinnotheres marioni* Gourret, 1888 (Gulf of Marseille, France, Mediterranean Sea). Since their first records, these inhabitants of sea squirts have rarely been mentioned in the literature and never carefully compared with the other European species. Therefore, the aim of the present study was to collect pea crabs from a wide variety of hosts and to produce standardized comparative descriptions of the pinnotherid species distributed along the coasts of Europe, using characters, which are preferably consistent for both sexes and through all crab stages.

MATERIAL AND METHODS

The Research Institute and Natural History Museum Senckenberg (SMF) holds a large crustacean collection with plenty of pinnotherid material from the coasts of Europe from different localities and hosts. Even so, additional material had to be collected, in particular to provide reliable documentation of the hosts. The older material was compared with that freshly collected from different hosts and with the species' original descriptions. None of the type material of any of the European species is extant in the collections of Marseille (MHNM) or Paris (MNHNP); therefore, morphological comparisons are based on the original descriptions and drawings of *P. ascidicola* and *P. marioni*. *Pinnotheres pisum* was intensively collected on a regular basis from a population of horse mussels, *Modiolus modiolus*, in the Helgoland Trench during cruises to the German Bight with RV SENCKENBERG from 1985 to 1992 by hard-bottom dredge and beam trawl. Global positioning system data of samples range from 54°08.419′N–54°08.599′N to 07°50.921′E–07°53.431′E, the depth from 50 to 55 m. Additional material collected on later cruises is listed in detail in "Material examined".

In the Northern Adriatic Sea (Rovinj/Croatia) blue mussels, *Mytilus galloprovincalis*, edible oysters, *Ostrea edulis,* and solitary ascidians were hand-collected by scuba- and skin-diving in depths of between 1 and 35 m. Further samples were taken by beam trawl on trips with RV BURIN from the Institute Ruđer Bošković on different sample sites ranging from 45°02′N–45°07′N to 13°36′E–13°40′E. Since the giant Mediterranean pen, *Pinna nobilis,* is a protected species, it was only obtained as by-catch and from earlier collections. Sampling in Greece was

by scuba-diving around the northern part of the Island of Crete. Potential hosts were opened with a knife and carefully examined for inhabiting pea crabs. The material was pre-fixed in formaldehyde (3.5% in seawater), later rinsed with freshwater and transferred to 70% ethanol. Specimens were examined by stereo microscope Leica MZ8; drawings were prepared with the help of a camera lucida.

The nomenclature of seta types is based on the classification system of Garm (2004).

RESULTS

Nepinnotheres Manning, 1993

Nepinnotheres Manning, 1993, p 150–170, figures 18–30 (type species *Cancer pinnotheres* Linnaeus, 1758, by original designation, gender masculine)

Nepinnotheres pinnotheres (Linnaeus, 1758)

(Fig. 3.1D, E; 2A–D; 3A–C)

Cancer pinnotheres Linnaeus, 1758, p 628, types probably not extant, type-locality: "Habitat in Mari Mediterraneo and Asiatico"; Linnaeus, 1767, p. 1040.

Pinnotheres veterum Bosc, 1802, p. 243.

Pinnotheres ascidicola Hesse, 1872, p. 30–35 [newly synonymized].

Pinnotheres marioni Gourret, 1888, p. 186–187, plate 2 (fig. 5–9), plate 4 (fig. 6) [newly synonymized].

Pinnotheres pinnotheres: Balss, 1927, p 1022 [new combination]; Atkins, 1954, p 700–715, figures 8–17.

Nepinnotheres pinnotheres: Manning, 1993, p 150–170, figures 18–30 [new combination].

A detailed synonymy is presented in Schmitt et al. (1973).

Material examined

Northeast-Atlantic. France, Brittany: 1♂, 1♀ juvenile, host: *Ascidia mentula*, Morgat, 48° 13' N 4° 29' W, hand-collected, 21.03.2007, leg. A. Magdeburg (SMF 33403 – SMF 33406). 1♂, 1♀, host: *Ascidia mentula*, bay of Morlaix, Île le Cerf, le Colombier, 48° 36' N 3° 59' W, 29.03.1994, leg. E. Dumoulin (SMF 33411).

Mediterranean. Ligurian Sea: 1♂, Italian Riviera, Genova, Portofino 44° 18.312'N 9° 12.702' E, Oct. 1913, leg. L. Nick (SMF 5293). 2♂, 1♀, host: *"Phallusia"*, Italian Riviera,

Genova, Portofino, 44° 18' N 9° 12' E, 17.10.1913, leg. L. Nick (SMF 5294).

Tyrrhenian Sea: 1♂, host: *"Cynthia mentula"*, Italy, Campania, 13.03.1912, leg. L. Nick (SMF 5295). 1♀, Italy, Isola d'Elba, Aug. 1965, leg. J. Martens (SMF 5153). 4♀, host: *Pinna nobilis*, Strait of Bonifacio, Italy, Sardinia, Teresa di Gallura, 41°14'44'N 9°11'24'E, Aug. 1961, leg. M. Grasshoff (SMF 4907).

Northern Adriatic Sea, Italy, Trieste: 1♀, Isla Croce, 13.02.1914, leg. O. Löw-Beer (SMF 4925). 1♀, Isla Croce, 20.07.1969, leg. G. Pilleri (SMF 9867).

Northern Adriatic Sea, Croatia, Istria: 3♂, 4♀, 2♀, 1♀ juvenile hard stage, 4♀ ovigerous, Rovinj, Dvije Sestrice, hard bottom dredge, 10.09.1985, leg. RV BURIN (SMF 31505, SMF 31507, SMF 31509, SMF 34003). 1♂, Stat. 1 Ku, beam trawl, 05.09.1985, leg. RV BURIN, SMF 31506. 2♂, Rovinj, west of Crveni otok (Red Island), Stat. 5-1, 16.08.1989, leg RV BURIN (SMF 31513). 8♂, 1♀, 1♀ juvenile, 13♀ ovigerous, 2,8 nm W lighthouse San Giovanni in Pelago, Stat. Rov95-10, 45° 2.634' N 13° 32.646' E, hard bottom dredge, 05.09.1995, leg. RV BURIN (SMF 31508). 1♂, 2♀ juvenile hard stage, 1♀ ovigerous, host: *Ascidia mentula*, 1 nm SW Banjole, Stat. YU-87/7b-1, 45° 3.407' N 13° 35.158' E-45° 3.407' N 13° 35.158' E, beam trawl, 18.09.1987, RV BURIN, leg. D. Krämer (SMF 33811). 1♀ juvenile stage II, 1♀ juvenile stage III-IV, 2♀ ovigerous, 2 nm N Banjole, Stat. YU-87/7c, 18.09.1987, RV BURIN, leg. D. Krämer (SMF 33812). 1♀ ovigerous, Rovinj, 1 nm SW Banjole, Stat. YU-87/7b-2, 45° 3.407' N 13° 35.158' E-45° 3.407' N 13° 35.158' E, 18.09.1987, RV BURIN, leg. D. Krämer (SMF 33813). 2♂, 2♀, 1♀ ovigerous, host: *Ascidia mentula*, 1 nm SW Banjole, Stat. YU-87/7a-1, 45° 3.407' N 13° 35.158' E-45° 3.407' N 13° 35.158' E, 18.09.1987, RV BURIN, leg. D. Krämer (SMF 33814). 3♀, host: *Ascidia virginea*, Banjole, Stat. YU-87/3b-1, 45° 3.407' N 13° 35.158' E-45° 3.407' N 13° 35.158' E, 15.09.1987, RV BURIN, leg. D. Krämer (SMF 33815). 3♂, host: *Ascidia virginea*, Banjole, Stat. YU-87/3b-1, 45° 3.407' N 13° 35.158' E-45° 3.407' N 13° 35.158' E, 15.09.1987, leg. RV BURIN, SMF 34005. 1♂, 1♀ ovigerous, Rovinj, 16.08.1989, leg. RV BURIN (SMF 31511). 2♂, host: *Halocynthia papillosa*, Rovinj, Stat. Rov05, scuba-diving, 24.08.2005, leg. C. Becker (SMF 33806). 2♀, Rovinj (SMF 5291). 3♂, host: *Ascidia mentula*, Rovinj, scuba-diving, 16.08.1989 (SMF 33807). 1♂, Rovinj, leg. 16.08.1989 (SMF 31514). 1♂, Rovinj, leg. 1987 (SMF 31515). 1♀, Rovinj, leg. 1989 (SMF 31516). 1♀, host: *Pinna nobilis*, Rovinj, beam trawl, Dec. 2003, RV BURIN, leg. D. Brandis (SMF 33409).

Levantian Sea: 1♂ free living, NW-Greece, Jul. 1993, leg. C. d'Udekem d'Acoz, (SMF 33461).

Ionian See: 1♀ ovigerous, host: *Halocynthia papillosa*, Greece, Thesprotia, Syvota,

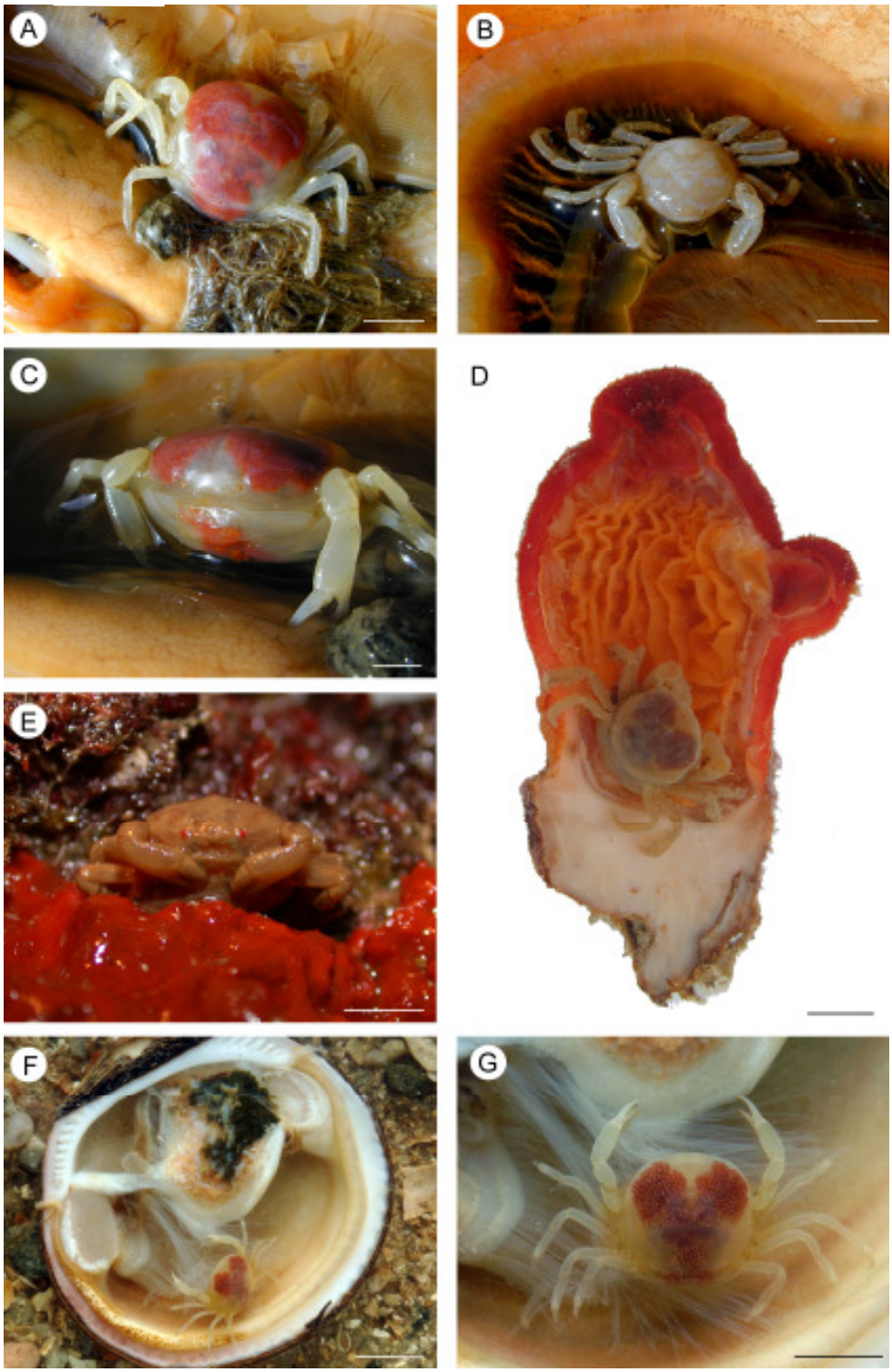

Figure 3.1. Living specimens in their bisected hosts. (**A**) Female of *Pinnotheres pisum* (dorsal view) in the horse mussel *Modiolus modiolus* from the North Sea. The mature ovary is red, shown through the translucent carapace. (**B**) Male of *P. pisum* (dorsal view) inside the Mediteranean pen shell *Pinna nobilis*, (Northern Adriatic Sea). The carapace is rigid with orange ornamentation. (**C**) Frontal view on female of *P. pisum* in *M. modiolus* (from 1A). The broad pleon reaches the front and holds ovary. (**D**) Female of *Nepinnotheres pinnotheres* in the gills gut of sea squirt *Halocynthia papillosa* (Northern Adriatic Sea). (**E**) Frontal view on female of *N. pinnotheres* from the Eastern Mediterranean (Crete, Greece) *H. papillosa*; (**F**) Female *Pinnotheres pectunculi* in the dog cockle *Glycymeris glycymeris*; (**G**) Close up on same female. Scale bars: 5 mm (A, B, D, E, G), 2 mm (C), 10 mm (F); photographs: C. Becker (A–E), S. Tränkner (F–G).

38° 37'N 20° 40'E, 15.07.1993, leg. C. d'Udekem d'Acoz (SMF 33410). 1♀ ovigerous, host: *Halocynthia papillosa*, Greece, Crete, Agia Pelagica, „Made", 35°24'3.41'N 25°2'1.70'E, scuba-diving, 18.01.07, leg. C. Becker, M. Schneider (SMF 33408).

Male

General description (fig. 3.2A). Color fawn to light brown. Carapace rounded, dorsally convex, strongly calcified, not translucent, without defined regions and lateral teeth. Carapace, as well as whole body surface, especially front, pilose, appearance dull owing to short pappose setae only noticeable under high magnification. Front pronounced, bilobed by narrow median notch. Eyes clearly visible in dorsal view, with bright red coloration in living specimens. Chelipeds (P1), relative length of articles of walking legs and third maxillipeds consistent with description of female given below. Second and third pair of walking legs (P3, P4) with swimming fringes: two rows of long pappose setae on distal articles. One running dorso-posteriorly on carpus and propodus, one ventro-anteriorly. Shorter pappose setae lining dorsal and ventral margins of all walking legs (P2–P5).
Size of males varies with host, maximum carapace width about 8 mm in specimens from giant Mediterranean pen, *Pinna nobilis*.

Pleon (abdomen) and sternum (fig. 3.2B). Male abdominal segments clearly separated. Belonging to thoracotremata, male gonopores located on sternum. Pleon narrow, roughly tongue-shaped, general form slightly triangular. Pleon tapering distally with segments 3–5 trapezoidal, every segment somewhat narrower than previous. Pleon broadening in distal part of segment 6 and in rounded telson. Whole outer margin of abdomen fringed with setae, entire surface of pleon pilose with short pappose setae.

First gonopod (G1) (fig. 3.2C, D). Paired copulatory organs, first gonopods, running parallel basally for three-quarters of total length, distal quarter strongly curved towards lateral outside: position of distal tip with opening of ejaculatory canal resulting in angle of about 90° to base. First gonopod slender, slightly flattened dorso-ventrally, gradually tapering distally. Next to long pappose setae on proximal base of gonopod, long simple setae along total length of first gonopod, particularly near its curve.

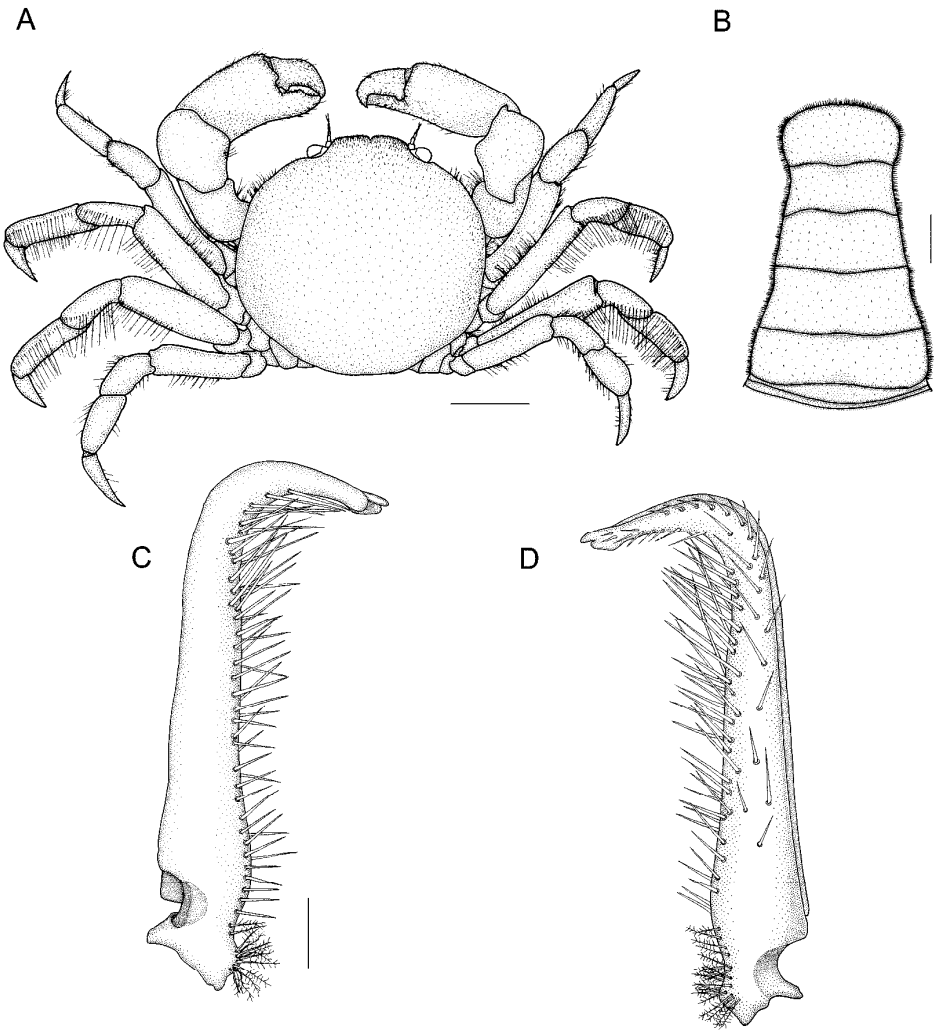

Figure 3.2. Male of *Nepinnotheres pinnotheres.* (**A**) Dorsal view on male. (**B**) Pleon, margin fringed with setae. (**C**) Ventral view on left first gonopod. (**D**) Dorsal view on left first gonopod. Scale bars: 2 mm (A); 500 µm (B); 250 µm (C), (D).

Female (adult)

General description (fig. 3.1D, E, 3.3A). Color fawn to light brown. Carapace subglobular or wider than long, especially in large females. Carapace soft, slightly translucent, surface setose, without defined regions. Front projecting a little, clearly bilobed by median incision. Eyes more or less visible in dorsal view, depending on size of specimen. Eyes with bright red coloration in living specimens. Surface of carapace pilose. Pleon very broad and rounded, covering whole ventral side, coxae of walking legs laterally, reaches buccal region anteriorly. Pleon's margin fringed with setae. Surface of pleon pilose, with short pappose setae. Juvenile hard stage females before metamorphosis consistent with description of male (except for pleopods). Carapace width of clearly adult (ovigerous) females from around 5 mm in small females inhabiting ascidians, up to 20 mm in *Pinna nobilis*.

Chelipeds and walking legs (fig. 3.3A, B). Cheliped, especially palm of chela, rather robust. Cutting edge of palm with one stout triangular tooth on movable finger (dactylus) interlocking into depression on fixed finger (propodus), latter with five to six additional blunt teeth. Palm with simple setae of different lengths and with pappose setae. Setae in higher densities around cutting edge and at base of fingers. Whole surface of cheliped and palm pilose owing to short pappose setae. Walking legs (P2–P5) with long, pointed, slightly curved dactyli. Dactyli of P2–P5 considerably more than half as long as propodus. Dactyli of equal length in walking legs P2–P4, slightly longer in P5: approximately as long as three-quarters of propodus. Swimming fringes of second and third walking legs present in juvenile females, reduced in adults.

Third maxilliped (fig. 3.3C). Third maxilliped with large completely fused merus-ischium-article. Dactylus of palp inserting underneath propodus (subterminally). Flagellum two-segmented with tuft of long simple setae originating from its tip. Third maxillipeds' inner margins densely fringed with long simple setae. Short pappose setae distributed over whole surface of maxilliped.

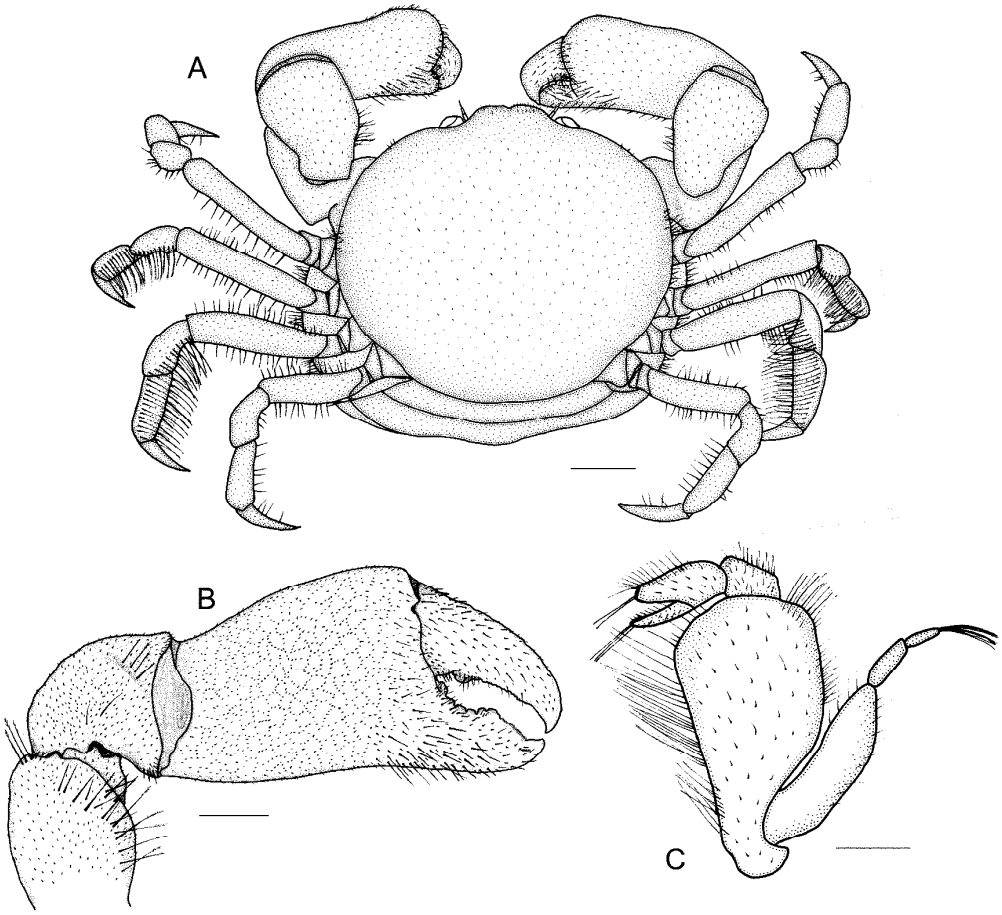

Figure 3.3. Female of *Nepinnotheres pinnotheres*. (**A**) Dorsal view on female, carapace setose. (**B**) Left cheliped with setose surface. (**C**) Exterior surface of left maxilliped. Scale bars: 2 mm (A); 1 mm (B); 500 μm (C).

Comments

Hesse (1872) was the first to describe specimens from sea squirts as a new species, namely *Pinnotheres ascidicola* from the northern French Atlantic coast around Brittany. Miers (1886) listed it as *P. ascidiicola* [sic] among a number of other *Pinnotheres*-species without giving any definition. One of Hesse's main reasons to assign these pea crabs to a separate species was their ascidian host. While he presupposed that the established species *P. pisum* and *N. pinnotheres* live exclusively in bivalves, he found his specimen in "l'ascidie phallusiennes (*Ascidia canina*)" and "*Ascidia intestinalis*", which are, according to the literature, synonyms of *Phallusia mammillata* (Cuvier, 1815) and *Ciona intestinalis* (Linnaeus, 1767).

Further arguments, brought forward by Hesse, were the differences in size and color, the carapace being less transparent, and his observation of *P. ascidicola* not being pilose, which is said to be the case in the other species, especially in *N. pinnotheres*. The general color was characterized as sepia; the eyes were red. Moreover, Hesse described antennae, chelae, walking legs and third maxillipeds, without any comparison to *P. pisum* or *N. pinnotheres*. He stated for example that the first antenna was composed of three articles, the second antenna of four articles and the third maxillipeds' flagellum of two articles, but he didn't mention that all these characters are consistent with *N. pinnotheres.*

Pinnotheres marioni is mentioned for the first time in Gourret (1884) as "*Pinnotheres* nov. spec" out of *Ascidia mentula* from the Gulf of Marseille in the French part of the Mediterranean Sea. Only one drawing of the telson of the zoea is shown. In 1888, Gourret gave a detailed description, naming the species *Pinnotheres Marioni* in honour to his Professor A.F. Marion in the article "Quelques Crustacés parasites des ascidies". Drawings were displayed for the male, the female's carapace as well as pleon and chelae of both sexes and zoea larvae. All features shown in these drawings are, again, absolutely consistent with our analysis and description of *N. pinnotheres*.

According to Gourret (1888), *P. marioni* differs from *P. pisum* and *N. pinnotheres* in being pilose and in the carapace not being translucent. The front of the male was described as pronounced with a median incision. He pointed out that this character clearly differs from *P. pisum,* but admitted that it resembles *N. pinnotheres.* The first antenna was said to differ from *P. pisum* in having three articles, which is also the case in *N. pinnotheres*. The characters described by Gourret were mainly compared by him with *P. pisum*, but not compared thoroughly with *N. pinnotheres*. In addition to this, Gourret was obviously not aware of Hesse's description of *P. ascidicola* 15 years earlier, otherwise he should have compared his supposed new species with that one. From the original descriptions of *P. ascidicola* and *P.*

marioni and the mentioned range of ascidian hosts, it becomes clear that Hesse and Gourret were talking about one and the same species. One feature, pointed out by Hesse and Gourret as an argument for the inhabitants of sea squirts being separate species, is their degree of pilosity respectively the non-pilosity of the whole body. While *P. ascidicola* was said to be smooth, *P. marioni* was stated to be pilose. But since both authors were already misjudging the pilosity of *P. pisum* and *N. pinnotheres* – Hesse having said both species were pilose, Gourret having maintained just the opposite – we assume that they probably just did not have adequate optical methods and the sufficient degree of magnification for a proper examination of very small setae. A study of the setae types by scanning electron microscopy (SEM) has been carried out by us for the European species, revealing that the carapace of *P. pisum* is smooth, while *N. pinnotheres* is pilose (Becker and Türkay, unpublished).

Pinnotheres Bosc, 1802

Pinnotheres Bosc, 1802 (type species *Cancer pisum* Linnaeus, 1767, subsequent designation by Latreille, 1810, gender masculine).

Pinnotheres pisum (Linnaeus, 1767)

(Fig. 3.1A–C; 3.4A–D; 3.5A–C)

Cancer pisum Linnaeus, 1767, p 1039 (type probably not extant, type-locality: “Barbarbia, North coast of Africa”); Herbst, 1783, p 95–96, plate 2, figure 21.
Cancer mytilorum albus Herbst, 1783, p 101, plate 2, figure 24.

Pinnotheres pisum: Bosc, 1802, p 243 [new combination]; H. Milne Edwards, 1837, p 31–32, plate 19, figure 1a–f; Atkins, 1926, p 475–493, plate 1–5, text-figures 1–4; Atkins, 1954, p 687–700, figures 1–7, 14, 16; Lebour, 1928, p 109–110, 114–115, plate 2, figures 1–6; ebour, 1928, p 553 (larval stages) Christensen, 1959, p 267–270, figures 1–2.
A detailed synonymy is represented in Schmitt et al. (1973).

Material examined

Northeast Atlantic. North Sea, Dogger Bank: 1♂, host: *Mactra stultorum*, Stat. DOGN-14 Ku, 54° 30.73' N 2° 40.352' E-54° 30.478' N 2° 39.246' E, 19.07.2006, beam trawl, RV SENCKENBERG, leg. K. Pietratus (SMF 34583). 1♂, 2♀, host: *Mactra stultorum*, Stat. DOGN-6 Ku, 54° 45.489' N 1° 43.853' E-54° 45.7' N 1° 42.443' E, 19.07.2006, beam trawl, RV SENCKENBERG, leg. K. Pietratus (SMF 34584 – SMF 34586). 1♂, 1♀ juvenile hard

stage, host: *Spisula solida*, Dogger Bank-West, Stat. DOGO-9 RD, 54° 59.635' N 1° 39.267' E-54° 59.635' N 1° 39.267' E, 01.08.2008, ring dredge, leg. RV SENCKENBERG, (SMF 34043). 1♀ juvenile hard stage, host: *Mactra stultorum*, Dogger Bank-West, Stat. DOGO-4 RD, 54° 28.71' N 1° 51.771' E-54° 28.71' N 1° 51.771' E, ring drdge, 30.07.2008, leg. RV SENCKENBERG, (SMF 34040). 1♀, host: *Mactra stultorum*, Dogger Bank-West, Stat. DOGO-5 Ku, 54° 37.473' N 1° 42.225' E 54°-37.307' N 1° 42.38' E, beam trawl, 30.07.2008, leg. RV SENCKENBERG (SMF 34007). 1♀, host: *Mactra stultorum*, Dogger Bank-East, Stat. DOGO-17 Ku, 54° 51.011' N 2° 5.168' E 54°-51.859' N 2° 5.222' E, beam trawl, 01.08.2008, leg. RV SENCKENBERG (SMF 34008). 1♂, host: *Mactra stultorum*, Dogger Bank-East, Stat. DOGO-26 Ku, 54° 50.798' N 2° 49.403' E-54° 51.625' N 2° 50.568' E, beam trawl, 01.08.2008, leg. RV SENCKENBERG (SMF 34009). 1♀ ovigerous, host: *Mactra stultorum*, Dogger Bank-East, DOGL-14 ku, 54° 31' N 2° 40.8' E-54° 30.8' N 2° 38.8' E, beam trawl, 03.08.2004, leg. RV SENCKENBERG (SMF 34006). 1♀, host: *Gari fervensis*, Dogger Bank-East, Stat. DOGN-40/8 Ku, 55° 27.544' N 4° 8.624' E-55° 27.768' N 4° 6.907' E, beam trawl, 24.07.2006, RV SENCKENBERG, leg. K. Pietratus (SMF 32742). 1♀, host: *Mactra stultorum*, Dogger Bank-East, Stat. DOGO-11 RD, 54° 45.396' N 2° 0.459' E-54° 45.396' N 2° 0.459' E, 01.08.2008, leg. RV SENCKENBERG (SMF 34041). 1♀ juvenile hard stage, free living, Dogger Bank-East, Stat. DOGO-29 Ku, 55° 8.026' N55° 8.323' N2° 41.64' E2° 43.195' E, 03.08.2008, leg. RV SENCKENBERG (SMF 34042). 1♂, 1♀ ovigerous, host: *Mactra stultorum*, Dogger Bank-East, Stat. DOGO-40/1 RD, 55° 27.453' N 4° 8.622' E-55° 27.453' N 4° 8.622' E, 04.08.2008, leg. RV SENCKENBERG (SMF 34044). 1♀ juvenile hard stage, host: *Donax vittatus*, Dogger Bank-East, Stat. DOGO-13a RD, 54° 27.113' N 2° 16.064' E-54° 27.113' N 2° 16.064' E, 30.07.2008, leg. RV SENCKENBERG (SMF 34045). 1♀ ovigerous, host: *Gari fervensis*, Dogger Bank-East, Stat. DOGO-40/1 Ku, 55° 27.612' N 4° 8.219' E-55° 27.525' N 4° 10.267' E, 04.08.2008, leg. RV SENCKENBERG (SMF 34046). 1♀ ovigerous, host: *Mactra stultorum*, Dogger Bank-East, Stat. DOGM-14 Ku, 54° 31.008' N 2° 40.609' E-54° 30.847' N 2° 40.535' E, hard bottom dredge, 03.08.2005, RV SENCKENBERG, leg. M. Türkay (SMF 34374).

North Sea, German Bight: 78♂, 239♀ stage I to stage V (in part ovigerous), host: *Modiolus modiolus*, Helgoland Trench, 54°08,419'N-54°08,599'N to 07°50,921'E – 07°53,431'E, beam trawl/hard bottom dredge, Jan. – Dec. 1985-1992, leg. RV SENCKENBERG, uncatalogued material. 1♀, Helgoland Trench, Stat. NR-45 Ku, 54° 8.56' N 7° 52.3' E-54° 8.52' N 7° 52.08' E, beam trawl, 13.08.1984, leg. RV SENCKENBERG (SMF 12887). 2♂ free living, Stat. D2007-27 Ku, 55° 17.046' N 6° 43.88' E-55° 17.55' N 6°

45.238' E, beam trawl, 07.08.2007, RV SENCKENBERG, leg. K. Pietratus (SMF 32743). 1♀, 1♂, host: *Spisula solida*, Loreley Bank, ring dredge, 14.05.1985, leg. RV SENCKENBERG, uncatalogued material. 1♀ ovigerous, host: *Mactra stultorum*, north of Juist, Stat. D2007-3 RD, 53° 47.161' N 7° 3.225' E-53° 47.161' N 7° 3.225' E, hard bottom dredge, 01.08.2007, RV SENCKENBERG, leg. M. Türkay (SMF 34375). 1♀, ovigerous, host: *Spisula elliptica*, Wangerooge, Stat. LR-060728-2 Ku, 53° 49.395' N 7° 52.498' E-53° 48.899' N 7° 53.863' E, 28.07.2006, beam trawl, RV SENCKENBERG, leg. K. Pietratus (SMF 34587). 1♀, Norderney, Stat. V53-078 Ku, 53° 49.51' N 7° 13.64' E-53° 49.72' N 7° 12.13' E, beam trawl, 18.02.1987, RV Valdivia (SMF 32676). 1♂, 1♀, host: *Mactra stultorum*, Stat. WH287 Stat. 398, 01.05.2006, beam trawl, RV WALTER HERWIG, leg. K. Pietratus (SMF 32741).

North Sea, other locations: 1♂, host: *Mytilus edulis*, Netherlands, close to the German boarder, mussels from a restaurant,15.09.2006, leg. C. Becker, SMF 34582. 3♂, 1♀, host: *Mytilus edulis*, North Sea, Ireland, supermarket "Metro" in Frankfurt, Germany, 2005, leg. S. George (SMF 32744).

France, Brittany: 1♀, host: *Mytilus edulis*, Roscoff, 01.04.1990-31.05.1991, leg. J. Klein (SMF 34382). 1♀ ovigerous, host: *Mytilus galloprovincialis*, 48° 34' N 2° 24' W, 08.10.1994, leg. C. d'Udekem d'Acoz, SMF 33457. 1♂, 1♀ juvenile hard stage, host: *Spisula solida*, Région de Dinard: "Les Haches", ring dredge, Apr. 1993, leg. C. d'Udekem d'Acoz (SMF 34001). 1♀ juvenile, host: *Mytilus edulis*, Brest (supermarket), Apr. 1991, leg. J. Klein (SMF 34318). 1♀, host: *Mytilus edulis*, France, Brittany, Brest (supermarket), 17.4.1991, leg. J. Klein (SMF 34319). 1♀, host: *Mytilus edulis*, France, Brittany, Vannes (supermarket), 17.4.1991, leg. J. Klein (SMF 34320).

France, other locations: 9♂, 17♀, host: *Mytilus edulis*, France, Haute-Normandie, 49° 19' N 0° 22' W, 08.10.1994, leg. C. d'Udekem d'Acoz, SMF 33456. 2♀, host: *Mytilus edulis* from the market hall in Frankfurt/Germany, origin: France, Bay of Biscay, Oléron, 06.08.2005, leg. C. Becker (SMF 34576). 2♂, 11♀, host: *Mytilus edulis* from the market hall in Frankfurt/Germany, origin: France, Oléron, 17.02.2007, leg. C. Becker (SMF 34581).

Mediterranean. Northern Adriatic Sea, Croatia, Istria: 1♀ ovigerous, Rovinj, mole in front of the Institute Ruđer Bošković, Stat. Rov93, 01.09.1993 (SMF 31503). 1♂, 1♀, host: *Mytilus galloprovincialis*, Rovinj, in front of the old town, leg. 19.08.1989 (SMF 31519). 1♀, host: *Ostrea edulis*, Rovinj, Big Figarola, skin-diving, 01.09.2005, leg. C. Becker & S. Kalscheid (SMF 34579). 1♀ ovigerous, host: *Mytilus galloprovincialis*, Rovinj, Sv. Katarina, 09.07.1986, leg. U. Pettke (SMF 31512). 1♂, 2♀, 1♀ juvenile stage III-IV, 5♀ ovigerous, host: *Pinna nobilis*, Rovinj, bay of Bale, 1982, leg. U. Pettke (SMF 33808). 1♀ ovigerous,

host: *Pinna nobilis*, Rovinj, bay of Bale, Jul. 1982, leg. U. Pettke (SMF 33810). 2♀, Rovinj, Sv. Katarina, SE-coast, Stat. Rov01-02, 45° 4.651' N 13° 37.891' E-45° 4.651' N 13° 37.891' E, leg. 13.08.2001 (SMF 31500). 1♂, 1♀, host: *Pinna nobilis*, Rovinj, bay of Bale, Jul. 1982 (SMF 31501). 1♀, 1♂, host: *Pinna nobilis*, Rovinj, bay of Bale, Jul. 1982 (SMF 31502). 1♂, 1♀ ovigerous, host: *Pinna nobilis*, Rovinj, bay of Bale, Jul. 1987, leg. D. Krämer (SMF 33809). 8♂, 3♀, 3♀ juvenile, 5♀ ovigerous, host: *Mytilus galloprovincialis*, Rovinj, Monte Mulini, leg. 21.08.1989 (SMF 31517). 2♂, 2♀ juvenile, 6♀ ovigerous, Rovinj, Val Salina, leg. 23.08.1989 (SMF 31518). 1♀, host: *Pinna nobilis*, Rovinj, Dec. 2003, leg. D. Brandis (SMF 34577). 1♂, 1♀ ovigerous, host: *Pinna nobilis*, bay of Kolone, Jul. 1983 (SMF 31504). 1♀, host: *Ostrea edulis*, Limski Fjord, skin-diving, 29.08.2005, leg. C. Becker (SMF 34580). 1♀ juvenile stage II, host: *Mytilus galloprovincialis*, Limski Fjord, 21.08.1989 (SMF 31520). 3♀, Limski Fjord, Aug. 1968, leg. Kinzelbach (SMF 5099).

Tyrrhenian Sea: 1♀, Italy, Isola d'Elba, leg. S. Rau (SMF 5303).

Ionian Sea: 1♂, 1♀, host: *Pinna nobilis*, Greece, Ionian Islands, Island Marathonisi in front of Island Zakynthos, 36°45'13' N 22°34'25'E, Aug. 1979, leg. B. Kurlemann (SMF 16285).

Sea of Marmara: 3♂, 1♀ ovigerous, host: *Pinna nobilis*, Turkey, Princes' Islands, Büyükada, Jul. - Aug. 1966, leg. M. Türkay (SMF 4451). 2♂, 1♀ ovigerous, host: *Pinna nobilis*, Turkey, Princes' Islands, Kınalıada, Jun. 1964, leg. M. Türkay (SMF 4455). 2♀, host: *Pinna nobilis*, Turkey, Princes' Islands, Büyükada, Jul. 1964, leg. M. Türkay (SMF 4467).

Male

General description (fig. 3.1B, 3.4A). Color light, nearly white to ivory with orange ornamentation on dorsal surface of carapace, chelipeds and walking legs. Carapace rounded, dorsally very convex, strongly calcified, not translucent, without defined regions and lateral teeth. Surface plain and smooth owing to lack of setae. Front very pronounced, slightly acuminated in middle. Eyes clearly visible in dorsal view, with light orange coloration in living specimens. Chelipeds (P1), relative length of articles of walking legs and third maxillipeds consistent with description of female given below, but chelipeds generally much stronger and stouter than in adult females.

Second and third pair of walking legs (P3, P4) with swimming fringes: two rows of long pappose setae on distal articles. One running dorso-posteriorly on carpus and propodus, one ventro-anteriorly. Shorter pappose setae lining dorsal and ventral margins of all walking legs (P2–P5). Largest males, found in *Pinna nobilis,* with carapace width of about 7 mm.

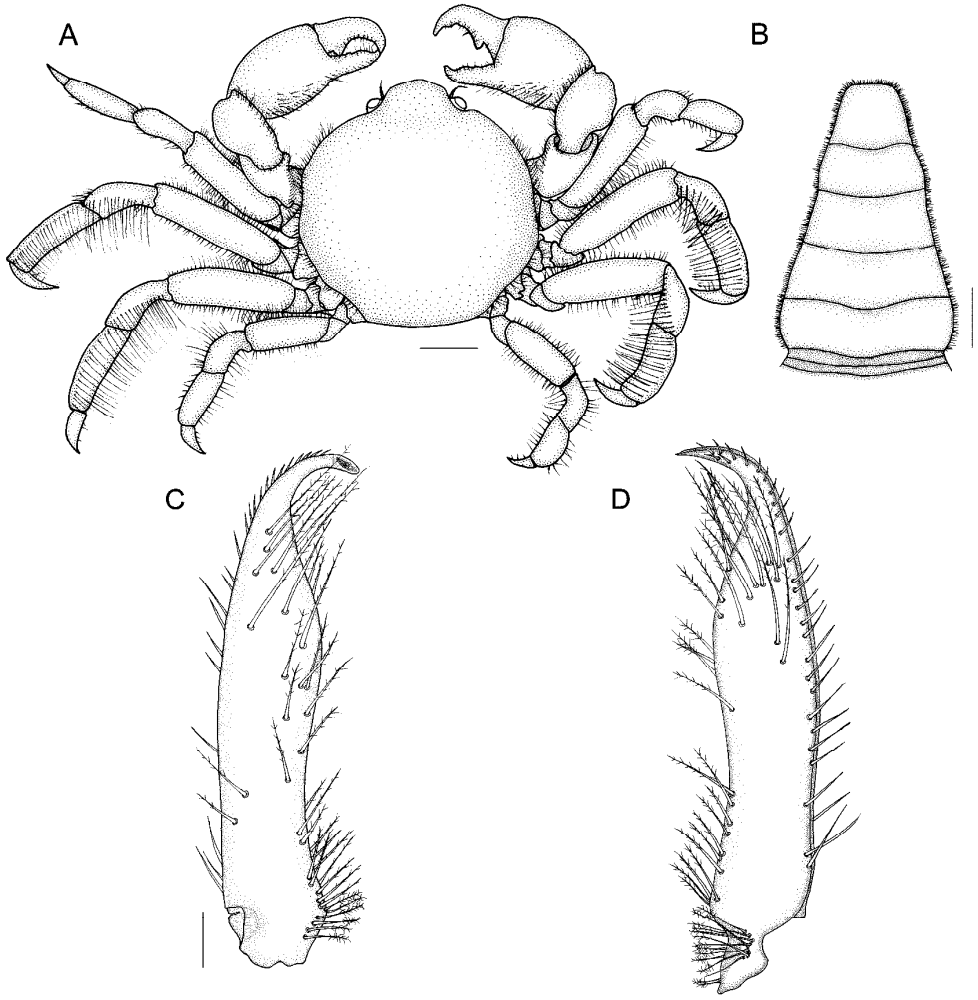

Figure 3.4. Male of *Pinnotheres pisum.* (**A**) Dorsal view. (**B**) Pleon (abdomen). (**C**) ventral view on left first gonopod. (**D**) Dorsal view of same. Scale bars: 2 mm (A), 500 µm (B), 250 µm (C)**.**

Pleon (abdomen) and sternum (fig. 3.4B*).* Male abdominal segments clearly separated. Belonging to Thoracotremata, male gonopores on sternum. Pleon narrow, general shape triangular. Pleon tapering distally from segment 3 to segment 6, lateral margins of segment 3 slightly rounded, shape of segment 4 and 5 trapezoid, margins of segment 6 slightly rounded, shape of telson trapezoid. Whole outer margin of pleon setose, remaining surface smooth.

First gonopod (G1) (fig. 3.4C, D). G1 straight over almost total length, strongly flattened dorso-ventrally. Distal part narrowed abruptly with light curve in last 7/8. Distal openings of ejaculatory canal oriented slightly towards lateral side of body. Gonopods with long pappose setae on proximal base and along total length, setae more numerous in curve of distal part.

Female (adult)

General description (fig. 3.1A, C, 3.5A). Color light ivory, nearly white. Carapace subglobular or slightly wider than long, especially in large females. Carapace very soft, translucent through decalcification, without defined regions and lateral teeth. Eyes hardly visible in dorsal view, especially in large females. Eyes of living specimen with light orange coloration. Carapace and whole body surface smooth, shiny owing to lack of setae. Pleon very broad, rounded, covering whole ventral side including coxae, reaching front anteriorly. Pleons' margin setose, outer surface smooth. Maximum body size of adult (ovigerous) females about 18 mm in carapace width in specimens from *Pinna nobilis*, minimum about 4 mm in small ovigerous females inhabiting *Mytilus edulis*. Juvenile females consistent with the description of males (except for pleopods).

Chelipeds and walking legs (fig. 3.5A, B). Cheliped (P1), especially palm, slender in adult females (stouter in juvenile females and males). Cutting edge of claw with one single pointed tooth on movable finger (dactylus) and one single tooth on fixed finger (propodus). Inner and outer surface of palm rather smooth, only scattered setae. Simple and plumose setae located near angle of propodus and dactylus and on cutting edge of claw. Field of long pappo-serrate setae forming dense comb on bottom side of claw.
Walking legs (P2–P5) with short, pointed, curved dactyli. Dactyli of P2–P5 considerably less than half as long as propodus. Dactyli almost of equal length in all walking legs. Swimming fringes of second and third walking legs (P3, P4) present in juvenile females, reduced in adults.

Third maxilliped (fig. 3.5C). Third maxilliped with large completely fused merus-ischium-article. Dactylus of palp inserting underneath propodus (subterminally). Flagellum two-segmented with tuft of long simple setae originating from tip. Third maxillipeds' inner margins densely fringed with long simple setae, surfaces of merus-ischium, carpus, and propodus smooth.

Comments

In contrast to data in the literature (Schmitt et al. 1973), we found *Pinnotheres pisum* exclusively in bivalves, but never in ascidians.

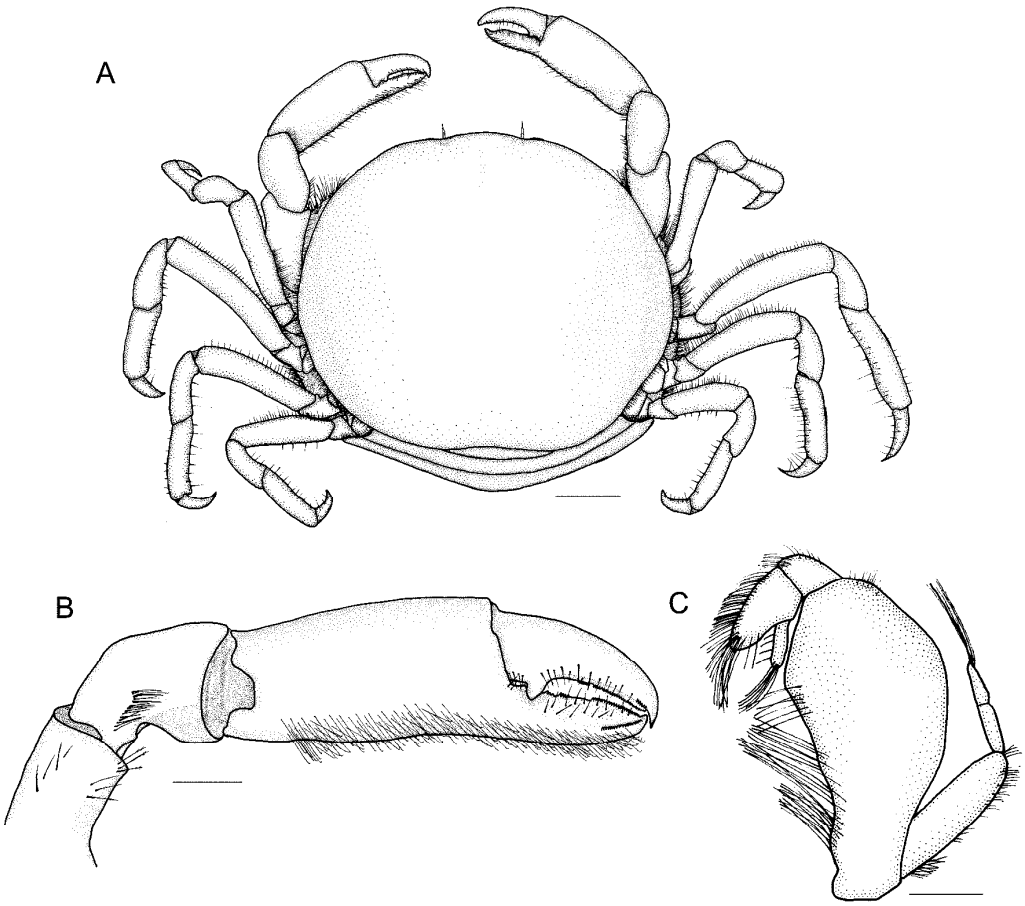

Figure 3.5. Female of *Pinnotheres pisum*. (**A**) Dorsal view. (**B**) Left cheliped with comb of setae; (**C**) exterior of left maxilliped. Scale bars: 2 mm (A), 1 mm (B), 500 µm (C).

Pinnotheres pectunculi Hesse, 1872

(Fig. 3.1F, G; 3.6A–D)

Pinnotheres pectunculi Hesse, 1872, p 36–38 (no depository of types, type-locality: coast of Brittany, France); d'Udekem d'Acoz, 1988, p 195–201, figures 24–25, 28, 30, 32–33; *Pinnotheres pisum* forma *pectunculi*: Bourdon, 1965, p 32, 40

Material examined

Northeast Atlantic. France, Brittany: 1♀, host: *Glycymeris glycymeris*, region of Dinard, east of "des Haches", 48° 38' N 2° 3' W, Apr. 1993, leg. C. d'Udekem d'Acoz (SMF 33459). 1♀, host: *Glycymeris glycymeris*, Pointe de Bilfot, Plouézec, 48° 45' N 2° 56' W, 09.04.1993, leg. C. d'Udekem d'Acoz (SMF 33460). 3♀, host: *Glycymeris glycymeris*, Bay of Morlaix, Île Callot, 11.04.1986, leg. C. d'Udekem d'Acoz (SMF 34002). 40♀, 38♀ ovigerous, host: *Glycymeris glycymeris*, Roscoff, ring dredge, May 1990, leg. J. Klein (SMF 34252-SMF 34294, SMF 34390-SMF 34399, SMF 34460-SMF 34484). 48♀, 3 juvenile stage III-IV, 2♀

ovigerous, host: *Glycymeris glycymeris*, Bay of Morlaix, ring dredge, May 1991, leg. J. Klein (SMF 34332). 1♂, 3♀ juvenile, 3♀ juvenile hard stage, 1♀ juvenile stage II, host: *Glycymeris glycymeris*, Roscoff, ring dredge, Apr. 1991, leg. J. Klein (SMF 34295-SMF 34303). 3♀ juvenile, host: *Venus verrucosa*, Roscoff, ring dredge, Apr./May 1990/1991, leg. J. Klein (SMF 34304-SMF 34306). 1♂, 10♀, host: *Venus verrucosa*, Roscoff, ring dredge, Apr./May 1990/1991, leg. J. Klein (SMF 34307 - SMF 34313, SMF 34383). 1♂, 1♀, host: *Circomphalus casina*, Roscoff, ring dredge, Apr./May 1990/1991, leg. J. Klein (SMF 34314, SMF 34315). 1♀, 1♀ juvenile hard stage, host: *Clausinella fasciata*, Roscoff, ring dredge, Apr./May 1990/1991, leg. J. Klein (SMF 34316, SMF 34517). 3♂, 1♀ juvenile, 3♀ juvenile hard stage, 1♀ juvenile stage II, 1♀ juvenile stage II-III, host: *Glycymeris glycymeris*, Roscoff, ring dredge, Apr./May 1990/1991, leg. J. Klein (SMF 34321 – SMF 34328, SMF 34384-SMF 34386). 2♂, 1♀ juvenile hard stage, host: *Clausinella fasciata*, Roscoff, ring dredge, Apr. 1991, leg. J. Klein (SMF 34327-SMF 34329). 2♂, host: *Circomphalus casina*, Roscoff, ring dredge, 1991, leg. J. Klein (SMF 34330, SMF 34331). 9♀, host: *Circomphalus casina*, Roscoff, ring dredge, Apr. 1991, leg. J. Klein (SMF 34335-SMF 34373). 1♂, 1♀, host: *Circomphalus casina*, Roscoff: Stat. 9, beam trawl, Apr. 1991, leg. J. Klein (SMF 34387, SMF 34388). 1♀, host: *Clausinella fasciata*, Roscoff: Stat. 9, beam trawl, Apr. 1991, leg. J. Klein (SMF 34389). 56♀, host: *Glycymeris glycymeris*, Roscoff, May 1990, leg. J. Klein (SMF 34485-SMF 34540). 20♀, host: *Glycymeris glycymeris*, Roscoff, Oct. 2005, leg. W. Thomas, Station biologique de Roscoff (SMF 34333). 63♀ ovigerous, 1♀ stage IV, host: *Glycymeris glycymeris*, Roscoff, 21.05.2008, leg. L. Lévèque, Station biologique de Roscoff (SMF 34334). 1♀, larvae zoea 1, host: *Glycymeris glycymeris*, Roscoff, 01.08.2005, leg. T. Wehe (SMF 34578).

France, unspecified locations: 1♀ juvenile, 15.04.1987, leg. C. d'Udekem d'Acoz (SMF 33458). 1♀, host: *Glycymeris glycymeris*, France, fishmarket close to La Sausaie, Feb. 1982, leg. H. Nesemann (SMF 34004).

Male

General description. Very similar to *P. pisum* (fig. 3.4A). Males of *P. pectunculi* rarely found inside host. Present study based on preserved material, therefore no information on general color, possible ornamentation and coloration of eyes available. Carapace rounded, dorsally very convex, strongly calcified, not translucent, without defined regions and lateral teeth. Surface of carapace smooth, shiny, without setae. Front very pronounced, slightly acuminated in middle. Eyes clearly visible in dorsal view. Chelipeds, walking legs and third

maxillipeds generally consistent with description for female given later, but chelipeds generally much stronger and stouter than in adult females. Second and third pair of walking legs (P3, P4) with swimming fringes: two rows of setae on distal articles: one runs dorso-posteriorly on carpus and propodus, another ventro-anteriorly. Dorsal and ventral margin of walking legs (P2–P5) lined with shorter setae.
Maximum carapace width of males about 5 mm in the dog cockle *Glycymeris glycymeris*.

Pleon (abdomen) and sternum (fig. 3.6B). Male abdominal segments clearly separated. Belonging to thoracotremata, male gonopores located on sternum. Pleon tapering from proximal to distal segments, tapering stronger, less gradual than in *P. pisum*. Shape of pleon less triangular than in *P. pisum*, rounded, especially telson. Outer margin of pleon lined with setae, remaining surface smooth.

First gonopod (G1) (fig. 3.6C, D). Gonopods flattened dorso-ventrally, tapering by degrees from base to tip. Curvature towards lateral side of body gradual from proximal base of gonopod to distal opening of ejaculatory canal. Whole limb sickle-shaped. Tapering to distal opening gradual, regular along total length. With same plumose setae as in *P. pisum*, but highest density less distal to tip, more concentrated in flexed region.

Female (adult)

General description (fig. 3.1F, G). Very similar to *P. pisum* (fig. 3.5A). General color light ivory, nearly white. Carapace subglobular or slightly wider than long, especially in large females. Carapace very soft, translucent through decalcification, without defined regions and lateral teeth. Eyes hardly visible in dorsal view, especially in large females. Eyes with light orange coloration in living specimens. Carapace, as well as whole body surface, smooth and shiny due to lack of setae. Pleon very broad, rounded, covering whole ventral side and coxae, reaching front anteriorly. Pleons' margin setose, outer surface smooth.
Maximum carapace width around 10 mm in adult females from *Glycymeris glycymeris*, minimum carapace size in ovigerous females from *Circomphalus casina* about 5 mm. Juvenile females consistent with description of males (except for pleopods).

Chelipeds and walking legs (fig. 3.6A). Palm of cheliped very slender in adult females, appearing generally slightly more slender than in *P. pisum*. Chelipeds of juvenile females stouter, number and arrangement of teeth, setae types and their distribution identical to *P. pisum* (fig. 3.5B). Movable finger (dactylus) with one single pointed tooth. Fixed finger

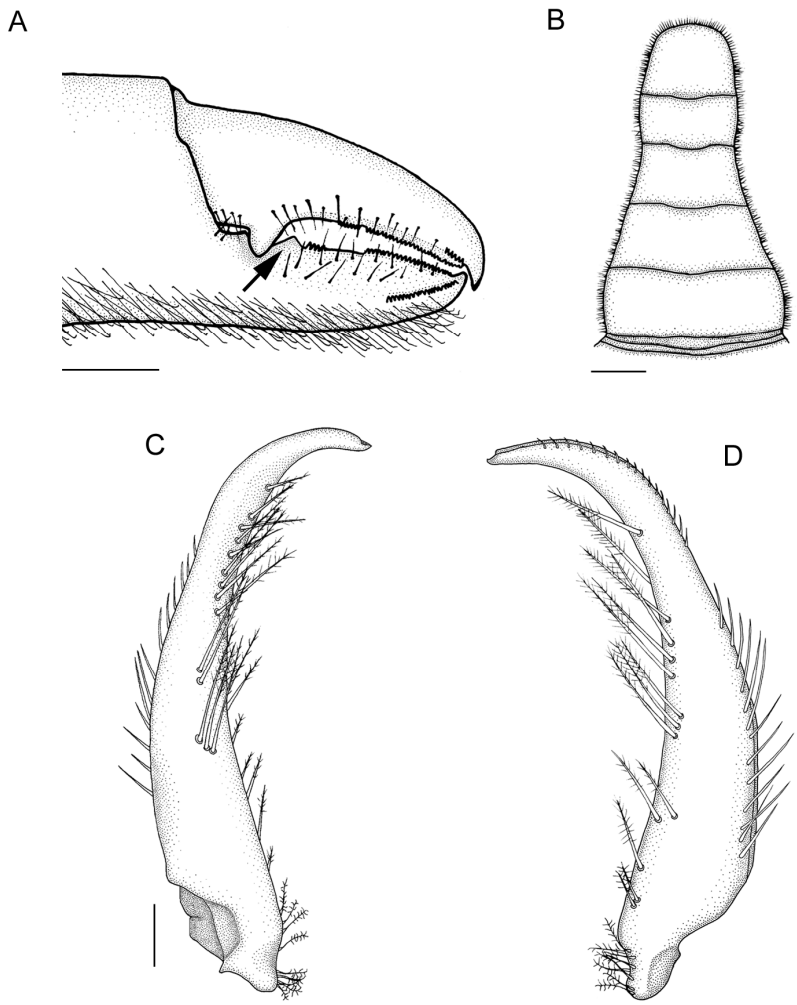

Figure 3.6. *Pinnotheres pectunculi* from the dog cockle *Glycymeris glycymeris*. (**A**) Left cheliped of female. Arrow points on small triangular tooth on the fixed finger. (**B**) Pleon of male. (**C**) Ventral view on left male first gonopod. (**D**) Dorsal view on left male first gonopod. Scale bars: 1 mm (A), 500 µm (B), 200 µm (C).

(propodus) with one tooth followed by additional small tooth. Small tooth is the only feature to distinguish females of *P. pectunculi* from *P. pisum*. Setae types and grouping on claw identical. Principal part of palm plain and smooth, showing only scattered setae. Simple and pappose setae located on cutting edge of claw and near angle of propodus and dactylus. Bottom side of claw with field of long pappo-serrate setae forming dense comb. Walking legs (P2–P5) slender with short, pointed and curved dactyli, considerably less than half as long as propodus. Dactyli of almost equal length in all walking legs. Swimming fringes of second and third walking leg (P3, P4) present in juvenile females, reduced in adults.

Third maxilliped. Third maxilliped similar to *P. pisum* (fig. 3.5C), with large completely fused merus-ischium-article. Dactylus of palp inserted underneath propodus (subterminally). Flagellum two-segmented with tuft of long simple setae originating from its tip. Third

maxillipeds' inner margins densely fringed with long simple setae, but surfaces of merus-ischium-article, carpus and propodus smooth. Juvenile females very similar to males, except for slight differences in shape of pleon and abdominal appendages.

Comments

The first description of *Pinnotheres pecunculi* was done by Hesse (1872). From the fact that the specimens found in the dog cockle, *Glycymeris glycymeris*, were much smaller than *Pinnotheres pisum* from the blue mussel, *Mytilus edulis*, Hesse already concluded there must have been two separate species. Furthermore, Hesse described that the first antenna had five articles and that the male carapace exhibited an orange ornamentation.

Bourdon (1965) mentioned specimens from *Glycymeris glycymeris* and indicated that these might have been a new species, but since he had pointed out the need for further investigations, he carefully called his specimens *Pinnotheres pisum* forma *pectunculi.* Bourdon described the additional tooth on the fixed finger of the claw in the female and mentioned that the walking legs were more pilose than in *P. pisum* (1965). In addition to this, he indicated the infection rate for *P. pectunculi*, but he didn't give any statement for the incidence of males.

Bourdon also mentioned the presence of *N. pinnotheres* in *Ascidia mentula* and in *Ostrea edulis*, while he was claiming that *P. pisum* did not inhabit *Ostrea edulis*, but was also found in ascidians. Our present study suggests that both is not accurat: while we never found *N. pinnotheres* in *Ostrea edulis*, *P. pisum* was not present in ascidians (see examined material).

The origin and relationship of *P. pectunculi* is not yet clear. Since its distribution and host range seems to be very restricted so far, it might be a relatively new species. Next to its similarities with *Pinnotheres pisum*, it also resembles *Zaops ostreum* (Say, 1817) from the Northwest Atlantic. *Z. ostreum* was redescribed by Stauber (1945) and especially its chela and the males' first gonopods look very similar to *P. pectunculi's*, but it can still be separated by the relative length of the dactyli of the walking legs and by differences in the existence of dorsal and lateral spines of the larvae. The larvae of *Z. ostreum* were described by Sandoz and Hopkins (1947).

DISCUSSION

The following European species can be distinguished by morphology: *Nepinnotheres pinnotheres* (Linnaeus, 1758), *Pinnotheres pisum* (Linnaeus, 1767) and *Pinnotheres*

pectunculi Hesse, 1872. On further examination *Pinnotheres marioni* Gourret, 1888 and *Pinnotheres ascidicola* Hesse, 1872 are junior synonyms of *N. pinnotheres.* According to our study there are no differences between the material collected from sea squirts and *N. pinnotheres* from the giant Mediterranean pen, *Pinna nobilis* – except for size and color. Furthermore, we cannot make out any differences from the descriptions and the figures of *P. ascidicola* published by Hesse (1872) and *P. marioni* by Gourret (1888). It is obvious that the first authors of *P. ascidicola* and *P. marioni* did not carefully compare their "new species" to *N. pinnotheres*. The description of characters and the emphasis on differences are mainly given in comparison with the more abundant species *P. pisum*. The main problem with the original descriptions of *P. ascidicola* and *P. marioni* is the overvaluation of size and color. As a matter of fact the size of adult pinnotherids can vary strongly within one species according to host size, which was obvious in the present study and had also been demonstrated in literature by Palmer (1995) and Pregenzer (1978).

Earlier, it was often presupposed that pinnotherids are host-specific. Therefore, new records of hosts often led to the description of new species without accurate comparison with those already described. According to our study, the European pinnotherids have a specific host range instead of being specific in just one single host-species.

ACKNOWLEDGEMENT

We thank Richard G. Hartnoll (Port St. Mary, Isle of Man, UK) for streamlining our English and for many helpful suggestions. We also thankfully acknowledge captains and crews of RV SENCKENBERG as well as many individual collectors mentioned in the material lists. They have largely contributed to the success of this study.

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Morphology of the Female Reproductive System of European Pea Crabs (Crustacea, Decapoda, Brachyura, Pinnotheridae)

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ABSTRACT

Commensal pea crabs inhabiting bivalves have a very high reproductive output due to the great extension and fecundity of the ovary. We studied the underlying morphology of the female reproductive system in the Pinnotheridae *Pinnotheres pisum*, *Pinnotheres pectunculi* and *Nepinnotheres pinnotheres* by histological methods and transmission electron microscopy (TEM).

Eubrachyura have internal fertilization: the paired vaginas enlarge into storage structures, the spermathecae, which are connected to the ovaries by oviducts. Sperm is stored inside the spermathecae until the oocytes are mature. The oocytes are transported by the oviducts into the spermathecae, where fertilization takes place.

In the investigated pinnotherids, the vagina is of the 'concave pattern' (sensu Hartnoll 1968): musculature is attached alongside flexible parts of the vagina-wall that controls the dimension of its lumen. The genital opening is closed by a muscular mobile operculum.

The spermatheca can be divided into two distinct regions by function and morphology. The ventral part includes the connection with vagina and oviduct and is regarded as the zone where fertilization takes place. It is lined with cuticle except where the oviduct enters the spermatheca by the 'holocrine transfer tissue'. At ovulation, the oocytes have to pass through this multi-layered glandular epithelium performing holocrine secretion. The dorsal part of the spermatheca is considered as being the main sperm storage area. It is lined by a highly secretory apocrine glandular epithelium.

Thus, two different forms of secretion occur in the spermathecae of pinnotherids. The definite role of secretion in sperm storage and fertilization is not yet explored, but it is notable that structure and function of spermathecal secretion are more complex in pinnotherids, and probably more efficient, than in other brachyuran crabs.

Keywords: *Pinnotheres*, histology, ultrastructure, spermatheca, holocrine secretion, apocrine glandular epithelium.

INTRODUCTION

Pinnotherids are small crabs that live in association with other invertebrates. The studied European species *Pinnotheres pisum* (Linné, 1767) and *Pinnotheres pectunculi* Hesse, 1872 live inside bivalves, while *Nepinnotheres pinnotheres* (Linné, 1758) inhabits solitary sea squirts and the Mediterranean pen shell *Pinna nobilis* (Becker and Türkay 2010).

The pea crabs feed on the mucus produced by the gills of their host and accumulated food particles (Orton 1920). This commensal mode of life is at a cost to the host. In several cases, pinnotherids are considered as truly parasitic and they can have a negative impact on commercially exploited bivalves like mussels and oysters (Berner 1952, Bierbaum and Ferson 1986, Bierbaum and Shumway 1988).

Pea crabs have a quite complex life history. The dispersal of larvae is planktonic, as in most crustaceans. Adult males are found inside hosts but also free-living (Christensen 1959). As juveniles, both sexes inhabit hosts temporarily, being good pelagic swimmers by paddling with their setose second and third pairs of walking legs (Hartnoll 1972). For females, this applies only up to the juvenile "stage I" or "hard stage" (sensu Atkins 1926). Mating actually occurs in this female juvenile hard stage. At that time, the ovaries are not yet developed and the female is still several stages away from the moult of puberty (Hartnoll 1969). This precocious mating is rather exceptional for true crabs (Brachyura Linné, 1758). From the moment copulation has taken place, the female remains in its final host definitively and passes through a metamorphosis that leads to a conspicuous sexual dimorphism (Atkins 1926). With every subsequent moult, the females' cephalothorax and pleon grow faster compared to walking legs and chelipeds. Simultaneously, the carapace decalcifies and becomes soft and translucent so that the internal organs show through. As a result of this metamorphosis, the female totally adapts to its parasitic phase of live and never leaves the host again.

The reproductive investment of female pinnotherids is very high compared to other brachyurans (Hartnoll 2006). The result is an outstanding reproductive output as shown by Hines (1992). Moreover, the females' gonads have an exceptional extension and productiveness (Hines 1992). However, the fundamental structures have not been studied to date. So, we here investigate the morphology of the female reproductive system of the species named above by histological methods and transmission electron microscopy (TEM).

Within Brachyura, internal fertilization has developed (fig. 4.1). In Eubrachyura Saint-Laurent, 1989, the paired vaginas enlarge into storage structures, the seminal receptacles or

so-called spermathecae. These have an interior connection with the ovaries by oviducts. Male's sperm masses are received during copulation and stored inside the spermathecae until the oocytes are mature. Afterwards, they are transported through the oviduct into the spermatheca, where the oocytes come in contact with the sperm mass and fertilization takes place. The fertilized eggs are extruded via the vagina and are retained under the females' pleon until the larvae hatch.

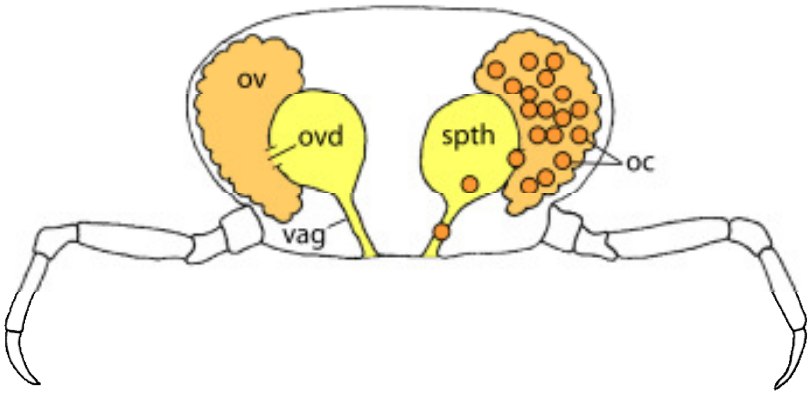


Figure 4.1. Overview on the female reproductive system. Model of transverse section through female eubrachyuran crab showing the reproductive structures. The internal fertilization is realized by oviducts being connected to spermathecae storing the sperm mass. oc = oocytes; ov = ovary; ovd = oviduct; spth = spermatheca; vag = vagina.

The mating strategies of Brachyura and their reproductive morphology has to date mainly been observed for species of commercial interest within the group Heterotremata Guinot, 1977. The most relevant studies on female reproductive morphology, including histological data on spermathecae, are listed in table 4.1. Such studies are essential to understand reproductive strategies and therefore an important contribution for sustainable fishery management of exploited crabs (e.g. Elner and Beninger 1992, 1995). Beyond that, the diversity of the reproductive structures is of great phylogenetic relevance since the evolution of brachyuran mating systems has been scarcely studied to date, especially for higher Brachyura, the Thoracotremata Guinot, 1977, to which pinnotherids belong.

MATERIAL AND METHODS

The present study was conducted from 2004 – 2009 in the Research Institute Senckenberg in Frankfurt/Germany and at the University of Heidelberg/Germany. The research complied with the institutional guidelines of animal ethics and adhered to the local legal requirements.

Pinnotheres pisum was collected from a population of the horse mussel, *Modiolus modiolus*, in the Helgoland Trench during cruises to the German Bight with RV SENCKENBERG in

Table 4.1. Relevant studies on the histology of spermathecae (synonyms used in original publications in brackets).

	Heterotremata	
Cancridae	*Metacarcinus (Cancer) magister*	Jensen et al. 1996
	Cancer pagurus	George 2004
Eriphiidae	*Eriphia verrucosa*	George 2004
Majoidea	*Libinia spinosa*	Sal Moyano et al. 2009
	Inachus phalangium	Diesel 1989, 1990, 1991
	Hyas coarctatus	Hartnoll 1968
	Hyas coarctatus	Lanteigne et al. 1996
	Hyas araneus	Hartnoll 1968
	Chionoecetes opilio	Beninger et al. 1988, 1993
		Lanteigne et al. 1996
		Sainte-Marie and Sainte-Marie 1998
Portunoidea	*Carcinus maenas*	Spalding 1942
		Hartnoll 1968
	Callinectes sapidus	Johnson 1980
	Portunus sanguinolentus	Ryan 1967b
	Portunus pelagicus	Bawab and El-Sherief 1988, 1989
	Portunus trituberculatus	Xuan et al. 2009
Potamidae	*Potamon spp.*	Brandis et. al 1999
	Sinopotamon yangtsekiense	Wang and Li 1999
Gecarcinucidae	*Spiralothelphusa hydrodroma*	Sudha Devi and Adiyodi 2007
	Thoracotremata	
Grapsidae	*Cyclograpsus integer*	Hartnoll 1968
	Eriocheir sinensis	Lee and Yamazaki 1990
	Neohelice (Chasmagnathus) granulata	López Greco et al. 1999
Ocypodoidea	*Ocypode quadrata*	López Greco et al. 2009
	Ocypode ceratophthalmus	Sudha Devi and Adiyodi 2008
	Ucides cordatus	Sant'Anna 2006
		Sant'Anna et al. 2007
	Uca spp.	Lautenschlager et al. 2010

2004 and 2005 by hard bottom dredge and beam trawl. Global positioning data of samples range from 54°08.419'N - 54°08.599'N to 07°50.921'E - 07°53.431'E, the depths from 50 to 55 m. *Pinnotheres pisum* and *Nepinnotheres pinnotheres* were collected in the Northern Adriatic Sea (Rovinj/Croatia) from different hosts in 2005 and 2007. Partly hand-collected by scuba- and skin-diving in depths ranging from 1 to 35 m, partly by beam trawl on trips with RV BURIN from the Institute Ruđer Bošković on different sample sites ranging from

45°02N– 45°07N to 13°36E – 13°40E. *Pinnotheres pectunculi* was collected from the dog cockle, *Glycymeris glycymeris*, around Roscoff (Brittany/France) in 2007 and 2008. Additional material of the species *P. pisum* was obtained from the commercially traded host *Mytilus edulis* bought in fish markets in Germany from 2007-2009.

For histology, 12 specimens were used. Tissue was fixed in 'Susa Heidenhain' (Romeis 1989) and embedded in paraffin. Histological sectioning was done with a microtome (Leitz 1515) at 8-10 µm. For general tissue differentiation, the 'trichromatic Masson-Goldner staining light green' was used (Romeis 1989).

For transmission electron microscopy (TEM), 16 specimens were used. TEM and tissue preparation were done at the Zoological Institute of the University of Heidelberg/Germany and in the EM-laboratory of Goethe-University in Frankfurt/Germany. Fresh tissue was fixed in 4% glutaraldehyde in cacodylate-buffer (pH 7.4) and washed with the same buffer. The tissue was postfixed with 1% osmium tetroxide for 2 hours. Cacodylate and maleate buffer (pH 5.2) washing steps were followed by en-bloc staining with 1% uranyl acetate overnight. After dehydration through a graded series of ethanol, the tissue was infiltrated and embedded in Spurr's, respectively Araldite resin. Semi-thin sections (1 –2 µm) were made with glass knives on an ultramicrotome (by Reichert-Jung) and stained with 'Richardson's blue' (after Richardson et al. 1960). Ultrathin sections (75 nm) were prepared with a diamond knife. Sections were collected on meshed copper grids and contrasted with aqueous lead citrate for 1 min. Electron micrographs were taken on a Zeiss EM10 transmission electron (University of Heidelberg). Photographs of semi-thin sections and paraffin sections were taken under light microscope Leica Diaplan with camera CamScan® (software ProgRes®).

RESULTS

Overview

The female reproductive system has a uniform morphology, histology, and ultrastructure in the investigated species *Pinnotheres pisum*, *Pinnotheres pectunculi*, and *Nepinnotheres pinnotheres*. Histological sections of the females' inner organisation were combined to construct a model of the female spermatheca shown in figure 4.2.

The spermatheca can be divided into two distinct areas by function and morphology. The dorsal part is considered as the main 'sperm storage area'. It is lined by an apocrine glandular epithelium (fig. 4.2). The ventral part of the spermatheca includes the adjacent junctions with the vagina and the oviduct, wherefore it is regarded as the 'fertilization area'. It is lined with

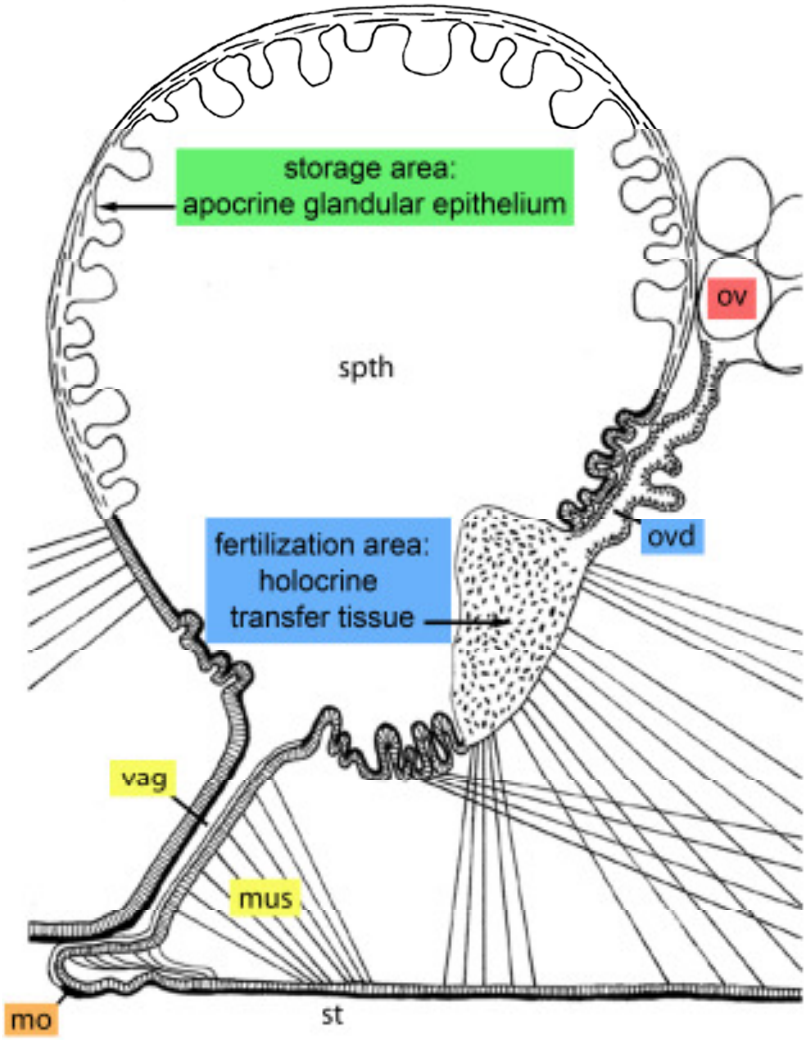


Figure 4.2. Model of the spermatheca of European pinnotherids (right spth). The ventral fertilization area and the vagina are lined with cuticle. The oviduct enters the spermatheca by a holocrine transfer tissue. The dorsal sperm storage area is lined by an apocrine glandular epithelium. mo = mobile operculum; mus = musculature; ov = ovary; ovd = oviduct; spth = spermatheca; st = sternum; vag = vagina.

cuticle, except for the part, where the oviduct enters the spermatheca by the 'holocrine transfer tissue' (fig. 4.2). The ventral part of the spermathecal wall, including vagina and mobile operculum, conform to the integument of crabs, being lined with cuticle overlying a columnar epithelium. The spermathecal wall is ventrally strongly folded (fig. 4.2). The spermatheca is externally coated by connective tissue. Muscle bundles are attached to it externally running in several directions (fig. 4.2). All the investigated females, except one juvenile hard stage before metamorphosis, had filled spermathecae. The sperm mass inside the spermatheca was homogenous, without distinct layers, and spermatozoa were found to be free rather than enclosed in spermatophores.

Ovary

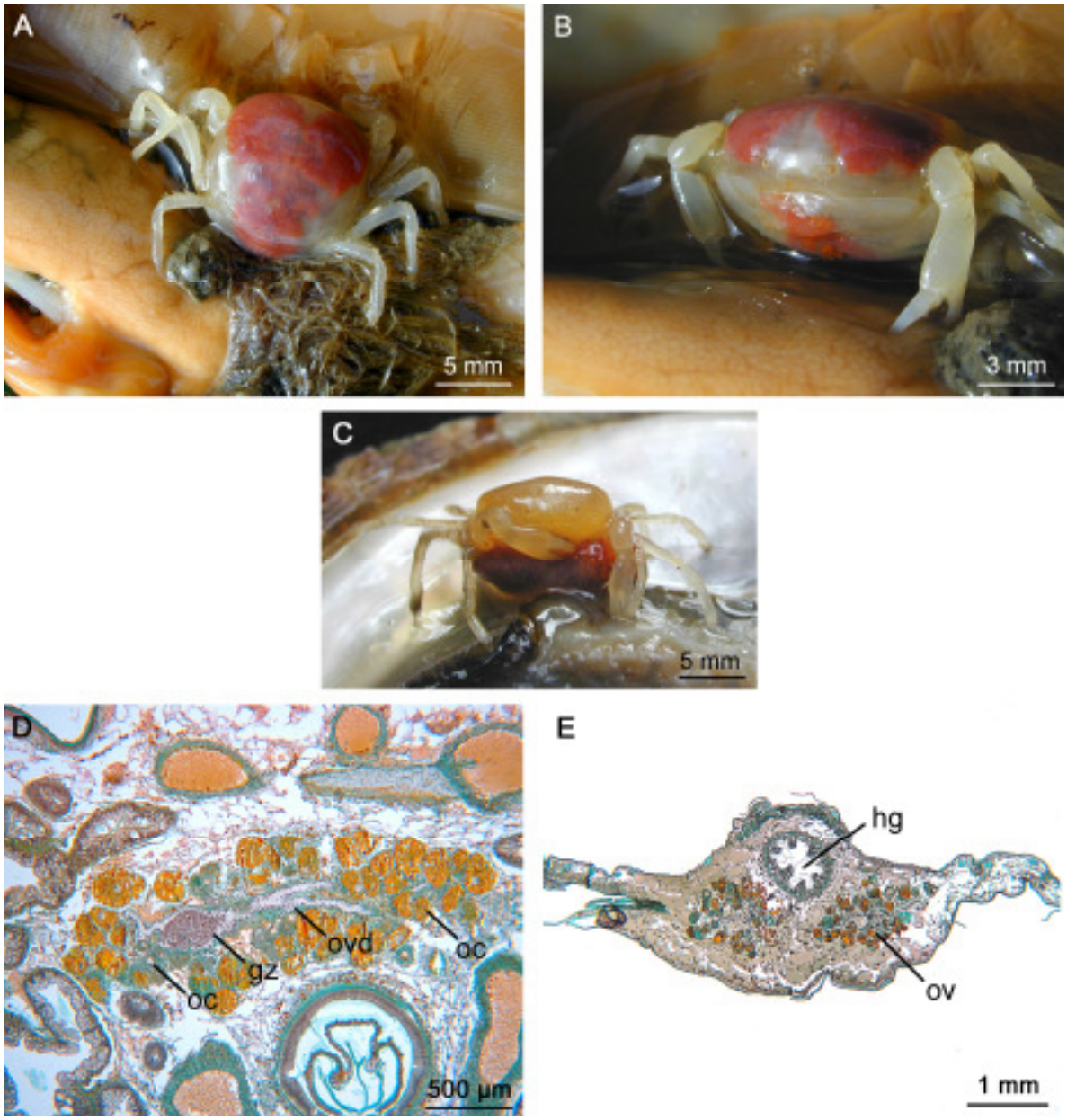


Figure 4.3. Ovary –general morphology. (**A**) Dorsal view on female *Pinnotheres pisum* in the horse mussel, *Modiolus modiolus*. The carapace is decalcified and translucent whereby the red ovary shines through. (**B**) The ovary extends into the broadened pleon that overlaps the mouthparts in adult females. (**C**) Ovigerous female of *P. pisum* in a European oyster, *Ostrea edulis*. (**D**) Section through cephalothorax showing a subunit of the ovary. (**E**) Section through pleon with the two posterior ovary ropes running along both sides of the hindgut. gz = germinative zone; hg = hindgut; oc = oocytes; ov = ovary; ovd = oviduct.

The ovaries are very expanded internally, and even extend into the broad pleon (fig. 3A, 3B, 3E). The clutch-size is accordingly great (fig. 4.3C). The general form of the ovary is H-or X-shaped, being organized into two subunits connected by a central bridge in the region of the heart. The subunits of each body half run left and right alongside the gut (fig. 4.3D). Each side comprises an anterior and posterior strand. The anterior strands run to the front, are strongly coiled laterally and posteriorly. The two posterior strands are connected to the spermathecae

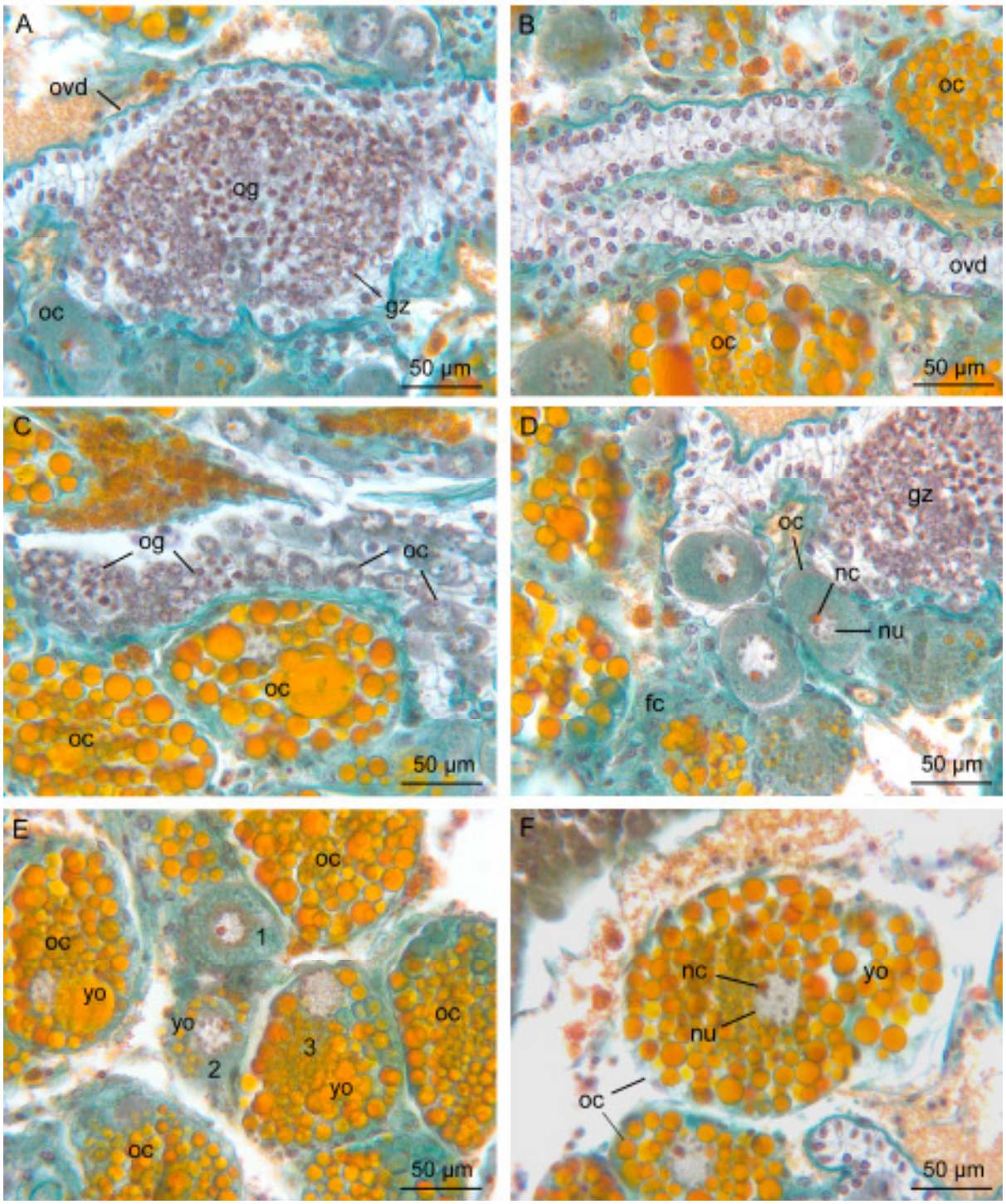


Figure 4.4. Ovary – proliferation and yolk accumulation. (**A**) Germinative zone of the ovary where cell division takes place and oogonia are produced. (**B**) The proximal oviduct is a two layered epithelium outlined by connective tissue. (**C**) Section shows oogonia developing into oocytes in the periphery of the germinative zone. (**D**) The oogonia increase in size while they are transported to the ovarian lobes. (**E**) Different stages of vitellogenesis with the ongoing accumulation of yolk in three contiguous oocytes (1, 2, 3). (**F**) Full-grown mature oocytes densely filled with yolk droplets. gz = germinative zone; nc = nucleolus; nu = nucleus; oc = oocyte; og = oogonia; ov = ovary; ovd = oviduct; yo = yolk.

and extend into the pleon (fig. 4.3B, E). The coiled ovary strands nearly fill the cephalothorax. This remarkable extension is obvious in living specimens, because of the transparency of the carapace (fig. 4.3A, B). The histological sections of the ovary and its

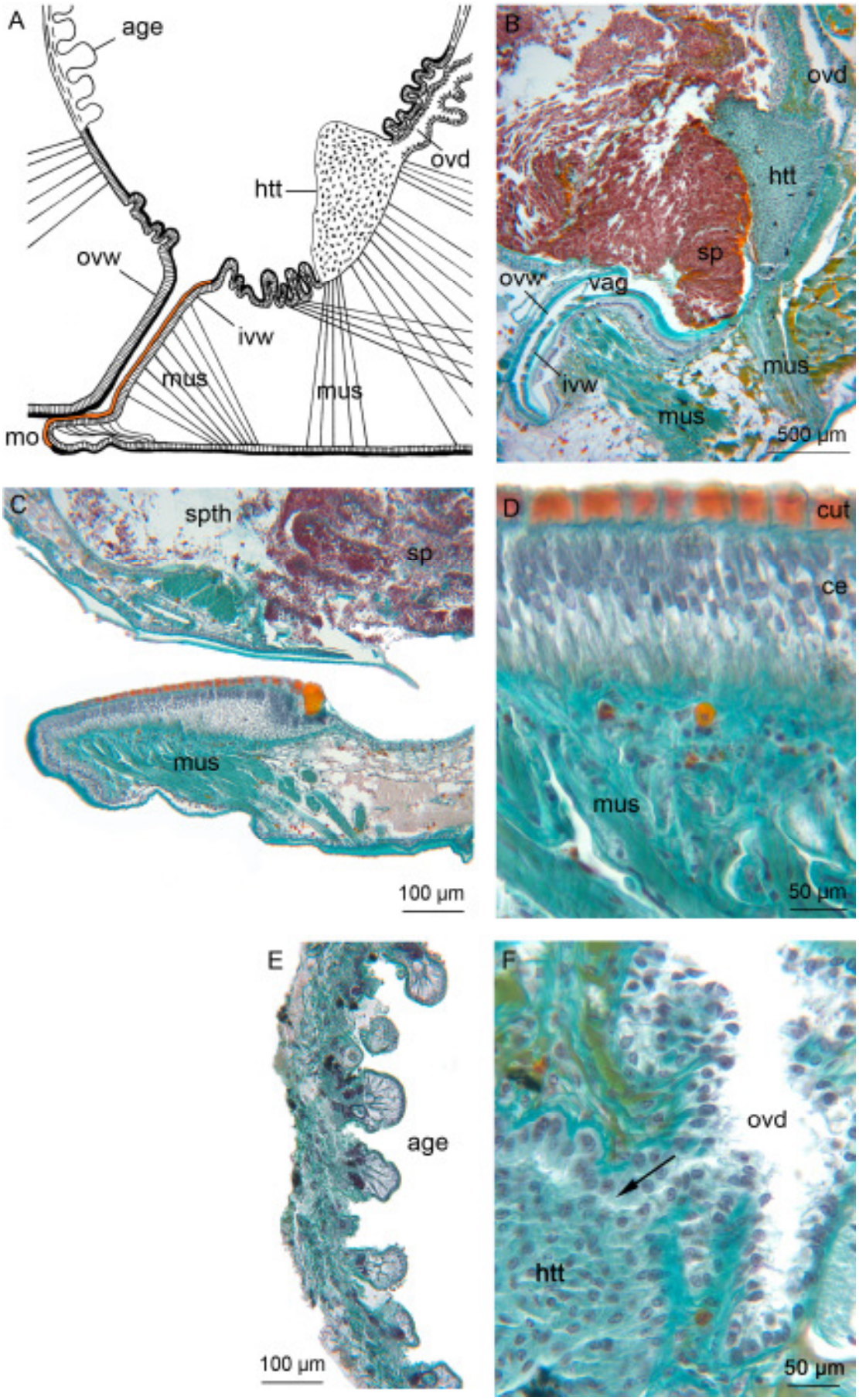
corresponding oviduct show that they are surrounded by a thin layer of connective tissue that stains turquoise (fig. 4.4A, B). Ovary and oviduct are structurally not separable. The ovarian lobes are continuous with the oviduct. The proximal oviduct holds the germinative zones and transports the oocytes to the lobes in the periphery of the ovary where they are stored and mature until ovulation. This part is conventionally termed 'ovary', while only the distal part that runs to the spermatheca is designated as 'oviduct'.

Figure 4.4A-F shows different stages of proliferation. In the germinal zone, cell division takes place and the oogonia develop into previtellogenic oocytes (fig. 4.4A, C). The oocytes are ripening during their transport from the central germinal zone to the ovaries' periphery. They grow with the increasing distance from their origin in the germinal zone (fig. 4.4C, D). Figure 4.4D shows the full-grown previtellogenic oocytes. The ongoing accumulation of yolk is shown in figure 4.4E. Mature oocytes are densely filled with orange staining yolk droplets (fig. 4.4F). All stages of oocyte maturation were found in the ovaries of adult females. Mature oocytes were also present in ovigerous, freshly spawned females.

Spermatheca

Vagina (fig. 4.5A, B). The vagina is a short duct lined with cuticle overlying a columnar epithelium (fig. 4.5A). It follows the 'concave pattern' (sensu Hartnoll 1968). Transverse sections through the vagina appear crescent-shaped, because its lumen is narrowed by one side of the wall being collapsed into the other (fig. 4.5B). The collapsed part of the wall is flexible by musculature attached longitudinally along it, running to the sternum (fig. 4.5A, B). This flexible part is termed the 'inner vagina-wall', the non-flexible, rigid part the 'outer vagina-wall' (sensu Diesel 1989). With a contraction of the muscle strands attached alongside the inner vagina-wall, the lumen of the vagina extends to an open passage leading into the spermatheca.

Figure 4.5 (page 65). Ventral fertilization area of spermatheca. (**A**) Model of the fertilization area: the ventral part of the spermatheca including the connection with the oviduct and the vagina enclosed by a mobile operculum. The vagina is built by an outer vagina-wall and an inner vagina-wall which is collapsed into the outer. Musculature is attached to the inner vagina-wall. The flexible cuticle of the inner vagina-wall and of the mobile operculum is colored red. (**B**) Histological section of fertilization area. The vagina appears crescent shaped in histological transverse sections. A contraction of the musculature opens its lumen. (**C**) Section through the muscular mobile operculum that covers the entrance into the vagina. The flexible cuticle parts stain different (deep orange) from the nonflexible parts (turqoise). (**D**) Closer view on the flexible cuticle underlined by a columnar epithelium the muscle strands run to. (**E**) Histological section through spermathecal wall lined with apocrine epthelium. (**F**) The distal oviduct connects the spermatheca by the holocrine transfer tissue. Arrow on the oviduct leading into the holocrine transfer tissue. age = apocrine glandular epithelium; ce = columnar epithelium; cut = cuticle; htt = holocrine transfer tissue; ivw = inner vagina wall; mo = mobile operculum; mus = musculature; ovw = outer vagina wall; ovd = oviduct; sp = sperm mass; spth = spermatheca; vag = vagina.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While the cuticle of the outer vagina-wall and the whole integument stains turquoise in paraffin sections, the cuticle that lines the flexible inner vagina-wall stains deep orange to light red (fig. 4.5A).

Mobile operculum (fig. 4.5C, D). Rigid sternal projections overlap mobile opercula that cover the paired genital openings (fig. 4.5A C). The term 'mobile operculum' was defined by Hartnoll (1968) as an operculum that is flexible by musculature. Just like in the vagina, the cuticle of the non-flexible parts (sternal projections) stain turquoise while the cuticle of the flexible parts in the mobile opercula attached to musculature stains bright red (fig. 4.5C, D).

Oviduct and holocrine transfer tissue (fig. 4.5F, 4.6, 4.7). The oviduct is a two layered epithelium of columnar cells with oval nuclei surrounded by a thin layer of connective tissue (fig. 4.4B). In the proximal oviduct, the two cell layers rest onto each other (fig. 4.4B). Only the distal part of oviduct, close to its connection with the spermatheca, shows a lumen formed between either epithelia (fig. 4.5B, F, 4.6A).

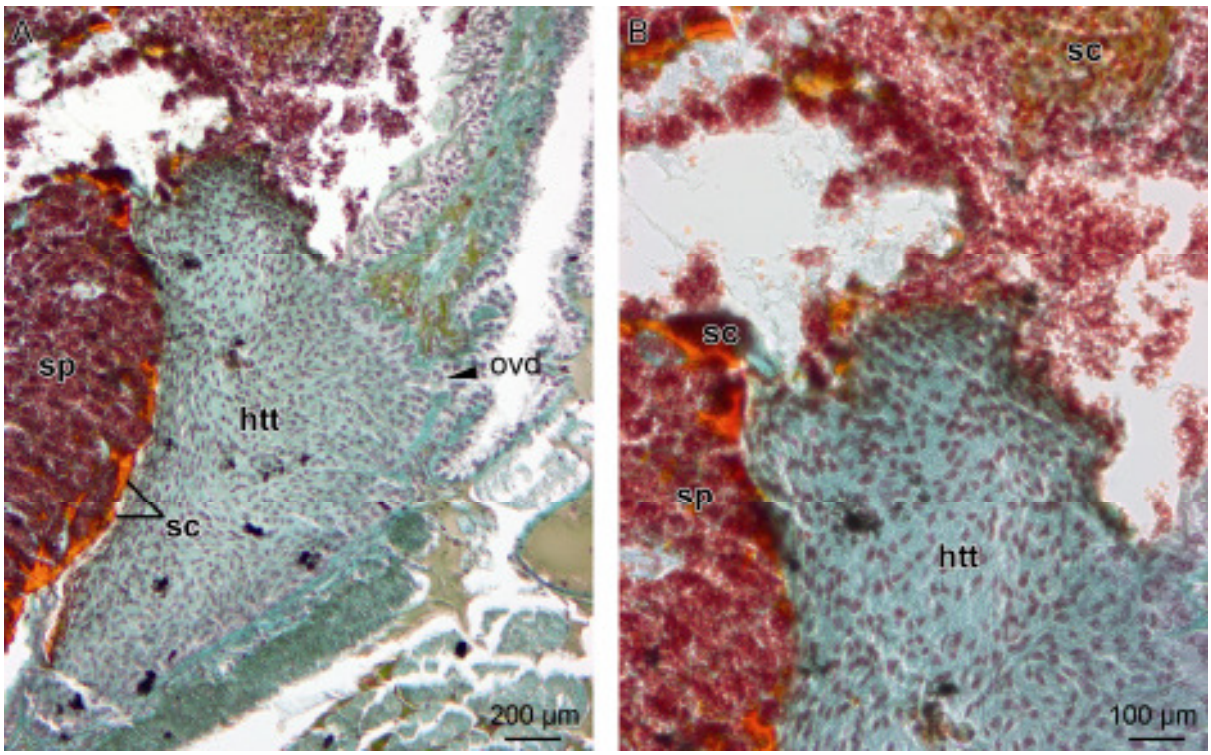

Figure 4.6. Histology of the secretory transfer tissue connecting the oviduct to the spermatheca. (**A**) An orange staining secretion is often found close to the periphery of the holocrine transfer tissue. The sperm mass is densely packed in this region. (**B**) Closer view on the holocrine transfer tissue with arrow on secretion. htt = holocrine transfer tissue; ovd = oviduct; sc = secretion; sp = sperm mass.

At its junction with the spermatheca, the oviduct opens into a special tissue (fig. 4.5B, F, 4.6A), which consists of densely packed cells with oval nuclei (fig. 4.5F, 4.6, 4.7). There is no open transition passing through this tissue into the lumen of the spermatheca. Where the

special tissue expands into the spermathecal lumen, a homogenous substance is always present that stains orange in histological sections (fig. 4.6). The histological sections in fig. 4.6 and the semi-thin section in fig. 4.7A reveal how the substance is secreted. The outer cells transform into secretion and are sloughed off into the lumen of the spermatheca. This secretory mechanism is called holocrine because whole cells are dissolved into secretions, hence we name the tissue connecting oviduct and spermatheca the 'holocrine transfer tissue'. The ultrastructure in fig. 4.7B shows the decondensed nuclei in the tissue which is characteristic of secretory and therefore highly active cells.

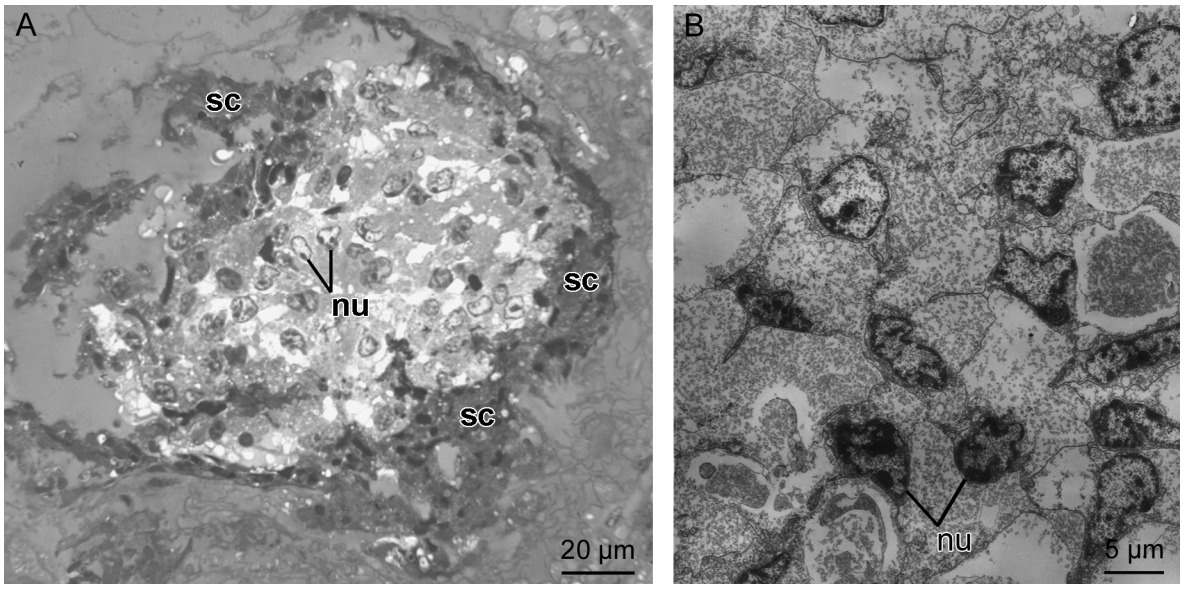

Figure 4.7. Holocrine mode of secretion in transfer tissue. (**A**) Semi-thin section. Outer cells are dissolving into secretion. (**B**) Ultrastructure of the holocrine transfer tissue with nuclei. sc = secretion; nu = nucleus.

Apocrine glandular epithelium (fig. 4.5E, 4.8, 4.9). The dorsal part of the spermatheca, considered as a storage area, is not cuticularized, but lined with a one-layered glandular epithelium, underlaid by layers of connective tissue (fig. 4.5E, 4.8A, B). The glandular cells are large, 150 µm and more. The nucleus is located in the cellular base; it is strongly lobed and condensed (fig. 4.8C). Owing to the strong folding of the nucleus, it can appear, that several nuclei are present in one cell (fig. 4.8B, 4.8C), but by the identification of the nucleolus in a series of sections, we ascertained that we have just sectioned different parts of one nucleus. The cell boundaries interdigitate basally (fig. 4.8D); the cell body elongates freely into the lumen of the spermatheca (fig. 4.8B, 4.9A). Large fields of rough endoplasmatic reticulum are present in the bases of glandular cells (fig. 4.9A). The free cellular surface forms microvilli (fig. 4.9B). The central part of the cell body is densely packed with mitochondria (fig. 4.9D - F), which indicates the high oxygen consumption in the glandular cells, necessary for its high secretory activity. Secretory vesicles are also present in

the cell body, accumulating distally and fusing to large bodies of secretion (fig. 4.9C, F). The secretions are released into the spermathecal lumen by dissolving the apical part of the cell, wherefore the mechanism is called apocrine (fig. 4.9F). The basal part of the cell, where secretions are produced, remains intact.

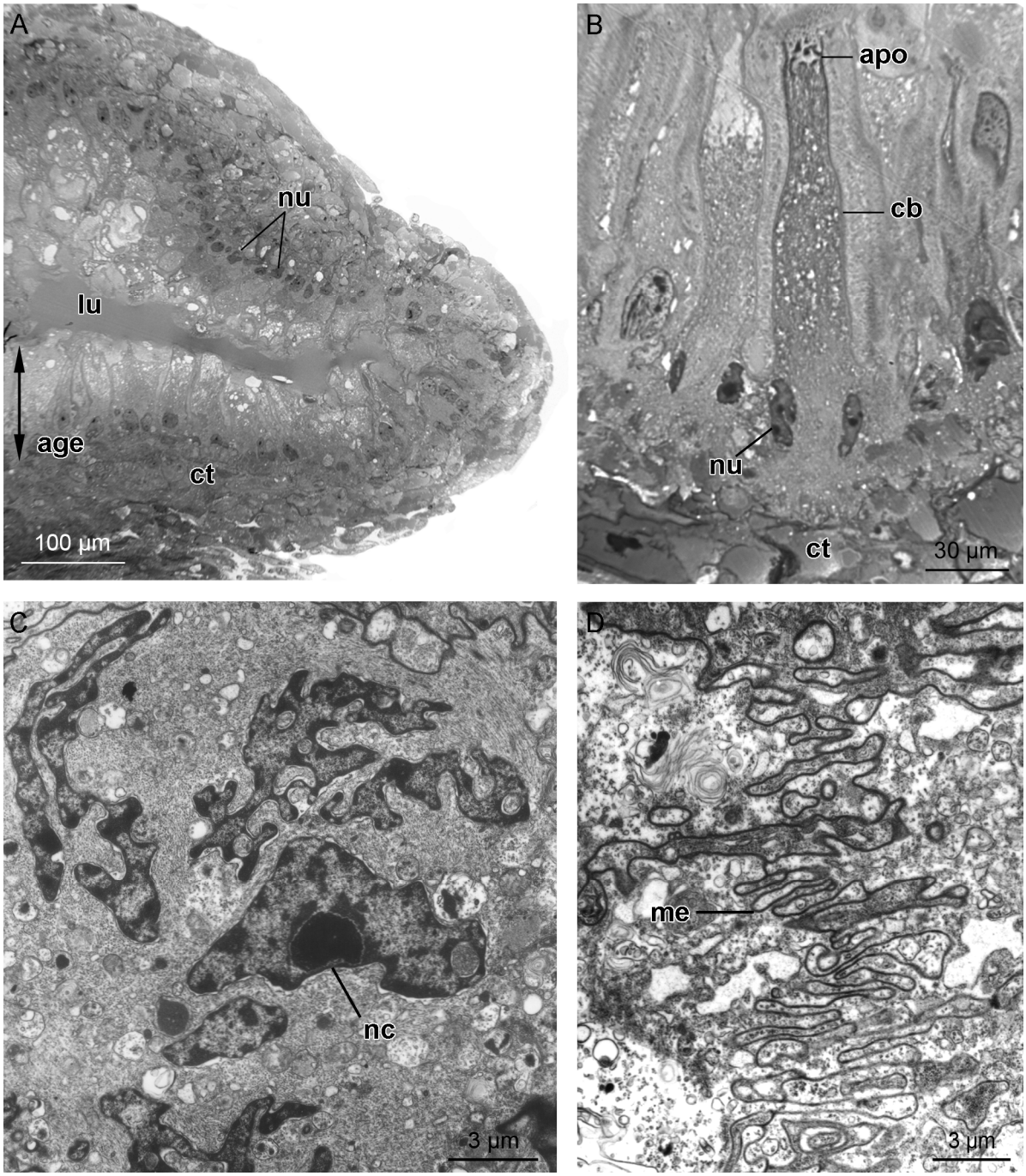

Figure 4.8. Spermatheca - dorsal sperm storage area lined with apocrine glandular epithelium (Histology, TEM). (**A**) Semi-thin section through dorsal spermatheca. The spermatheca is embedded in connective tissue; internally it is lined by an apocrine glandular epithelium. The cells project into the spermathecal lumen. (**B**) Single glandular cell with nucleus located basally. While the nucleus remains during the secretion process, the apical part of the cell releases secretion. (**C**) The nucleus is strongly lobed and condensed. (**D**) Membranes of basal cell boundaries are interdigitating. age = apocrine glandular epithelium; apo = apocrine secretion; cb = cell body; ct = connective tissue; lu = lumen of spermatheca; me = membranes; nc = nucleolus; nu = nucleus.

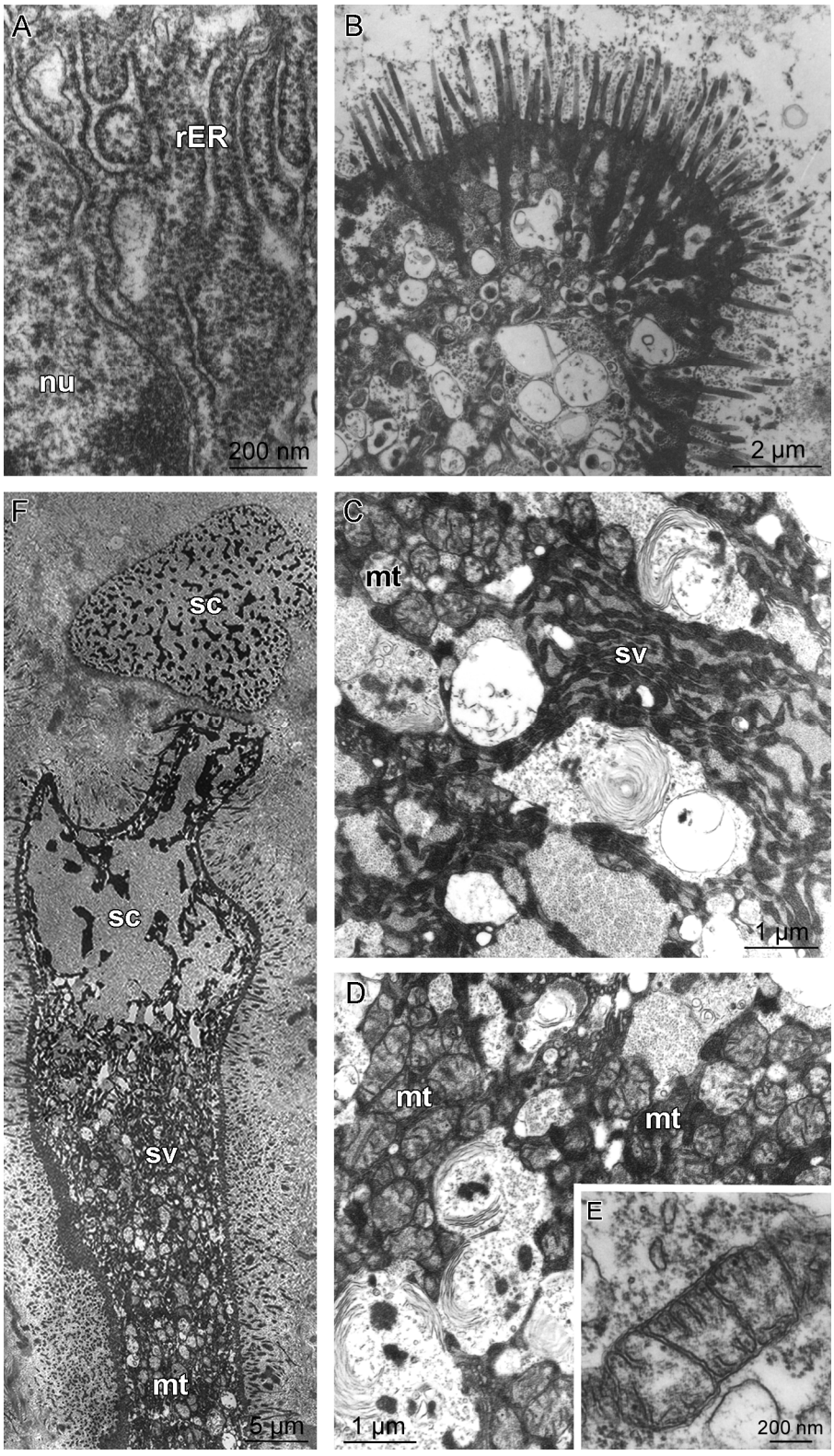

Figure 4.9. Ultrastructure of the apocrine glandular epithelium. (**A**) Regions with rER are found in the cellular base. (**B**) The cell membrane forms microvilli. (**C**) Secretory vesicle in the cell body. (**D**) Mitochondria are accumulated in the body of the cell. (**E**) Inset: closer look on single mitochondrium. (**F**) Apocrine breaking of cell: organelles are dissolved in the distal part where secretion is released. mt = mitochondrion; mv = microvilli; nu = nucleus; rER = rough endoplasmatic reticulum; sc = secretion; sv = secretory vesicle.

DISCUSSION

Overview

The spermatheca of the investigated pinnotherids conforms to most other eubrachyurans in its ventral part being lined with cuticle, and the dorsal part with glandular epithelium.

The question of the probable origin of these epithelia is relevant to understanding how internal fertilization has evolved in Eubrachyura. From the histological structure of the spermatheca, Bauer (1986) and Krol et al. (1992) reason that the ventral cuticular part of the spermatheca, including the vagina, is a sternal integumental invagination, while the dorsal glandular part is formed by the oviduct.

The location where the oviduct opens into the spermatheca can vary among brachyuran species. In the swimming crab *Callinectes sapidus*, the oviduct enters dorsally (Johnson 1980), while in the spider crabs *Inachus phalangium* (Diesel 1989) and *Chionoecetes opilio* (Beninger et al. 1988) it enters ventrally - similar to the studied pinnotherids.

I. phalangium (Diesel 1989), *C. opilio* (Beninger et al. 1988) and the swimming crab *Portunus trituberculatus* (Xuan et al. 2009) are examples of crabs that show a spatial division of the spermatheca into two chambers. A muscular diaphragm separates the 'dorsal sperm storage chamber' and the 'ventral insemination chamber' (sensu Diesel 1989).

The spermatheca of pinnotherids has a functional division indicated by the different nature of the spermathecal wall. Dorsally, the 'sperm storage area' is lined with glandular epithelium, whilst ventrally the predominantly integumental 'fertilization area' includes the connections with vagina and oviduct.

Different stages of oocyte maturation, present in the ovaries of adult females, show the simultaneous gestation of several generations without seasonal changes in the ovary. This is rather unusual compared to other crabs wherein ovarian maturation is often synchronized with the season or other periodic cycles (e.g. Keunecke et al. 2009, Minagawa et al. 1993, Peres de Souza and Feitosa Silva 2009, Weitzman 1966). Nevertheless, ovigerous females of the investigated pinnotherid species are not recorded throughout the whole year, but mainly in summer (Haines et al. 1994; pers. obs.), whereas several broods can be produced by the female (Hines 1992, Hartnoll 2006).

Vagina, Mobile Operculum, and Mating Behaviour

The vagina of the 'concave type', as found in pinnotherids, is present in various other crabs, e.g. *Hyas araneus*, *Pachygrapsus marmoratus*, and *Cyclograpsus integer* (Hartnoll 1968).

The histology and function of a concave vagina was shown by Diesel (1989) for *I. phalangium*. In contrast to the concave vagina, the vagina of the 'simple pattern' is a rigid rounded tube surrounded by longitudinal musculature (Hartnoll 1968). The 'simple' vagina occurs in *Carcinus maenas* (Spalding 1942), *Callinectes sapidus* (Johnson 1980), *Metacarcinus magister* (Jensen et al. 1996), and many other species.

In the primitive Brachyura, the Podotremata Guinot, 1977, vaginas are always of the simple pattern, in Heterotremata, both patterns are represented and in the most advanced Brachyura, the Thoracotremata, e.g. Ocypodidae, Grapsidae and Pinnotheridae, vaginas are always of the concave pattern (Hartnoll 1968). Based on this arrangement of types of vagina distributed in the brachyuran groups, it is most likely that the simple type is the primitive form, while the concave type should be the secondarily derived form, which might have evolved from the simple type once or several times (Hartnoll 1968).

In the studied pinnotherids, we found a 'mobile operculum' (sensu Hartnoll 1968) occluding the vagina. It is controlled by muscular activity hence it allows copulation in intermoult. In contrast to this, the genital openings of Brachyura can also be calcified. These are termed 'immobile opercula' (sensu Hartnoll 1968). They were supposed to be only passable in the soft-shelled form directly after moult (Hartnoll 1968). But for most species with calcified opercula, it turned out that it can also become mobile temporarily in intermoult due to local decalcification (Hartnoll 1969). This occurs in the ocypodids *Macrophthalmus hirtipes* (Jennings et al. 2000) and *Ilyoplax pusilla* (Henmi and Murai 1999).

The soft-shelled mating of crabs with an immobile operculum often involves foreplay, the pre-copulatory embrace: the male guards a pre-moult female close to ecdysis to assure he is the first the female mates with, as soon as the moult occurs (Hartnoll 1969). A post-copulatory courtship often follows mating to protect the female and the offspring from predators until the female has hardened again. The post-copulatory courtship can play an important role in male-male competition by preventing competitors from subsequent inseminations. *Cancer pagurus* is an example of a crab with a long courtship, having both, the pre- and post-copulatory embrace (Edwards 1966).

In pinnotherids, mating behaviour and courtship have not been observed so far. And even though we collected numerous pairs from hosts, we never saw pea crabs during copulation. Thus, we draw conclusions based on the morphology of vagina and mobile operculum. Both indicate an active role of the female during copulation, since the penetration by male copulatory organs is controlled by muscular activity of the female. Initially the mobile operculum has to uncover the genital openings. Then the musculature attached along the

vagina enlarges the normally constricted lumen to form a free passage for insemination. Due to this exclusively muscular control of the female ducts in pinnotherids, mating could presumably take place in the hard-shelled form, independent of moult-cycles.

It is not known whether mating in pinnotherids takes place in- or outside the host, nor whether it happens more than once in a female's lifetime. However, the first copulation clearly occurs in the juvenile hard stage female before the onset of metamorphosis, since all subsequent stages already have sperm-filled spermathecae (Atkins 1926; pers. obs.). So, if females with empty spermathecae do not undergo metamorphosis, mating seems to be the trigger for its initiation. This is reasonable, since it would pose a risk for the reproductive success of a female to pass through metamorphosis and settle in its final host without being inseminated. Our observation on the field (chap. 2) has shown that the density of pinnotherids in relation to their hosts, can be very patchy in some habitats. Consequently, it is hard to explain how the two sexes manage to find each other. However, as long as the female is in the hard stage before metamorphosis and still able to swim (Hartnoll 1972), it can seek other habitats and switch to hosts more frequented by males.

To find male and female together in couples in the host and the assumption that insemination triggers metamorphosis (and therewith the onset of the female's parasitic phase of life) suggest that copulation takes place inside the host.

The question of whether adult females copulate again after their precocious mating, arises, among other things, due to the sexual dimorphism in the adults. The female is, in the majority of cases, considerably bigger than the male (pers. obs.), hence copulation between the adults might seem implausible. The main reason for expecting that the highly fecund female copulates just once in the juvenile hard stage, is the observation that pinnotherids retain sperm inside the spermatheca over several moults anyway.

Sperm Retention

Moulting is antagonistic to sperm storage in female crabs with completely cuticular spermathecae. This is the case in the primitive Podotremata: with every moult, the spermathecal content is completely shed (Hartnoll 1975).

Trans-moult sperm retention, however, appears in Eubrachyura such as *Metacarcinus magister* (Shirley and McNutt 1989) and *Menippe mercenaria* (Cheung 1968), whose spermathecae are cuticular only in the ventral part.

The process of trans-moult sperm retention is not yet resolved. At least, sperm retention does not only depend on the nature of the spermathecal wall. In crabs with non-integumental

spermathecae, the content is still shed with the moult in *Rhithropanopeus harrisii* (Morgan et al. 1983) and *Neohelice granulata* (Lopéz Greco et al. 1999). However, in *M. mercenaria*, females fertilized more than ten broods without subsequent mating after their last moult (Cheung 1968).

In pinnotherids, the sperm has to be stored over several moults, because of their precocious mating. Ovigerous females are not found until the metamorphosis is completed which implies that the sperm is retained over at least four moults from stage I to V (Atkins 1926).

Spermathecal Secretion

Holocrine secretion is characterised by transforming whole cells into secretion (Ude and Koch 1994). Holocrine glandular epithelia are common in brachyuran spermathecae. They were described for the cancrids *Metacarcinus magister* (Jensen et al. 1996) and *Cancer pagurus* (George 2004); for portunids *Portunus sanguinolentus* (Ryan 1967b), *Callinectes sapidus* (Johnson 1980) and *Portunus pelagicus* (Bawab and El-Sherief 1989) and for the majids *Inachus phalangium* (Diesel 1989) and *Chionoecetes opilio* (Beninger et al. 1993). In the above, the holocrine glandular epithelia line the dorsal part of the spermatheca while the ventral part is cuticular underlined by a columnar epithelium.

The histology of the 'holocrine gland cells' in the spermathecae of *I. phalangium* (Diesel 1989) and the 'stratified epithelium' in *Carcinus maenas* (Hartnoll 1968) conforms with the histology and function of the holocrine transfer tissue observed in this study, but the glandular epithelia of the examples named above are always lining the dorsal part of the spermatheca. In pinnotherids however, the epithelial tissue is located where the oviduct connects to the spermatheca. It extends into the lumen of the spermatheca wherein the secretory cells are sloughed off. The histological sections clearly demonstrate that the oviduct leads into the holocrine transfer tissue (see 5A, 6A). Lautenschlager et al. (2010) found an identical tissue connecting oviduct and spermatheca in ocypodids of the genus *Uca*. They also observed ducts leading through that tissue. Moreover, Lee and Yamazaki (1990) already described the same structure for the Chinese mitten crab *Eriocheir sinensis*, as 'valve-like tissue', location and histology were identical to our results. Lee and Yamazaki studied changes in this tissue during the reproductive cycle and they observed the transfer of oocytes through it. The aim of their study was to clarify the actual site of fertilization. They interpreted the role of the valve-like tissue as a barrier to prevent sperm inside the spermatheca from entering oviduct and ovaries. They also observed the degeneration of cells in the periphery of the valve-like tissue facing the lumen of the spermatheca, but they did not discuss its secretory function, nor did

they mention the presence of secretions inside the spermatheca. The degeneration of cells and their regeneration is a regular process in epithelia, but the high activity of the tissue observed here and by Lee and Yamazaki (1990) clearly indicates a process of secretion.

In contrast to the widely distributed holocrine epithelia in brachyuran spermathecae, similar structures like the apocrine glandular epithelium, lining the dorsal sperm storage area in pinnotherids, have only recently been described for ocypodids: *Ucides cordatus* (Sant'Anna 2006, Sant'Anna et al. 2007), *Ocypode quadrata* (López-Greco et al. 2009) and *Uca* spp. (Lautenschlager et al. 2010). The glandular epithelium described by Lautenschlager et al. (2010) is consistent with our findings of interdigitating cell boundaries, the shape of nuclei, the cellular surface forming microvilli, and the high secretory activity of the cells shown by their high density of mitochondria and secretory vesicles.

Lautenschlager et al. (2010) describe two different types of glandular epithelia: a multi-layered one in *U. ecuadoriensis* and a mono-layered form in *U.* c.f. *forcipata*. *Uca tangeri* was recorded with both types. So, there might be divers differentiations of apocrine glandular epithelia among different crab-species. But since the detailed mode of function and the respond to periodic changes are not yet entirely understood, it is also possible that Lautenschlager et al. (2010) recorded different modes of activity or stages of the reproductive cycle in the same apocrine glandular epithelium.

While the species *U. ecuadoriensis* and *U.* c.f. *forcipata* only have a small part of the spermatheca lined with glandular epithelium, *U. tangeri* has a larger portion lined with glandular epithelia (Lautenschlager et al. 2010). The apocrine epithelium we observed in pinnotherids lines the whole dorsal area and, thus, a much larger portion of the spermatheca.

López-Greco et al. (2009) investigated the spermatheca of *Ocypode quadrata* and illustrate sections of a 'mesodermic secretor epithelium', which seems to be similar to the findings for pinnotherids in the present study and for ocypodids (Lautenschlager et al. 2010). However, the main focus of the study of López-Greco et al. (2009) is not on the secretion of the spermatheca. Again, no data on ultrastructure are presented and the magnification of the histological figures does not allow detailed comparison.

In the spermatheca of the ocypodid *Ucides cordatus* (Sant'Anna 2006, Sant'Anna et al. 2007) a highly secretory epithelium was recorded as well and the histochemistry of the secretions was investigated (see below).

Sudha Devi and Adiyodi (2007) found an apocrine epithelium in the spermatheca of the gecarcinucid *Spiralothelphusa hydrodroma*. Its fine structure is different from the apocrine epithelium described in the present study in consisting of multinucleolated cells. The

observed spermathecal secretory activity reached its peak close to breeding season (Sudha Devi and Adiyodi 2007). Again, a study on secretion inside the spermathecae of the freshwater crab *Sinopotamon yangtsekiense* defined an epithelium with 'topcrine gland cells' (Wang and Li 1999). Unfortunately, like similar interesting research on brachyuran spermathecae (e.g. Cheng et al. 2000, Xuan et al. 2009), it is not published in English and the quality of the figures does not allow for detailed histological comparison.

More work has to be conducted on brachyuran spermatheca to clarify whether the apocrine glandular epithelium is characteristic only for pinnotherids and ocypodids or for thoracotremes in general, or if it occurs independent of taxonomic affinity. Furthermore, the location and extension of the apocrine glandular epithelia among different species is of great interest, along with their ultrastructural differentiation (e.g. mono- and multi-layered types, postulated by Lautenschlager et al. 2010).

Function of Secretion

Two different modes of secretion are present in the pinnotherid spermatheca, from the holocrine transfer tissue and the apocrine glandular epithelium. The role of the spermathecal secretion in sperm storage and fertilization is still under debate and several functions are discussed in the literature for the holocrine epithelia, which are generally located dorsally in brachyuran spermathecae.

Even though several bio- and histochemical studies have been carried out (Beninger et al. 1993, Anilkumar et al. 1996, Lanteigne et al. 1996, Wang and Li 1999, Sant'Anna et al. 2007), the function of secretion is not fully explored.

Bawab and El-Sherief (1989) consider the secretion in context with the formation of a sperm plug enclosing the female genital ducts after copulation. Spalding (1942) stated that secretions might conversely be involved in the dissolution of sperm plugs. Both can certainly be excluded for the investigated pinnotherids, since sperm plugs were never present inside vaginae or spermathecae. Spermathecal secretions may also function in the dehiscence of spermatophores (Adiyodi and Anilkumar 1988, Diesel 1989).

Sant'Anna et al. (2007) proposed that secretion may promote the movement of gametes to the right location for fertilization in the ocypodid *Ucides cordatus*. Moreover, they found the secretions to form a glycoprotein matrix that is supposed to be supportive to the spermatophores, which are stored over long periods in *U. cordatus*.

The secretion could also defend against harmful agents or bacteria (Jensen et al. 1996) or provide a milieu for the maintenance of bacterial populations that are protective against other

harmful microbes (Beninger et al. 1993). Benhalima and Moriyasu (2001) disagree with the latter since they found an increase in bacteria colonies inside spermathecae of old and barren females, which have weak anti-microbial protection. Moreover, they stated that these bacteria actually may harm spermatozoa.

Anilkumar et al. (1996) conducted biochemical assays of the spermathecal content and observed spermatozoal oxygen uptake, which supports their hypothesis of an aerobic metabolism of spermatozoa inside the spermatheca in the crab, *Metapograpsus*. They characterized the lipids and proteins inside the spermatheca and hypothesize that these are the substrates the spermatozoa metabolize. Secretion my also directly function in the trans-moult sperm retention.

The probable functions of the spermathecal secretion are very diverse and sometimes contradictory. Studies on the function of secretion are difficult since the biochemical situation in the spermatheca has to be observed according to histological and cellular processes throughout the whole reproductive cycle. That female secretion mixes with male secretion, such as seminal fluids, and probably with sea water, further complicates the interpretation.

The actual function might be more complex than involving just one of the postulated processes. In fact, we agree with Johnson (1980) who assumes that the secretions must be in some way important for the 'maintenance of sperm', but probably also for processes in ovulation, fertilization, and spawning.

Conclusions

The pinnotherid apocrine mode of secretion conducted by vast areas of the spermathecal wall lined with glandular epithelium does not necessarily imply a function totally different from the holocrine epithelia, which are already known for many brachyurans. We suppose that the efficiency of secretion by the apocrine glandular epithelium must be very high compared to holocrine epithelia. This is also supported by the huge amounts of secretions inside the investigated spermathecae. A comparable specialization of spermathecal secretion has only been found in *Uca tangeri* by Lautenschlager et al. (2010) but was not discussed in terms of function and reproductive strategies.

Sperm storage over a long period of time is presumably an important issue for the investigated pinnotherids. Due to their precocious mating they have to store sperm over several moults anyway. Moreover, it is not known whether pinnotherids copulate repeatedly instead of inseminating all broods with the sperm that they have obtained from their first mating. The latter is definitely conceivable considering the fact that the chances of meeting

males to mate with are limited due to the parasitic mode of life. Pinnotherids can be regarded as true specialists in reproduction, which is also known for other parasites. The exploitation of the host as a food source and for protection is at the cost of mobility and the ability of meeting many potential partners to mate with. Thus, the resulting reproductive output of parasites is always limited by the need of finding an appropriate host.

Accordingly, the reproductive investment of pinnotherids is considerably higher than in free-living brachyurans (Hartnoll 2006). Hines' (1992) comparative study on the reproductive output in Brachyura has shown that the ovary mass of pinnotherids is about 70-90 % of the females' body weight, compared to an average of 10 % in other crab species. Only further comparative studies on the morphology of thoracotreme reproductive systems will reveal if the pinnotherids' outstanding spermathecal secretion has to be regarded in context of long term sperm storage and of the outstanding reproductive output, which are considered necessary due to the pinnotherids' parasitic mode of life.

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The Male Reproductive System of European Pinnotheridae (Crustacea, Decapoda, Brachyura)

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ABSTRACT

The male reproductive morphology of the European pinnotherid species *Pinnotheres pisum*, *Pinnotheres pectunculi* and *Nepinnotheres pinnotheres* was investigated by histological methods, scanning and transmission electron microscopy, and confocal laser scanning microscopy.

The male internal reproductive structures consist of paired testes and corresponding vasa deferentia. The sperm morphology conforms in the main to the thoracotreme type but specific differences are present between *Nepinnotheres pinnotheres* and *Pinnotheres pisum*. Spermatozoa become enveloped into spermatophores in the secretory proximal vas deferens. The medial vas deferens is strongly enlarged and stores spermatophores that are embedded in a matrix of seminal plasma. At the distal part of the vas deferens tubular appendices extend into the ventral cephalothorax but also slightly into the pleon, which is exceptional among the Brachyura. These expansion produce and store vast quantities of seminal plasma.

The brachyuran copulatory system is formed by paired penes and two pairs of abdominal legs, the gonopods, which function in sperm transfer. In pinnotherids, the long first gonopods transfer the sperm mass into the female ducts. The first gonopod holds the ejaculatory canal inside that opens basally and distally. The second gonopod is solid, short and conical. During copulation, the penis and the second gonopod are inserted into the basal lumen of the first gonopod. While the penis injects the sperm mass, the second gonopod functions in the transport of sperm inside the ejaculatory canal towards its distal opening. The second gonopod is precisely adapted for the sealing of the tubular system in the first gonopod by its specific shape and the ability to swell. Longitudinal cuticle foldings of the second gonopod hook into structures inside the first gonopod. The second gonopod can interact with the penis during copulation by a flexible flap that separates the lumina in which the second gonopod and the penis are inserted.

The presented results are discussed concerning their function in reproduction and in respect of the systematic account.

Keywords: *Pinnotheres*, reproductive morphology, vas deferens, spermatozoa, gonopods, copulatory system.

INTRODUCTION

Among true crabs (i.e., Brachyura Linnaeus, 1758), internal fertilization has evolved. While, in primitive crabs ('Podotremata' Guinot, 1977) the male sperm mass is transferred to simple invaginations of the female's sternum, complex storage structures with an inner connection to the oviduct, the so-called spermathecae, have developed among Eubrachyura Saint-Laurent, 1980 (including Heterotremata and Thoracotremata Guinot, 1977). The male reproductive system has developed in co-evolution with the female genital ducts, probably strongly driven by the transition from external to internal fertilization and by carcinization (Hartnoll 1979, Guinot and Quenette 2005).

The internal reproductive structures consist of paired gonads, the testes, and paired vasa deferentia. Spermatogonia originate in the testes and develop from spermatocytes to spermatids (spermatogenesis) into mature spermatozoa (spermiogenesis) during their transport to the vas deferens (see Krol et al. 1992). Sperm morphology has been claimed to reflect phylogenetic relationships and, therefore, has often been used in decapod systematics (e.g., Jamieson et al. 1995, Jamieson and Tudge 2000, Tudge 2009, Klaus et al. 2009a).

Decapod spermatozoa are aflagellate and immotile. In the vas deferens, they become enclosed inside spermatophores and embedded into a matrix of seminal plasma. Prior to copulation, the sperm mass is stored inside the long and coiled vasa deferentia. These open ventrally on the 8th thoracomere (Balss 1944) by ejaculatory ducts that open through penes.

In 'Podotremata' and Heterotremata, penes with terminal gonopores are located on the coxae of the 5th pereiopods. In pinnotherids, as in all Thoracotremata, the gonopores open on the corresponding sternites. The elongated gonopores ('penes') transfer the sperm mass to the actual male copulatory structures, the so-called gonopods. Gonopods have evolved from the first two pairs of abdominal legs (pleopods), modified for sperm transfer during copulation. The first gonopod (G1) and the second gonopod (G2) of each body half form - together with the corresponding penis - a functional unit achieving the transfer of the sperm mass. By conducting experiments with excised gonopods, Ryan (1967a) demonstrated their essential role in sperm transfer.

Gonopods are important characters for the taxonomy of the Brachyura. Türkay (1975) underlined their systematic account based on the fact that gonopods are less exposed to ecological selection than other characters, because their evolution is determined by the intraspecific optimization of sperm transfer in dependence of both sexes.

Brocchi (1875) established the comparative gonopod morphology with regard to decapod systematics. He included gonopods, penes, and inner reproductive structures in his account. In

recent taxonomic studies, mostly G1s are described. Their diversity in form and fine structure is great among Brachyura (see Shen 1932, Stephensen 1946, Guinot 1966-1971, 1976, 1979, Martin and Abele 1986).

The gonopods of Brachyura and other decapod groups, such as Astacidea Latreille, 1802, comprise a proximal short protopodite articulated to the pleon and an elongated endopodite (Beninger et al. 1991, Minagawa 1993, Tsuchida and Fujikura 2000). The protopodite consists of coxa and basis (Balss 1944). The presumed plesiomorphic character state is found in the Norway lobster *Nephrops norvegicus*, whose endopodite of the G1 forms a longitudinal groove. In interaction, the G2 complements the grooved G1 to form a tube that carries the sperm mass. An 'appendix masculina' (sensu Balss 1944) is present in the endopodite of the G2, which functions in sealing the tube (Guinot 1979).

Among the Brachyura, the grooved G1 is rolled up longitudinally to form a tube, the ejaculatory canal, by a overlapping of the (formerly) lateral margins of the groove. A suture is therefore present alongside the G1, which can be more or less closed according to the degree of tubulation. A proximal and a distal opening are always present in the tubular G1. In contrast, the G2s are usually not tubular. During copulation, the G2 is inserted into the G1. Length and function of the G2s vary considerably among crabs. A long G2, which protrudes from the distal opening of the ejaculatory canal inside the G1, can directly transfer the sperm mass into the female genital ducts. In contrast, a short G2 does not come in contact with the female ducts during copulation, and therefore only functions inside the G1. Both pairs of gonopods are essential for sperm transfer, though, either the G1 or the G2 serves as the actual copulatory organ inserted into the female gonopores. For instance, in the primitive copulatory system of the Podotremata, the G1 is a hardly closed tube (Hartnoll 1975). The proximal opening is (still) wide, the distal opening narrow. In the sponge crab, *Dromia personata*, the G1 is shorter than the G2. The endopodite of G2 is long, thin and flexible and is directly involved in sperm transfer (Hartnoll 1975). Again, in the frog crab, *Ranina ranina*, the G2 is shorter than the G1 and, consequently, works inside it (Minagawa 1993, Hartnoll 1979, Guinot 1979). Actually, the distribution of character states among podotreme crabs is so divers (see Guinot 1979) that phylogenetic conclusions based on gonopod morphology are impossible, which reflects the paraphyletic status of 'Podotremata' Guinot, 1977 (Spears et al. 1992, Schram 2001, Ahyong et al. 2007, Scholz and McLay 2009).

In eubrachyuran copulatory systems, two evolutionary trends can be observed: the advancing tubulation in the G1 and the shortening of the G2 (Hartnoll 1975). The G2s of Hetereotremata Guinot, 1977 are variable in length and specific function, whereas in Thoracotremata Guinot,

1977, G2s are always clearly shorter than G1s. While numerous heterotreme copulatory systems have been studied (Spalding 1942, Cronin 1947, Ryan 1967a, Diesel 1989, Beninger et al. 1991, Neumann 1996, Brandis et al. 1999), only marginal data on thoracotreme copulatory systems is represented in the literature (Lautenschlager 2010). In particular the short G2 of the Thoracotremata and its specific function in sperm transfer is not entirely understood.

In the present study, we investigate the morphology of the pinnotherids' copulatory system and the internal reproductive structures by histological methods, scanning electron microscopy (SEM), transmission electron microscopy (TEM), and confocal laser scanning microscopy (CLSM). Based on the results we infer possible functions of the reproductive structures and evaluate their use for systematics.

MATERIAL AND METHODS

Sampling of Specimens

Pinnotheres pisum was collected from a population of the horse mussel, *Modiolus modiolus*, in the Helgoland Trench during cruises to the German Bight with RV SENCKENBERG in 2004 and 2005 by hard bottom dredge and beam trawl. Global positioning system data of samples range from 54°08.419'N-54°08.599'N to 07°50.921'E-07°53.431'E, the depth from 50 to 55 m.

In the Northern Adriatic Sea (Rovinj/Croatia) *Pinnotheres pisum* and *Nepinnotheres pinnotheres* were collected from different hosts in 2005 and 2007. Partly hand-collected by scuba- and skin-diving in depths from 1 to 35 m, partly by beam trawl on trips with RV BURIN from the Institute Ruđer Bošković on different sample sites ranging from 45°02N–45°07N to 13°36E – 13°40E.

Pinnotheres pectunculi was collected from the host *Glycymeris glycymeris* around Roscoff (Brittany/France) in 2007 and 2008. Additional material of the species *P. pisum* was obtained from the commercially traded host *Mytilus edulis* bought on fish markets in Germany from 2007-2009.

Scanning Electron Microscopy (SEM)

The SEM-study was conducted at the Research Institute Senckenberg (Frankfurt, Germany). Six specimens of *P. pisum*, two of *P. pectunculi*, and four of *N. pinnotheres* were used for SEM-investigations. The material was fixed in 96% ethanol and cleaned in an ultrasonic bath

for 30 s to 2 min. Samples were dried by 'Balzor's CPD 030 critical point dryer' and sputter-coated with a gold/palladium-composite by 'Edwards S 150 B' sputter coater for 3 min (equivalent to a coating of 20 nm thickness). Samples were examined with a scanning electron microscope type 'CamScan (Elektronenoptik GmbH)'. Photographs were taken by means of software Orion®.

The description of setae types follows the nomenclature established by Garm (2004).

Histology

The histological work was carried out at the Research Institute Senckenberg and at the Morphisto GmbH (both Frankfurt, Germany). Three specimens of *P. pisum* and two specimens of *N. pinnotheres* were used for histology. Fresh tissue was fixed in 'Susa Heidenhain' (Romeis 1989) and embedded in paraffin. Histological sectioning was done with a microtome (type Leitz 1515) at 8-10 µm. For general tissue differentiation, the trichromatic Masson-Goldner stainings 'aniline blue' and 'light green' were used (after Romeis 1989).

Transmission electron microscopy (TEM) and semi-thin sections

Preparing techniques for semi-thin and ultra-thin sections were done in the EM-laboratory of Goethe-University (Frankfurt/Germany) and in the Zoological Institute of the University of Heidelberg (Germany). 12 specimens were used in total: six of *P. pisum* and six of *N. pinnotheres*. Fresh tissue was fixed in 4% glutaraldehyde in cacodylate-buffer (pH 7.4). The tissue was post-fixed with 1% osmium tetroxide for two hours. Cacodylate and maleate buffer (pH 5.2) washing steps were followed by en-bloc staining with 1% uranyl acetate overnight. After dehydration through a graded series of ethanol, the tissue was infiltrated and embedded in Araldite or Spurr's resin. Semi-thin sections (1 –2 µm) were made with glass knifes on a ultramicrotome (by Reichert-Jung) and stained with 'Richardson's blue' (after Richardson et al. 1960). Photographs of semi-thin sections and paraffin sections were taken with a light microscope Leica Diaplan with camera CamScan®; software ProgRes).

Ultra-thin sections (ca. 75 nm) were prepared with a diamond knife. Sections were collected on meshed copper grids and contrasted with aqueous lead citrate for 1 min. Electron micrographs were taken on a Zeiss EM10 transmission electron microscope at the University of Heidelberg (Germany).

Confocal Laser Scanning Microscopy (CLSM)

Confocal laser scanning microscopy (CLSM) and preparing techniques were conducted at the "Department of Cell Biology and Comparative Zoology" in the Institute of Biology at University of Copenhagen (Denmark) financed by a Synthesys-grant (project DK-TAF-4264). Six specimens (two of each species) were used. Samples were infiltrated and embedded in glycerine. Analyses were done using a confocal laser scanning microscope (CLSM) on a Leica DM IRBE microscope. Scan series were performed through whole objects. Single scans were combined in maximum projections of single photographs with help of Leica TCS NT software.

RESULTS

Internal Reproductive Structures

Overview

The reproductive system fills a large part of the male cephalothorax (fig. 5.1). The paired male gonads (testes) form long convoluted tubules on both sides of the gastric mill (fig. 5.1A-C). Each testis consists of germinative zones that provide early spermatogonia and seminiferous tubules where spermatids develop (fig. 5.2). The seminiferous tubules of the left and right body half are centrally connected, posterior to the gastric mill. Adjoining, they divide into the paired vasa deferentia.

The testis and all parts of the vas deferens are continuous and not strictly delimitable. Yet, we defined three parts of the vas deferens by its gross morphology and content (fig. 5.1A). Mature spermatozoa were present in each apron of the vasa deferentia.

The proximal (anterior) vas deferens is a strongly coiled tubule. The medial (middle) vas deferens is very wide in diameter and fills most of the cephalothorax (fig. 5.1A-D). The distal (posterior) vas deferens is a narrow, straight tubule that possesses several sac-like appendices or expansions before it opens to the outside by gonopores (fig. 5.1A). The voluminous appendices are situated ventrally in the cephalothorax (fig. 5.1E) and also slightly extend into the abdomen (pleon). Mature spermatozoa were present in each section of the vasa deferentia.

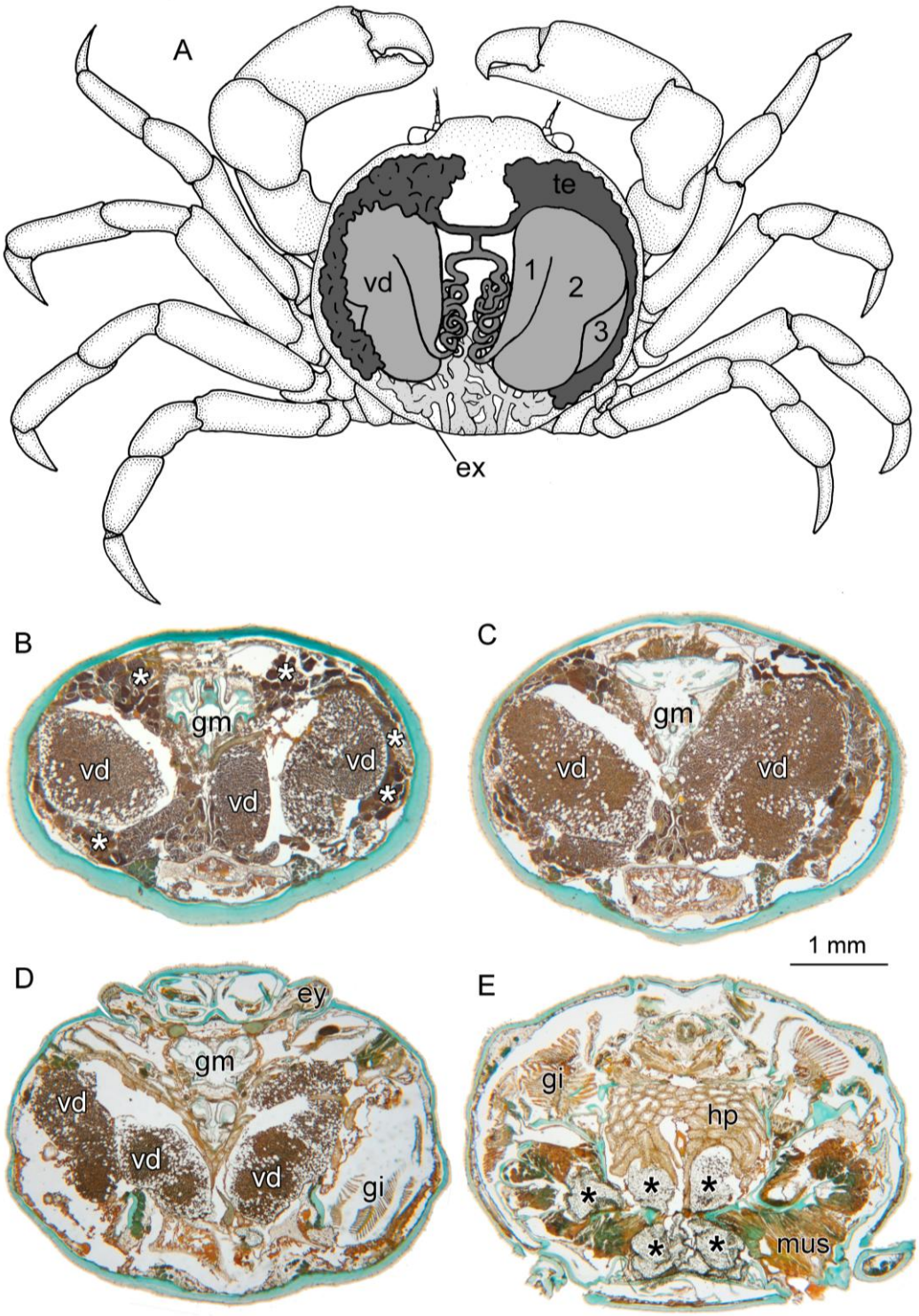

Figure 5.1. Overview on the male reproductive system (internal structures). Histological transverse sections of cephalothorax (B-E), from dorsally to ventrally (Masson-Goldner-staining 'light green'). (**A**) Schematic drawing of the paired testes and vas deferens with the testes overlying the vas deferens on the left side. On the right side, testes are in the background. Display of testes simplified, consisting of convoluted tubules. Coiled tubules of the proximal vas deferens centrally. In the vast medial vas deferens, several wide loops are overlaying (1, 2, 3). The most ventral loop is continuous with the narrow distal vas deferens (connection not shown), which opens into the gonopore (**B**) Section through dorsal cephalothorax showing testes (white asterisks) and vas deferens. (**C**) Vast medial vas deferens. (**D**) Medial vas deferens more ventrally (**E**) Most ventrally, expansions (black asterisks) of the distal vas deferens shown. ex = expansions; ey = eye; gi = gills; gm = gastric mill; hp = hepatopancreas; mus = musculature; te = testis; vd = vas deferens.

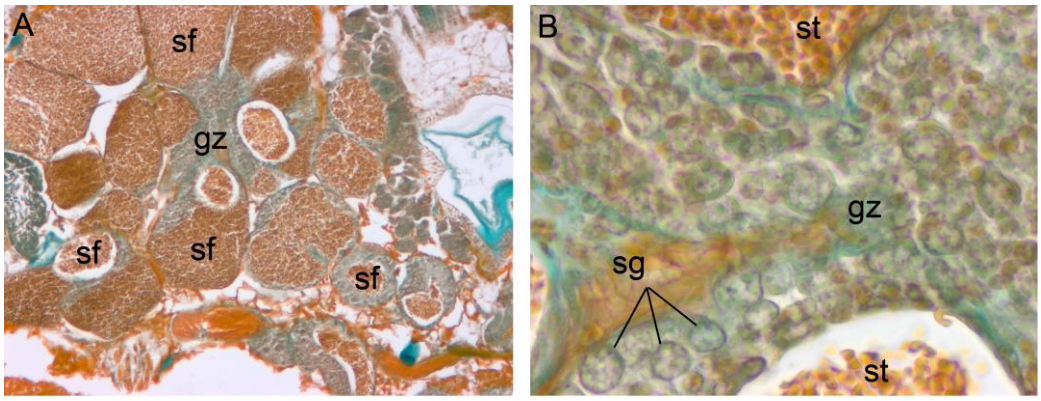

Figure 5.2. Histology of the testis (Masson-Goldner-staining 'light-green'). (**A**) Overview on testes with germinative zone and seminiferous tubules. (**B**) Germinative zone showing early spermatogonia. gz = germinative zone; sf = seminiferous tubules; sg = spermatogonia; st = spermatids.

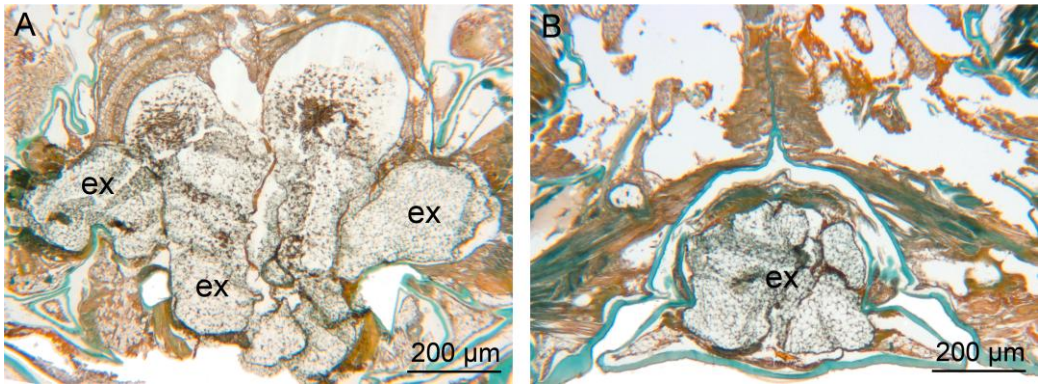

Figure 5.3. Histology of the expansions of the distal vas deferens (Masson-Goldner-staining 'light green'). (**A**) Sac-like expansions in the cephalothorax. (**B**) Expansions in the first pleomere. ex = expansions.

Sperm Morphology

The brachyuran spermatozoa consist of a spherical acrosome, surrounded by a cup-shaped nucleus. The cylindrical perforatorium is located centrally in the acrosome. The distal part of the acrosome is covered by an electron-dense operculum (fig. 5.4A, B). Several zones of distinct electron density can be observed in the operculum and its accessory structures (fig. 5.4C).

The spermatozoa of *Nepinnotheres pinnotheres* are shown in figure 5.4A-C, the ones of *Pinnotheres pisum* in figure 5.4D. In both species, the nucleus forms numerous nuclear arms (see fig. 5A) and the operculum possesses an apical button (fig. 5.4A-D). A circular structure of very low electron density is associated with the operculum of both species: a periopercular rim in *N. pinnotheres* (fig. 5.4A-C) and a subopercular rim in *P. pisum* (sensu Richer de Forges et al. 1997) (see fig. 5.4D). Furthermore, the concentric zonation of the acrosome distinguishes the species (compare fig. 5.4B and D).

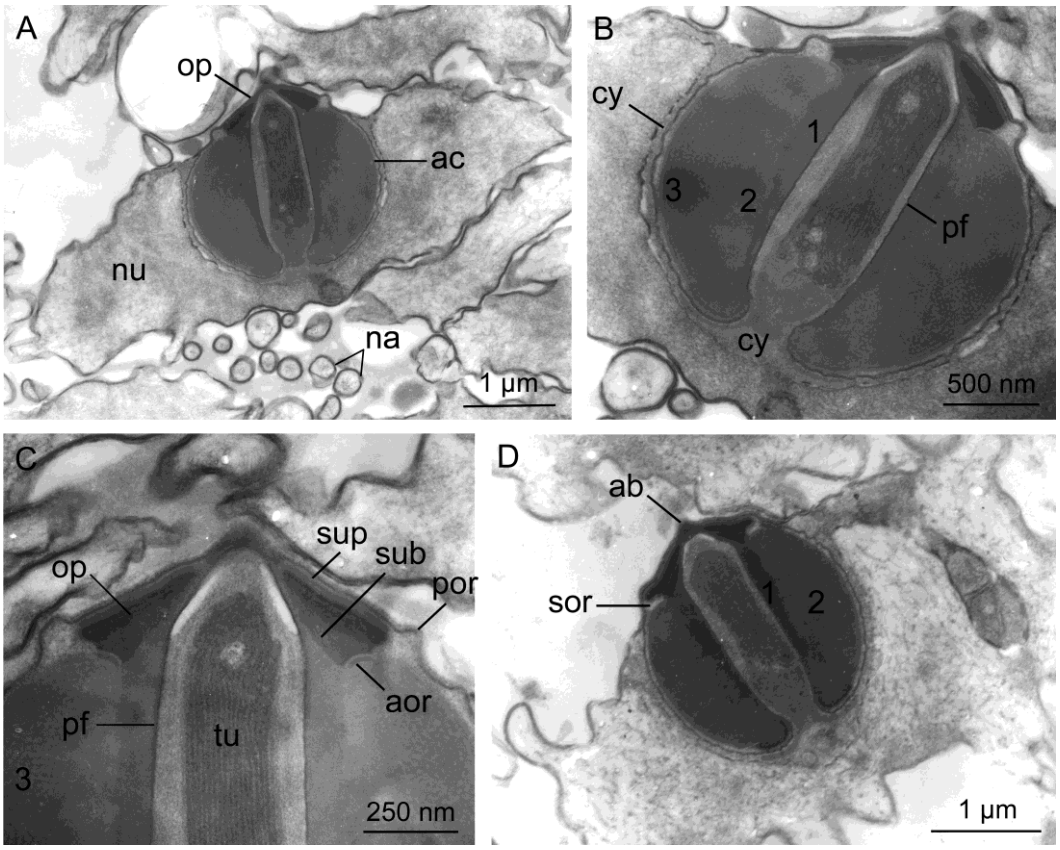

Figure 5.4. Spermatozoal ultrastructure of *Nepinnotheres pinnotheres* (A-C) and *Pinnotheres pisum* (D). (**A**) Cup-shaped nucleus with central acrosome covered by an operculum that forms a convex apical button. (**B**) Acrosome with the central perforatorium. Its base is continuous with the cytoplasma, which forms a thin layer around the acrosome. The acrosome is concentrically zoned with three distinct layers in *N. pinnotheres*: an inner layer of high electron density (1), an intermediate layer of lower electron density (2), and an outer granular layer (3). (**C**) Centrally, the perforatorium is fibrous by the presence of coiled tubular membranous structures. The operculum is composed by a median layer of high electron density and layers of lower electron densities: the supra- and subopercular material. An accessory opercular ring and a periopercular rim are present in *N. pinnotheres*. (**D**) In *P. pisum*, the acrosome shows two distinct concentric zones (1, 2) and a subopercular rim of very light electron density is present. ab = apical button; ac = acrosome; aor = accessory opercular ring; cy = cytoplasma; na = nuclear arms; op = operculum; pf = perforatorium; por = periopercular rim; sub = subopercular material; sup = supraopercular material; tu = tubular membranous structures.

Vas Deferens

The spermatophores are assembled in the anterior vas deferens, because they are already present in its distal section and in the medial vas deferens (fig. 5.6). A one-layered epithelium of flat cubic secretory cells lines the tubules of the proximal vas deferens (fig. 5.5A, B). It is enveloped by a thin layer of connective tissue without musculature (fig. 5.5A). The glandular epithelium secretes a substance of high electron density, which forms small droplets with diameters of 1 µm and less (fig. 5.5B). The secretions aggregate in the lumen of the most proximal vas deferens (fig. 5.5C).

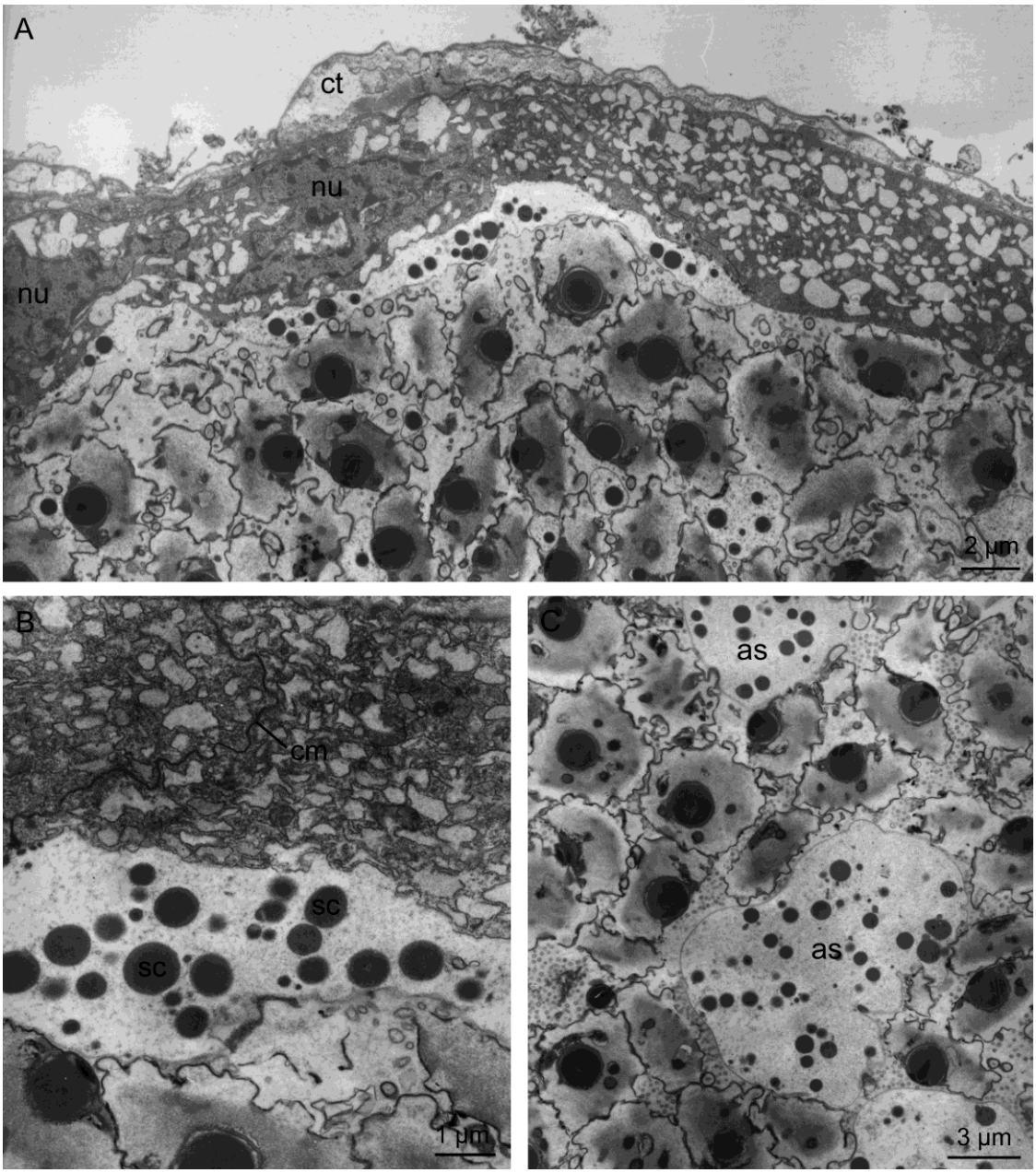

Figure 5.5. Proximal vas deferens holding free mature spermatozoa. (**A**) One-layered secretory epithelium of cubic cells. Note lobate nuclei. (**B**) An electron dense substance is secreted. (**C**) Aggregation of secretion amongst spermatozoa. as = aggregation of secretion; cm = cell membrane; ct = connective tissue; nu = nucleus; sc = secretion.

become increasingly mixed with large electron light vesicles holding seminal plasma (fig. 5.6A). The seminal plasma is produced in the medial vas deferens by a secretory epithelium. The anterior part of the medial vas deferens is filled with spermatophores. In the intermediate and posterior apron of the medial vas deferens, vesicles are more abundant, especially in its periphery close to the secreting epithelium. The distal vas deferens possesses several tubuliform appendices, which produce and store vast amounts of seminal plasma. The spermatophores are embedded in seminal plasma in the medial and distal vas deferens (fig. 5.6A and 5.7A), but absent from the appendices that only contain seminal plasma (fig. 5.7B,

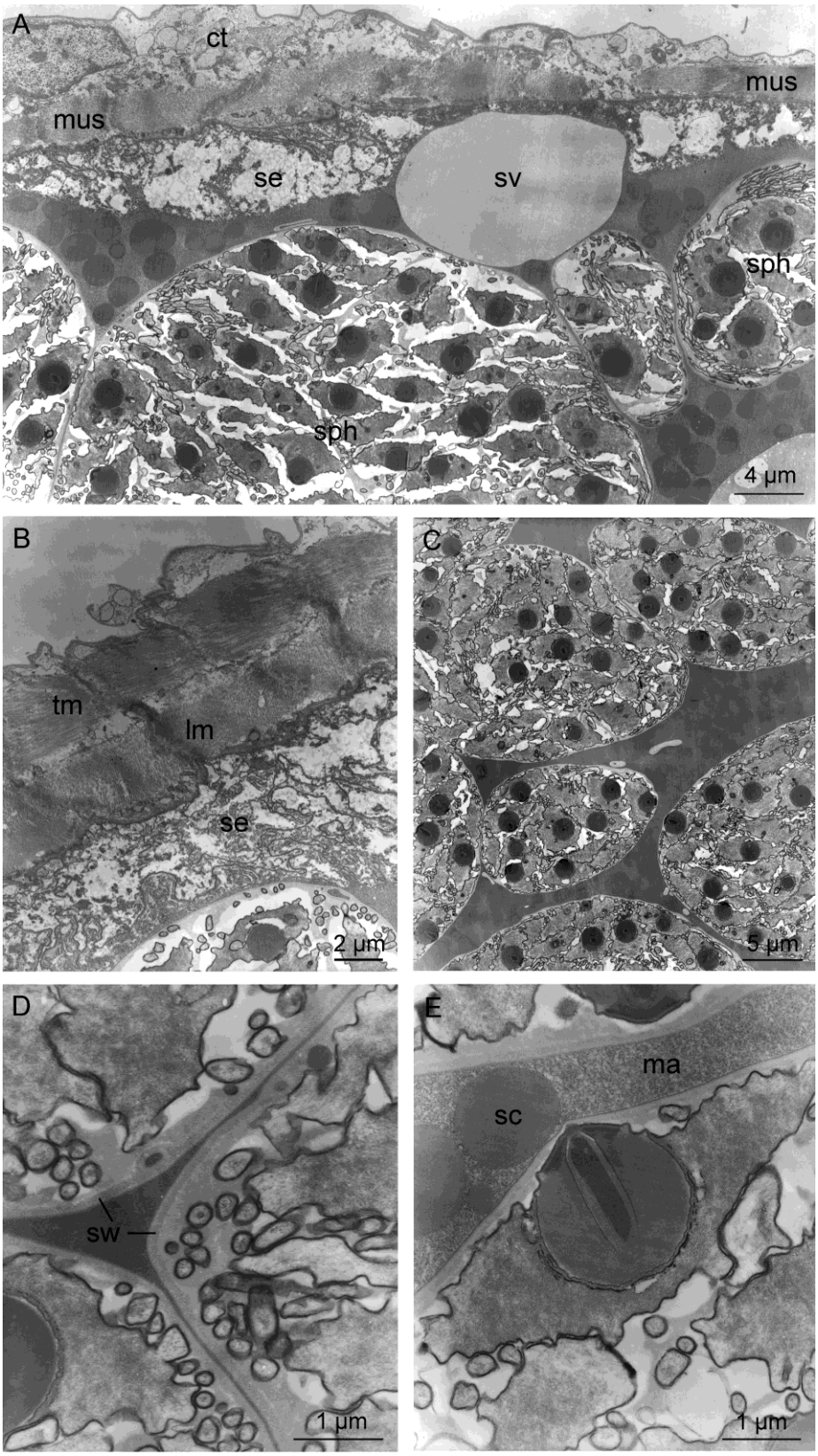

Figure 5.6. Medial vas deferens with spermatophores. (**A**) Secretory epithelium. Large vesicles of light electron density are present. (**B**) Outline of musculature. (**C**) Spermatophores envelop the spermatozoa. (**D**) The one-layered spermatophore wall. (**E**) Spermatophores embedded in matrix with small secretory droplets. ct = connective tissue; lm = longitudinal musculature; ma = matrix; mus = musculature; sc = secretion; se = secretory epithelium; sph = spermatophores; sv = secretory vesicle; sw = spermatophore wall.

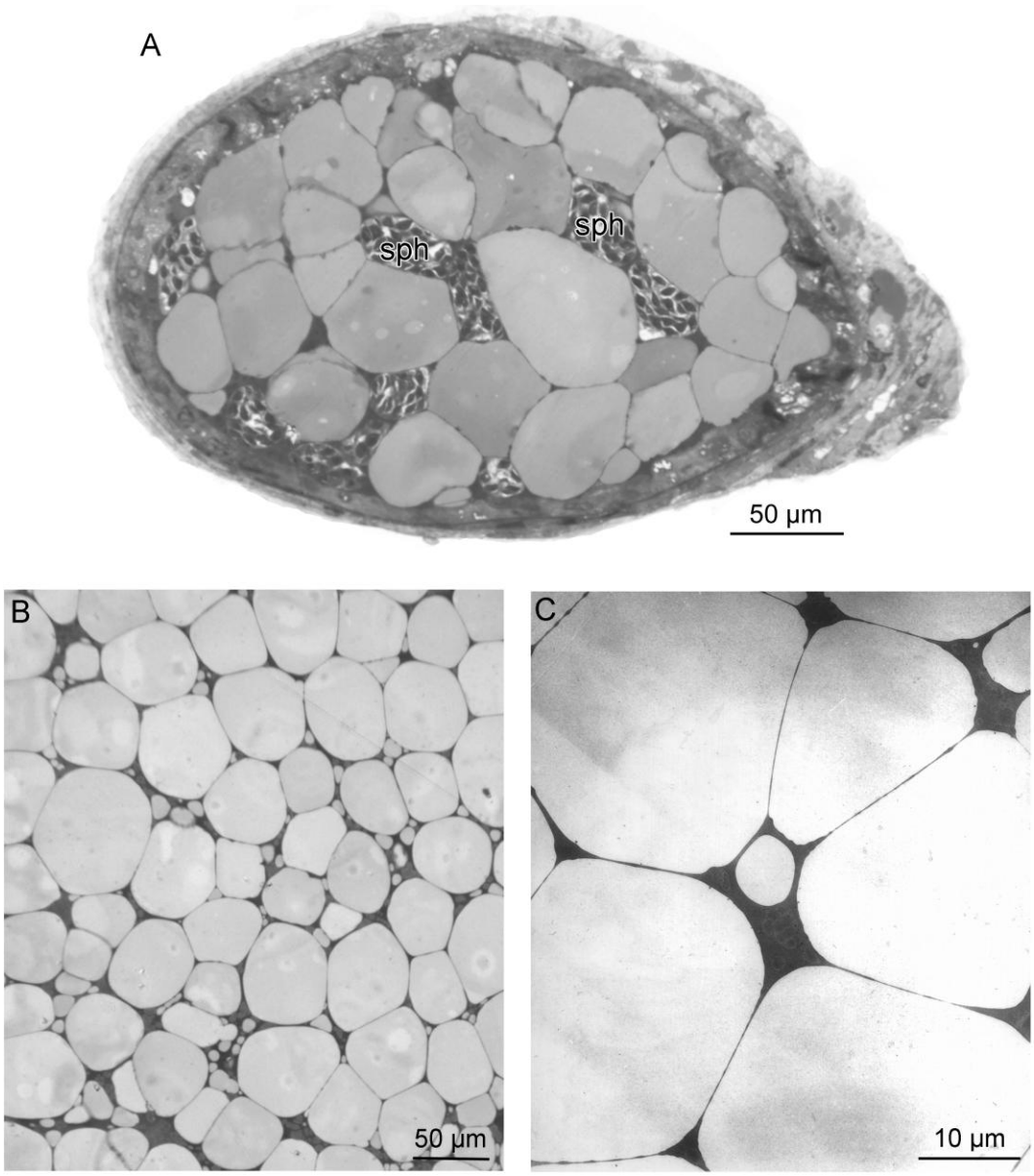

Figure 5.7. Histology and ultrastructure of the distal vas deferens with the appendices. (**A**) Transverse section through distal vas deferens showing seminal plasma and spermatophores (semi-thin). (**B**) Section through the appendices, exclusively holding vesicles of seminal plasma (semi-thin). (**C**) The vesicles of electron-light density are embedded in an electron-dense matrix (TEM). sph = spermatophores.

C). The most distal vas deferens is a muscular ejaculatory duct that terminates in a short penis on each side of the sternal depression.

No cyclic changes were observed in the vasa deferentia of the investigated pinnotherids. The size of the vas deferens did not vary considerably among specimens from different season and was always filled with spermatophores.

Copulatory System

Overview

The studied species *Nepinnotheres pinnotheres*, *Pinnotheres pisum*, and *Pinnotheres pectunculi* have a uniform copulatory system, consisting of a long first gonopod (G1) and a short second gonopod (G2) (see fig. 5.8). The gonopods have two articulated parts. The basal part corresponds to the protopodite, formed by the fused basis and coxa. The distal part corresponds to the endopodite. The endopodite of the G1 forms a tube with a wide basal opening and a narrow distal opening (fig. 5.9). The endopodite of the G2 is solid and coniform. The tube of the long first gonopod (G1) transfers the sperm mass to the female ducts. The European species differ in characters of the endopodite of G1 and in the form of the pleon, which is normally flexed into the sternal cavity to cover the gonopods (fig. 5.9).

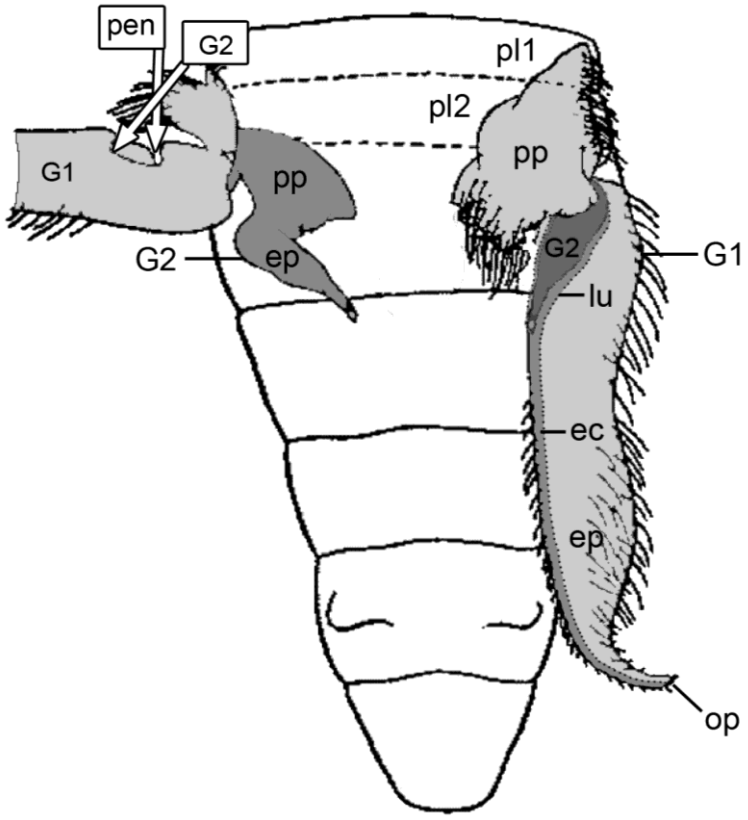

Figure 5.8. Overview on the male copulatory system (*Pinnotheres pisum*). Pleon opened. On the right, the tubular left G1 is folded backwards showing the ejaculatory canal and the introduced G2. On the left side the right G1 (only proximal part shown) is flapped towards the lateral body side whereby the G2 and the basal opening in G1 are exposed. During copulation, G2 and penis (not shown) are both inserted into the opening in G1 to arrive inside its basal lumen, which is continuous with the ejaculatory canal. ec = ejaculatory canal; ep = endopodite; G1 = first gonopod; G2 = second gonopod; pp = protopodite. (drawing after Atkins 1926).

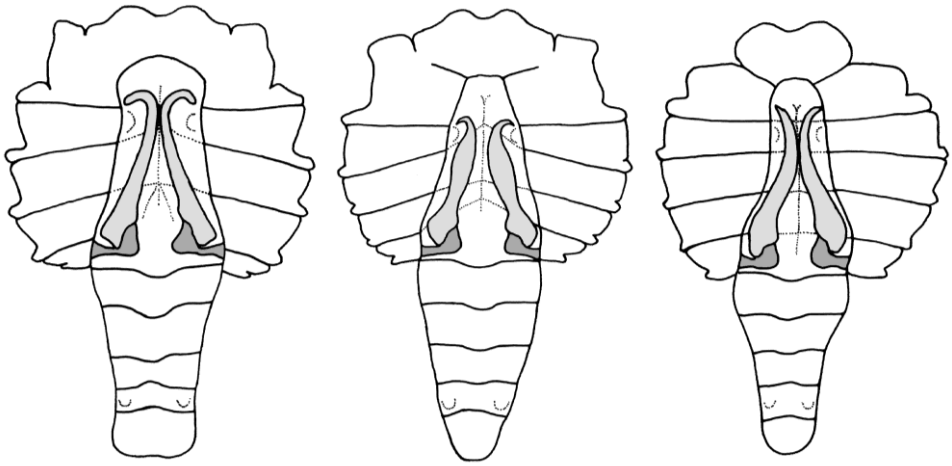

Figure 5.9. The copulatory systems of the European species. Ventral view on male sterna with pleons opened. The G1s shaded in light grey; The G2s (inserted) in dark grey; left = *N. pinnotheres*; middle = *P. pisum*; right = *P. pectunculi.*

First Gonopod (G1)

Nepinnotheres pinnotheres (fig. 5.9A, 5.10A, 5.12A, B). The G1 is long, slender, and slightly flattened dorsoventrally, gradually tapering distally. The endopodite runs straight, with its distal fourth being strongly curved towards the lateral body side. The position of the distal tip with the opening of the ejaculatory canal results in an angle of about 90° to the base. Next to long pappose setae on the proximal base of G1, long simple setae are present along most of its length, especially in its curve (fig. 5.12A, B). The distal opening of the ejaculatory duct forms two rounded lobes (fig. 5.12B).

Pinnotheres pisum (fig. 5.9B, 5.10B, 5.12C). The G1 of *P. pisum* is straight for almost its total length. It is strongly flattened dorsoventrally. The distal part of the G1 tapers abruptly, with a light bending in the most distal part. The terminal opening of the ejaculatory canal is slightly oriented towards the lateral body side. The G1 possesses long pappose setae in the proximal base and along its total length. Setae are more numerous in the distal curve (fig. 5.12C). The form of the opening of ejaculatory canal is simple (fig. 5.12C).

Pinnotheres pectunculi (fig. 5.9C, 5.10C). The G1 of *P. pectunculi* is flattened dorsoventrally and tapers gradually along its total length. The bending towards the lateral body side is gradual from the proximal base to the distal opening of the ejaculatory canal. The whole form of the endopodite is sickle-shaped. The pappose setae and the simple distal opening of the ejaculatory duct are the same as in *P. pisum* (see fig. 5.12C).

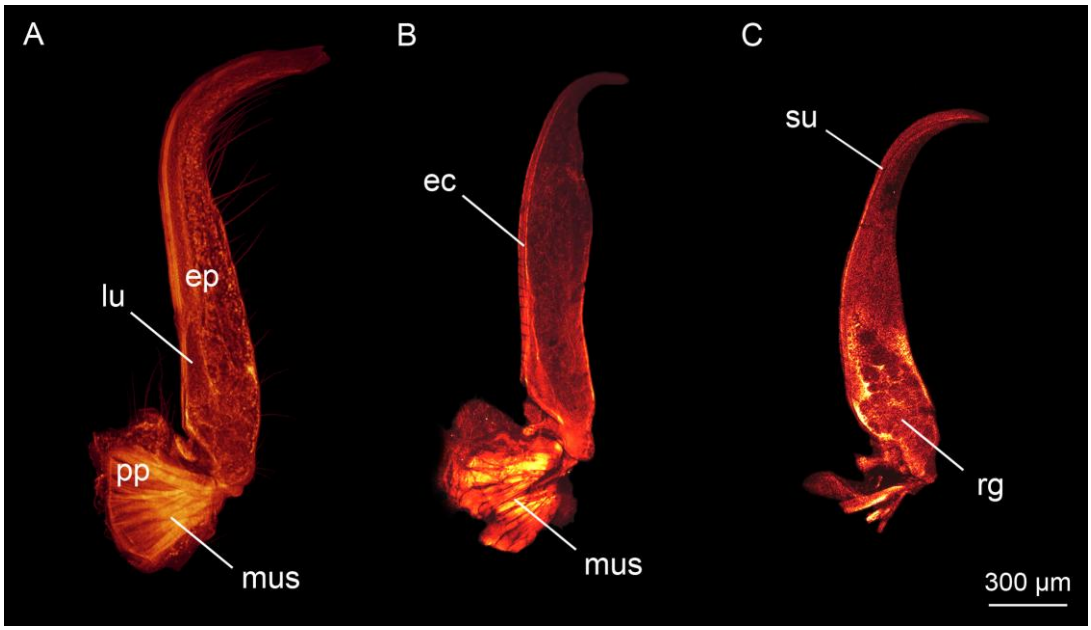

Figure 5.10. First gonopods of the European species (CLSM). (A) *Nepinnotheres pinnotheres*. (B) *Pinnotheres pisum*. (C) *Pinnotheres pectunculi*. ec = ejaculatory canal; ep = epipodite; lu = lumen; mus = musculature; pp = protopodite; PTG = pleopod tegumental glands; su = suture.

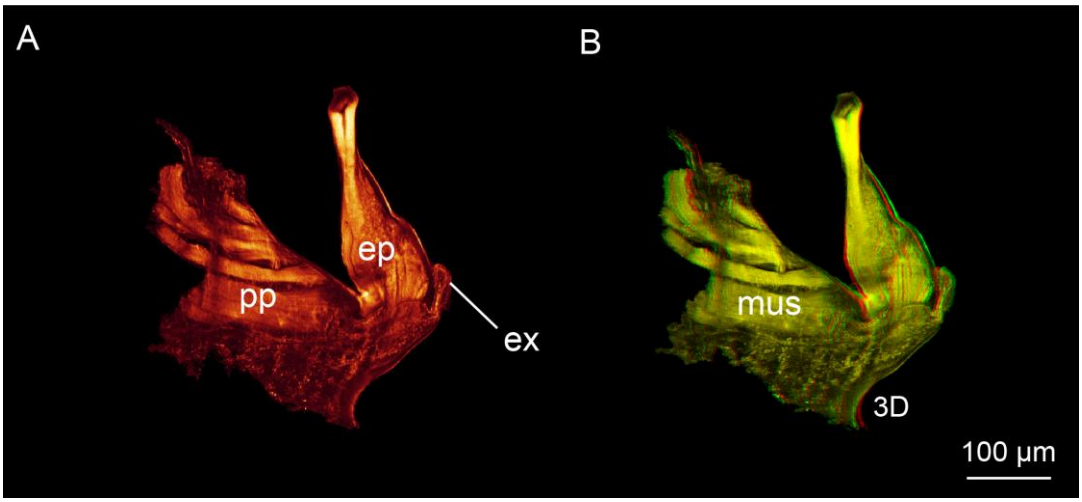

Figure 5.11. Second gonopod of *Pinnotheres pisum* (CLSM). Projection of scan series. (A) Protopodite with strong musculature; the endopodite possesses a rudimentary exopodite. (B) 3D-projection of same G2. ep = endopodite; ex = exopodite; mus = musculature; pp = protopodite.

The basal lumen in the endopodite of the G1 splits into two openings (fig. 5.13). The G2 is inserted from ventrally into a wide opening. The penis is inserted dorso-laterally through a narrow slit-like opening between the articulation of protopodite and endopodite fig. 5.13).

Second Gonopod (G2)

In the G2, protopodite and endopodite are almost fused, but still show a suture between the two articles (fig. 5.11). The protopodite is robust. It is oriented mesially from the lateral edges

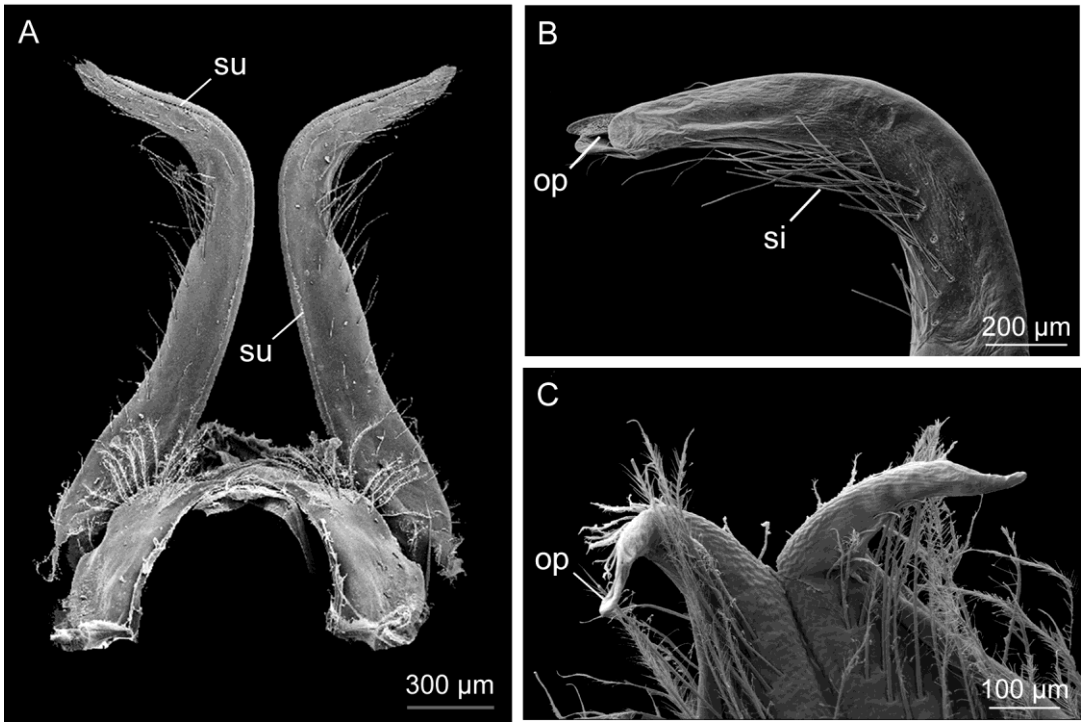

Figure 5.12. SEM-photographs of first gonopods. (**A**) Dorsal view on the paired G1s of *Nepinnotheres pinnotheres*, articulated to the first pleomere. (**B**) Ventral view on terminal joint of the same G1. The distal opening of the ejaculatory canal is formed in two rounded lobes. Simple setae are concentrated in the curve. (**C**) Paired G1s of *Pinnotheres pisum* with long pappose setae. The distal tip of G1 with a simple, slightly elongated opening of the ejaculatory canal. op = opening of ejaculatory canal; si = simple setae; su = suture.

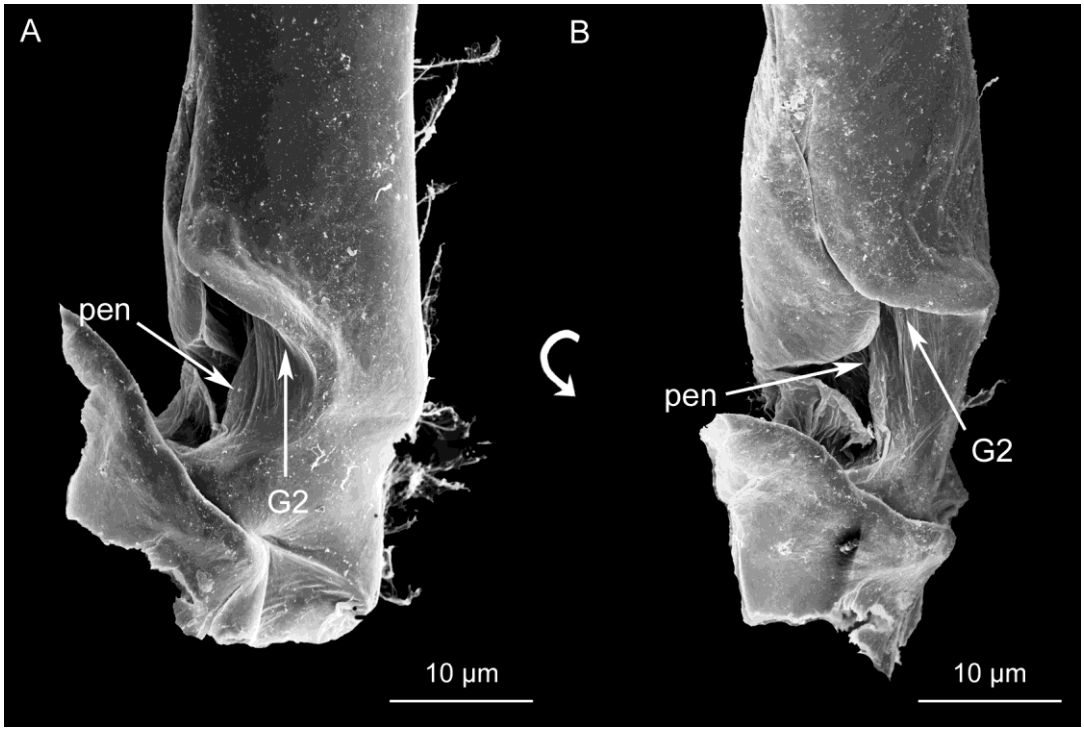

Figure 5.13. SEM-photograph of basal openings of the first gonopod (*Pinnotheres pisum*). The G2 is ventrally inserted; the penis from the dorso-lateral side (white arrows). (**A**) Ventral view. (**B**) Mesio-lateral aspect. G1 = first gonopod; G2 = second gonopod; pen = penis.

of the second pleomere. The endopodite is turned anteriorly towards the basal opening in G1. A small finger-shaped exopodite is present at the ventro-mesial face of the G2 (fig. 5.11). The endopodite is solid and basally somewhat flattened. Distally, it has a coniform or stump-like form (fig. 5.11, 5.14A-C). The dorsal and ventral side of the G2 possess longitudinal cuticle foldings (fig. 5.14A, B). The distal tip of the G2 has a circular swelling, the 'apical girdle' (*sensu* Beninger et al. 1991). The cuticle distal of the apical girdle is strongly folded and appears soft in the SEM-photographs (fig. 5.14A, C).

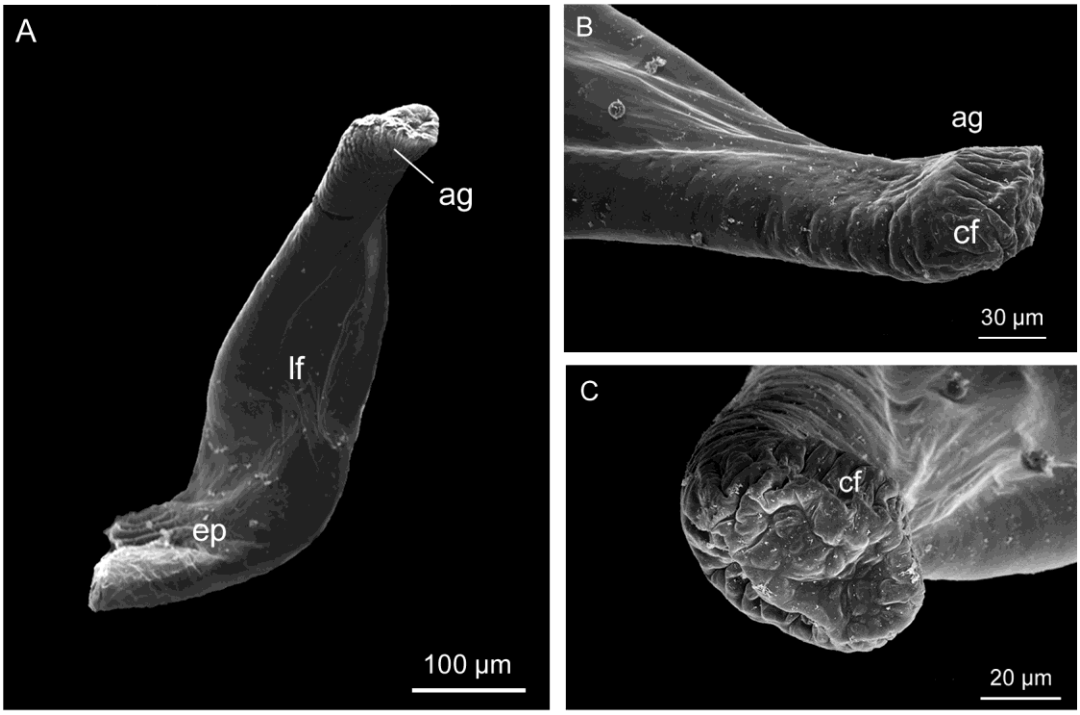

Figure 5.14. SEM-photographs of the second gonopod. (**A**) Dorsal face of endopodite with longitudinal folding. (**B**) Coniform tip in lateral view. (**C**) Apical girdle in frontal view with soft and wrinkled cuticle. ag = apical girdle; cf = cuticle folding; ep = endopodite; lf = longitudinal foldings.

Ejaculatory Duct and Penis

As diagnostic for all Thoracotremata Guinot, 1977, the pinnotherids' male gonopores open on the 8th thoracomere, located on the slope of the sternal depression.

The distal part of the vas deferens is muscular and termed the ejaculatory duct. The penis can be defined as the most distal part of the ejaculatory duct that opens on the body's outside and terminates by the gonopore. The paired penes are shown in figure 5.15. They are collapsed in SEM-observations (fig. 5.15) and in the histological sections (fig. 5.16C, D). The cuticle of the penis and the surrounding integument is thin and wrinkled, indicating flexibility (fig. 5.16A-D). The ejaculatory duct (inside the body) is shown in transverse sections (fig. 5.16A, B), the penis (outside the body) in longitudinal sections (fig. 5.16C, D). An inner layer of

longitudinal musculature and outer layer of transverse musculature allow peristaltic actions of the ejaculatory duct and penis (fig. 5.16B). The collapsed penis is short in fixed specimens and was never observed as being inserted into the G1. During copulation, the penis has to prolong to reach the opening in the G1.

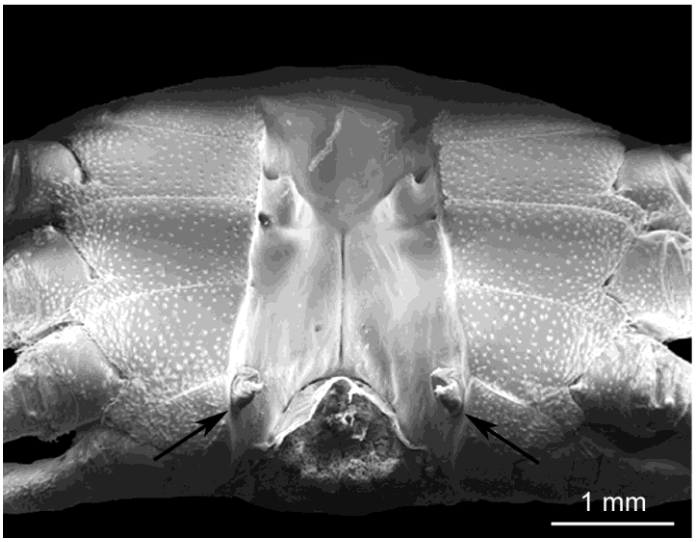

Figure 5.15. SEM-photograph of male sternum with paired penes (black arrows). Pleon removed.

Interaction of Gonopods and Penis

The histology of the G1 and the G2 is shown in figure 5.17. The G2 was always found to be inserted in the G1 in living and fixed specimens. The base of the G1 is filled with rosette-shaped pleopod tegumental glands (PTG), which are grouped around the basal lumen of the G1 (fig. 5.10C, 5.17B-D). Distally, they do not exceed further than the inserted G2 (fig. 5.10C, 5.17B-D). The coniform tip of the G2 with the apical girdle is shown in figure 5.17D. It dyes different from the remaining cuticle in the Masson-Goldner staining. The basal lumen of the G1, which is continuous with the ejaculatory canal, narrows abruptly after the proximal third of the G1 (fig. 5.10B, 5.17D, E).

The endopodite of the G2 is free of musculature. It possesses bold cuticle parts on the rounded lateral margins and thin cuticle on the ventral and especially on the dorsal face (fig. 5.17, 5.18). The form of the G2 follows the shape of the basal lumen in the G1 precisely (fig. 5.18B, C, E, F). Longitudinal cuticle foldings on the dorsal face of the G2 hook with the margin of the suture inside the G1 and, thus, arrest the G2 within the G1. The lumen of the G1 where the G2 is introduced is separated from the lumen, in which the penis is inserted, by a flexible flap with very thin cuticle.

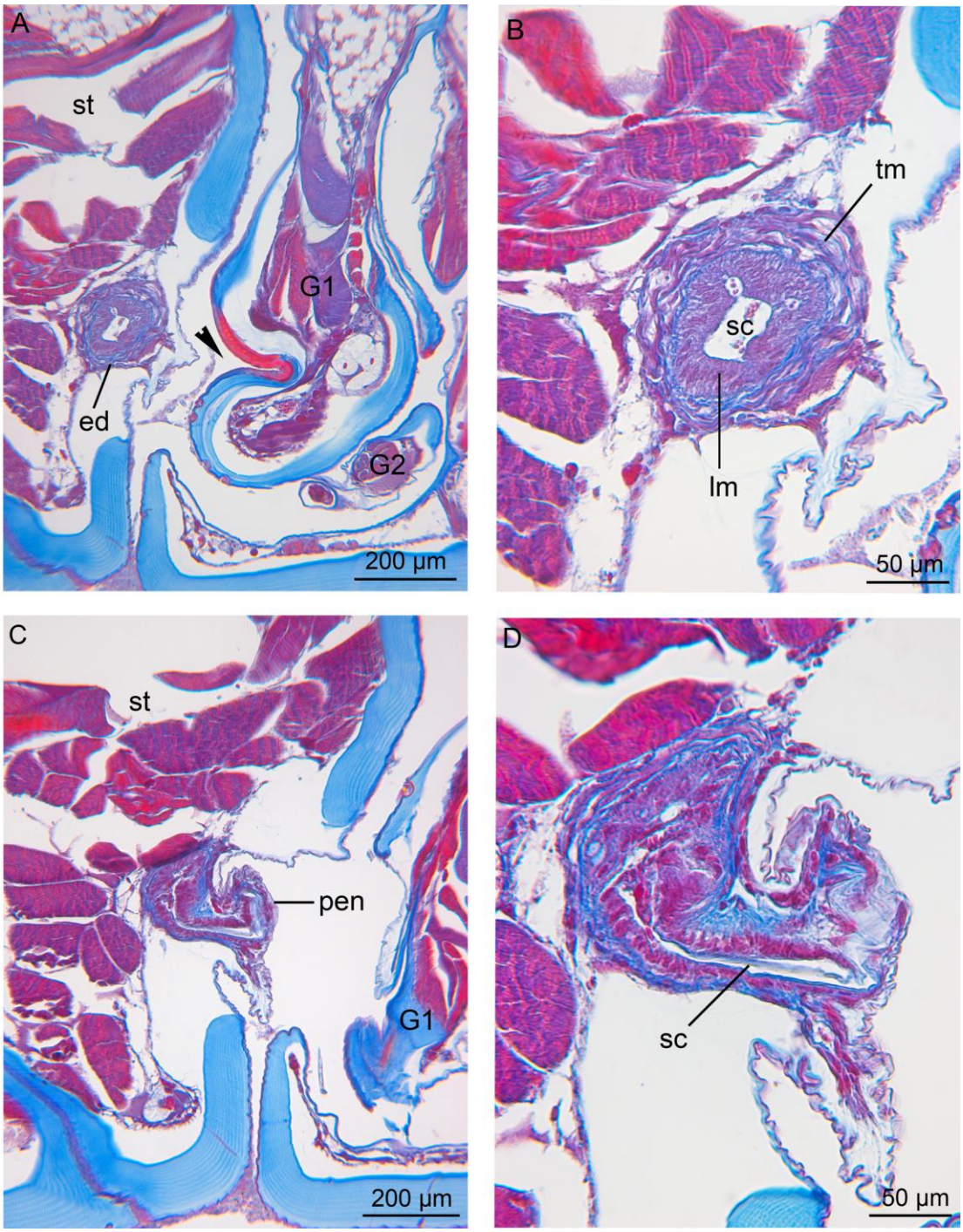

Figure 5.16. Histology of the ejaculatory duct and the penis (Masson-Goldner staining 'aniline blue'). (**A**) The Ejaculatory duct in the slope of the sterno-abdominal cavity in transverse section (inside the body). Base of the G1 met in longitudinal orientation. Arrow on the basal opening in the G1 (G2 not shown). (**B**) Higher magnification of transverse section showing an inner layer of longitudinal musculature and an outer layer of transverse musculature (staining magenta) separated by connective tissue (staining blue) around the central sperm canal (**C**) The penis in longitudinal section. (**D**) Closer view on the collapsed penis with the sperm canal. ed = ejaculatory duct; G1 = first gonopod; G2 = second gonopod; lm = longitudinal musculature; tm = transverse musculature; sc = sperm canal; st = sternum; pen = penis.

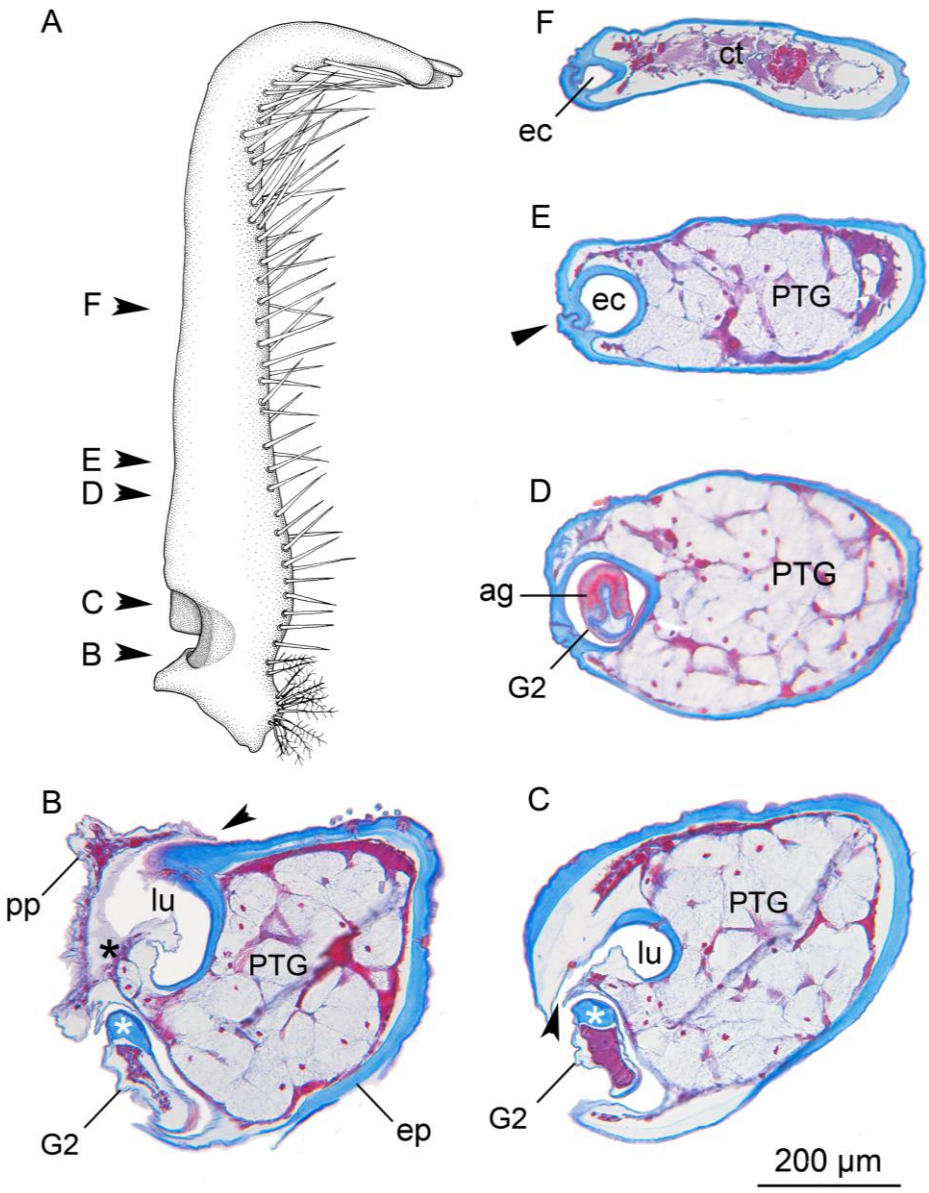

Figure 5.17. Histological transverse sections of the first gonopod (Masson-Goldner-staining 'aniline blue'). Pleopod tegumental glands are grouped around its basal lumen (B-E). (**A**) Drawing of the G1 of *Nepinnotheres pinnotheres* with arrowheads showing where histological sections (B-E) were taken from. G2 not shown. (**B**) Base of the G2 outside the G1. White asterisk on bold cuticle parts (staining blue); arrowhead on articulation of protopodite and endopodite. (**C**) G2 still outside G1. Arrowhead on insertion of the G2. Black arrow on central canal of PTG. White asterisk on bold cuticle. (**D**) G2 inserted. The cuticle of its apical girdle stains magenta. (**E**) The PTGs do not reach much further than the inserted G2. Arrowhead in suture of ejaculatory canal. (**F**) The basal lumen narrows into the ejaculatory canal. Arrowhead on suture. ag = apical girdle; ct = connective tissue; ec = ejaculatory canal; ep = endopodite; G2 = second gonopod; lu = basal lumen in G1; pp = protopodite; PTG = pleopod tegumental glands.

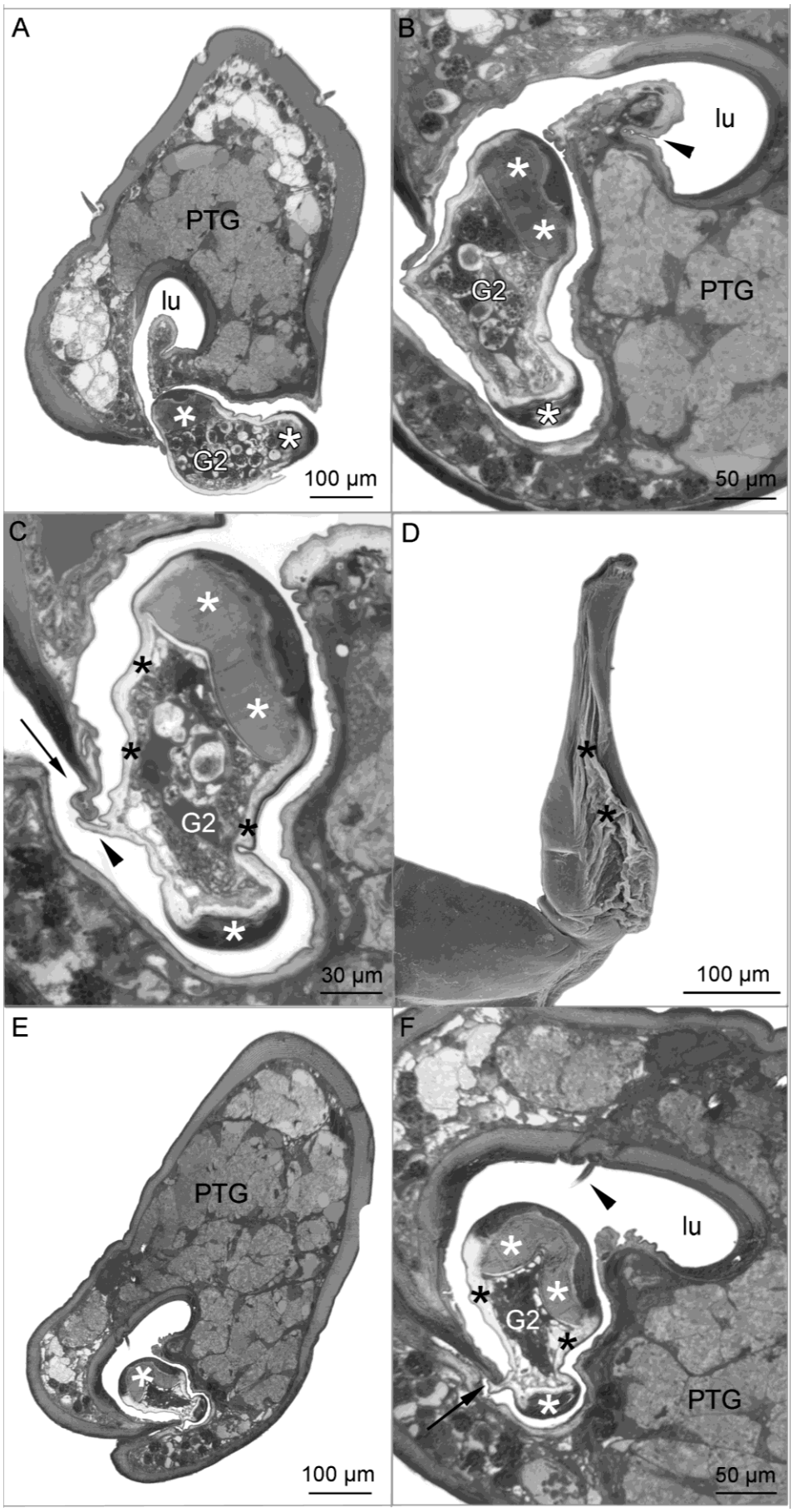
A
PTG
lu
G2
100 µm
B
lu
G2
PTG
50 µm
C
G2
30 µm
D
100 µm
E
PTG
100 µm
F
lu
G2
PTG
50 µm

Figure 5.18 (page 102). Basal lumen in the endopodite of the first gonopod with the second gonopod inserted. Transverse semi-thin sections, proximally to distally. (**A**) The lumen in the G1 is shown in which the penis is inserted during copulation. Proximal base of the G2 outside the G1. (**B**) Higher magnification of the G2 inside the G1. Strong cuticle parts face a flexible flap that separates the inserted G2 from the lumen where the penis is inserted. (**C**) The G2 inside the G1. Longitudinal cuticle foldings of the G2 interlocking with suture inside the tubular G1 (black arrowhead). The suture in the G1 is still open (black arrow). (**D**) Ventral face of the G2 showing longitudinal cuticle foldings (SEM). (**E**) The G2 inside the G1. (**F**) The G2 hooks with the margin of the suture inside the G1 (black arrow). Setae are present inside the tube of the G1 (black arrowhead). Black asterisks = soft cuticle parts. White asterisk: bold/rigid cuticle parts. G2 = second gonopod; lu = lumen for penis; PTG = pleopod tegumental glands; white asterisks = bold cuticle parts;

DISCUSSION

Review on Brachyuran Gonopods

The copulatory system of the investigated pinnotherids, i.e. a long first gonopod (G1) that transfers the sperm mass and a reduced, solid second gonopod (G2) is characteristic for thoracotreme crabs and represented among a number of higher heterotremes as well.

In the primitive heterotreme cancrids *Cancer pagurus* (Williamson 1900) and *Cancer gracilis* (Orensanz et al. 1995) a long G2 acts in transferring the sperm mass into the female gonopores. Their G2 is slender and flexible and its endopodite consists of two articles: a subterminal and a terminal joint (Williamson 1900, Orensanz et al. 1995). The G1 is comparatively short and robust with an open suture throughout most of its length. When the G2 is inserted, its terminal joint protrudes from the G1. Williamson (1900) observed the rigid G1 to be held firmly during copulation, while the flexible G2 can freely move back and forward inside the G1. By staining the sperm mass, Williamson (1900) showed that the pumping of the G2 inside the G1 forwards the sperm mass towards the distal opening of the ejaculatory canal. Since the G2 is not grooved in the mentioned *Cancer*-species, it remains unclear how the elongated part of the G2 is involved in sperm transfer and the accurate positioning into the female duct. So, the process of sperm transfer among Cancridae is supposed to be a rather unspecific kind of plugging (Elner et al. 1985, Williamson 1900).

Heterotreme freshwater crabs of the genus *Potamon* also have a copulatory system, which consists of short G1 and a long G2 that transfers the sperm mass (Brandis et al. 1999). The endopodite of the G1 is rigid. It is composed of two articles as well: a terminal and subterminal joint. These are connected by a flexible zone that can be bent during copulation. This is characteristic for many freshwater crabs. The longitudinal suture of the G1 is still open (Brandis et al. 1999, 2000). The endopodite of the G2 also consists of a subterminal and a terminal joint, with the latter protruding from the G1 if inserted. Different states of character are represented among Old World freshwater crabs. While the terminal joint of G2 forms a groove in Gecarcinucidae, a closed tube has developed in Potamonautidae and Potamidae

(Brandis 2002, Klaus et al. 2006, 2009b). In this case, the G2s are well adapted for sperm transfer. In the described examples, the tubulation of the G1 is not completed, thus, the longitudinal suture is still open. The long G2 is presumably inserted laterally into the G1 instead of being introduced by the basal opening. With an ongoing tubulation of G1 that results in a completely closed suture, a lateral insertion becomes impossible. As a result, the G2 has to be inserted through the basal opening in G1. This again, is only accomplished with a shortening in the G2. This is why the tubulation in the G1 and the shortening of the G2 are closely related (Hartnoll 1975).

In the hydrothermal vent crabs of the family Bythograeidae, the G2 can either be shorter than the G1 (about half its length or longer) (Tsuchida and Fujikura 2000) or protrude from it (Guinot and Hurtado 2003). In *Austinograea williamsi*, the G2 was always found outside the G1 (Tsuchida and Fujikura 2000). The basal opening in G1 is so small that Tsuchida and Fujikura (2000) suppose the G2 not to be inserted at all during copulation. They conclude that the G2 only acts as a sensor that guides the G1 to the right position in the female ducts.

In investigated species of the heterotreme groups Majoidea (Diesel 1989**,** Beninger et al. 1991, Neumann 1996), and Portunoidea (Spalding 1942, Cronin 1947, Ryan 1967a, Johnson 1980), the G1 forms a completely closed tube with a mesial suture, while the terminal joint of the G2 is shortened. As in the shore crab *Carcinus maenas*, the length of the G2 is one third of the G1 (Spalding 1942), while it is about one sixth in the spider crabs *Maja* spp. (Neumann 1996).

The role of a short G2 in sperm transfer is seen as a plunger or piston that accomplishes pumping movements inside the G1 and thereby drives the sperm mass inside the ejaculatory canal to its distal opening (Ryan 1967a, Bauer 1986, Diesel 1989, Beninger et al 1991). The pumping movement is achieved by a flexing of the pleon during copulation (Watson 1970, 1972, Diesel 1989, Elner and Beninger 1992). Even though the endopodite is free of musculature, the G2 can also move along its transverse axis by contracting the musculature in its protopodite.

For *Chionoecetes opilio*, several structures were described distally in the G2: the ’appendix masculina’, a ‘protuberance’ and an ‘apical girdle’ (sensu Beninger et al. 1991). Neumann (1996) followed this terminology to describe the G2s of *Maja* spp. The term ‘appendix masculina’ is deduced from pleopod features in male shrimps (see Balss 1944). In our opinion, the use of the term ‘appendix masculina’ on processes of brachyuran gonopods is hardely plausible, since it implies a homology of these appendices, which is very questionable.

The Pinnotherid Copulatory System

In the investigated pinnotherids, as in the thoracotreme Grapsoidea and Ocypoidea (pers. obs.), the G2 is reduced in length and appendices or protuberances are no longer present. However, we found the coniform tip of the G2 of the studied pinnotherids to form an apical girdle that conforms to the description by Beninger et al. (1991). The apical girdle is characterized by a circular cuticle swelling around the tip of the G2, distally followed by folded cuticle. The fine structure and histology of the endopodite of the G2 and especially of its apical girdle suggests a certain ability to swell. Due to the lack of musculature in the endopodite of the G2, a modification in form can only occur by the build up of hemolymph pressure. The whole endopodite of the G2 laterally possesses bold rigid cuticle parts and flexible, folded cuticle on the slightly flattened ventral and dorsal face. With a hemolymph swelling, the G2 is supposed to enlarge along the cuticle foldings. The ability to swell in the endopodite with its apical girdle and the precise adaptation of the specific shape of the G2 to the tube inside the G1 show that the G2 is really optimized to tightly seal the basal lumen, respectively the ejaculatory canal, inside the G1. Furthermore, the G2 seals the tubular system to the outside, which could minimize seawater influx and the loss of sperm. Observations on mating behaviour of *C. opilio* revealed the sperm transfer process to be kind of "leaky", which implies a partly loss of the sperm mass (Watson 1970, 1972). Beninger et al. (1988) stated that the transmitting process of sperm is sensitive in Brachyura because of the risk for spermatophores to come in contact with sea water during copulation, which infiltrates through the proximal openings in the G1 causing their dissolution. Furthermore, Beninger et al. (1991) supposed that a sealing of the ejaculatory canal in the G1 by the G2 is a precondition to enable the hydraulic pumping of seminal fluids inside the ejaculatory canal. Beninger et al. (1988) also observed an asymmetry in cuticle thickness in the G2 of *C. opilio* and supposed that it functions in breaking the sealing of the ejaculatory canal with every backward movement due to the asymmetrical forces.

The G2 of the investigated pinnotherids seems highly adapted for functioning in the hydraulic transport. We suggest that the G2 swells along its lateral faces and in the apical girdle with upward movements to tightly seal the ejaculatory canal, respectively the basal lumen (see fig 5.18). Thus, hydraulic pressure is built up, which drives the sperm mass distally. With backward movements, the hydraulic pressure is released by the G2's endopodite collapsing in its flexible parts. In addition to this, the G2 of the studied pinnotherids was observed to interlock with the internal suture of the G1. This clearly improves the sealing of the basal

opening and the internal suture of the G1 towards the outside. Again, the interlocking might also function in keeping the G2 in position while moving, by acting like a guide rail.
Furthermore, the G2 is supposed to interact with the penis, respectively with the lumen where the penis is inserted. The basal lumen in the G1 is split into two cavities for the G2 and the penis. The lumina where G2 and penis are inserted during copulation are only separated by a flexible flap. If the G2 moves inside the G1, it presumably presses with its bold cuticle face against that flap whereby the lumen of the penis is constricted. This might additionally contribute to the sealing of the hydraulic system, but it is also possible that the flexible flap directly interacts with the penis by pressing against it. Williamson (1900) observed such an interaction of the G2 and the penis in *Cancer pagurus*: the G2 pressed against the penis with every movement, whereby the sperm mass is conveyed into the tube of the G1.
The present results show, that the G2 of pinnotherids is reduced in size, but still essential for the function of the copulatory system in sperm transfer, since the G2 is precisely adapted to the basal lumen of the G1, it specifically interacts with. Among other thoracotremes, only sparse data on copulatory systems are available. The G1s of fiddler crabs of the genus *Uca* are figured in Crane (1975). Next to traditional taxonomic characters of the G1, she also displayed the course of the ejaculatory canal and cross-sections of the distal part of the G1. Lautenschlager et al. (2010) investigated the fine structure and histology of the G1 in *Uca* spp., Jennings et al. (2000) the fine structure of the G1 in *Macrophthalmus hirtipes*. However, comparative morphology of the G2s and their functional aspects is not presented in the literature.

Pleopod Tegumental Glands

Rosette-shaped ‘pleopod tegumental glands’ (PTG) have so far been found inside the first gonopods (G1s) of all investigated brachyuran crabs (Spalding 1942, Diesel 1989, Beninger and Larocque 1998, Brandis et al. 1999). The PGTs are ultrastructurally similar to functionally divers tegumental glands of crustaceans (Johnson and Talbot 1987, Schmidt et al. 2006). They are generally composed of a central cell, numerous secretory cells arranged in a rosette and one canal cell that leads though cuticle pores (Talbot and Demers 1993). The PTGs have been demonstrated to communicate with the ejaculatory canal via ducts passing through cuticular pores, and concluded that their secretions are involved in the process of sperm transfer (Spalding 1942, Beninger and Larocque 1998). As in the studied pinnotherids, the PTGs are generally grouped around the basal lumen of the G1. Distally, they extend as far as the inserted G2 (Spalding 1942, Diesel 1989, Beninger and Larocque 1998, Brandis et al.

1999). The function of the PTGs in the G1 of the Brachyura is still under debate. Spalding (1942) suggested that their secretions form the spermatophores, which is presently clearly disproved, since completed spermatophores are already found in the vas deferens (Adiyodi and Anilkumar 1988, Beninger et al. 1988, Diesel 1989). A prevalent idea is that secretions of the PTGs contribute to the formation of the so-called 'sperm plug' - a structure of hardened secretions often found to plug the female ducts after copulation (Williamson 1900, Spalding 1942, Bawab and El-Sherief 1989, Ryan 1967a, Cronin 1947). The sperm plug was supposed to prevent the loss of sperm or the entry of sea water in impregnated females (Williamson 1990), but it also plays a role in sperm competition by closing the female's genital openings in order to inhibit subsequent copulations. In the spider crab *Inachus phalangium*, the sperm received from several matings are separated by layers of sperm plugs inside the spermatheca, which suggests that males from consecutive copulations seal off rival sperm masses (Diesel 1990, 1991).

However, in the investigated pinnotherid species, sperm plugs were not present in the female vaginae or spermathecae (see Becker et al. 2011, chap. 4), which is also the case in a number of other crabs that still have the PTGs in their G1s. Johnson and Talbot (1987) identified at least two different types of secretory cells in the PTGs. Beninger and Larocque (1998) showed by biochemical assays that the composition of the substances secreted by the PTGs varies among species. They concluded that only some might contribute to the sperm plug and other function in the protection of spermatophores from opportunistic microbes. Furthermore, secretions may function directly in the transport of the sperm inside the G1. Beninger and Larocque (1998) proposed that they might act as a lubricant to reduce mechanical wear of the ejaculatory canal by G2 or by reducing the viscosity of the ejaculate as it enters the narrow ejaculatory canal. The secretions of the PTGs may also help in building up pressure inside the tubular system of G1, which is necessary for the transport of sperm. Besides, they may also just contribute to seminal plasma or somehow function inside the female spermatheca. But the spermatophores are already embedded in seminal plasma produced by the vas deferens and the PTGs secrete directly into the ejaculatory canal, respectively the basal lumen in the G2. That is why we rather suppose its function in the process of transmitting sperm.

Sperm Morphology

The spermatozoal ultrastructure of the studied pinnotherids conforms to typical thoracotreme spermatozoa in the nucleus forming numerous nuclear arms and a convex operculum that possesses an apical button (see Jamieson et al. 1995). The ultrastructure resembles the spermatozoa of *Pinnixa* sp., the only pinnotherid spermatozoa previously investigted (Reger 1970, Krol et al. 1992). However, an ‚onion ring' lamellation of the outer acrosome zone, which is also considered a typical thoracotreme character (Anilkumar et al. 1999), is missing in the studied European pinnotherids and in *Pinnixa* (Reger 1970, Krol et al. 1992).

The concentric zonation of the acrosome distinguishes the species *Nepinnotheres pinnotheres* and *Pinnotheres pisum*. In *N. pinnotheres* three zones are present, in *P. pisum* only two. Certainly, the acrosome zonation is also different in closely related species of the genus *Uca*. Cuartas and Sousa (2007) observed two divisible zones in *Uca uruguayensis*, while Benetti et al. (2008) found three zones in *Uca maracoani*, *U. thayeri* and *U. vocator*.

A circular structure of very low electron density is associated with the operculum in both species: a periopercular rim in *N. pinnotheres* and a subopercular rim in *P. pisum*. The ‘subopercular rim' was first described for the hymenosomatid *Odimaris pilosus* (Richer de Forges et al. 1997), but not applied in literature on spermatozoa since then. However, the periopercular rim was observed in the heterotremes *Potamonautes perlatus*, *Calocarcinus africanus* (Jamieson et al. 1995) and in the thoracotreme *U. uruguayensis* (Cuartas and Sousa 2007). Benetti et al. (2008) claim the absence of a periopercular rim in *Uca* spp.. Still, in the presented ultrastructure a subopercular ring of very light electron density is obvious, which is not mentioned nor discussed in their study. So, not only nomenclature of spermatozoel characters, but also their interpretation is diverse in literature. Klaus et al. (2009a) reasonably treated the peri- and subopercular rim as one structure of very light electron density, which can be beside and/or below the operculum. It is generally difficult to homologize spermatozoal characters. Also, the function of distinct structures is not yet understood. The same applies for the accessory opercular ring we found in both investigated pinnotherid species. It is also present in the heterotremes *Pilodius areolatus* and *Calocarcinus africanus* (Jamieson et al. 1995). Anilkumar et al. (1999) observed the accessory opercula ring in *Metopograpsus messor* and estimated its presence as a typical character for Grapsidae, which must be rejected with reference to our results. The uniform acrosomal morphology in the so far investigated pinnotherids apparently argues against its use as a diagnostic character on genus level within the Pinnotheridae. Moreover, the brachyuran acrosomal characters can be subject to convergent character evolution within families (Klaus et al. 2009a).

Spermatophores

Brachyurans have spherical spermatophores that contain numerous spermatozoa in most species, referred to as coenospermia (e.g. Krol. et al 1992). Cleistospermia, a rare case where spermatophores only include one sperm, is reported for some freshwater crabs (Guinot et al. 1997, Klaus et al. 2009a, Klaus and Brandis 2010). The spermatophore walls are acellular and smooth (Krol. et al 1992). The spermatophore wall consists of a varying number of layers of different electron densities, ranging from one to five (Spalding 1942, Subramonium 1993, Hinsch 1988b, Cuartas and Souza 2007, Klaus et al. 2009a). The spermatophore pellicle consist of mucopolysaccharid, the outer layer was reported to be chitinous in some species (Spalding 1942, Subramonium 1993). In *N. pinnotheres* and *P. pisum*, the spermatophore wall consists of only one layer, which is similar to *Pinnixa* sp. (Krol. et al. 1992) and the spider crabs *Libinia* and *Ovalipes* (Hinsch 1986). Again, in *Uca uruguayensis*, the spermatophore wall consists of two layers (Cuartas and Sousa 2007).

Internal Reproductive Structures

The vasa deferentia of crabs are mostly divided into three sections (George 1963, Hartnoll 1975, Hinsch and Walker 1974, Hinsch 1988a, Martins Garcia and Feitosa Silva 2006, Erkan et al. 2009, Simeó et al. 2009). However, some authors define more than three, up to 10 zones (see Ryan 1967a, Krol et al. 1992). Spermatophores are formed in the proximal part of the vas deferens, while the medial and distal vas deferens stores spermatophores and produces seminal fluids (Adiyodi and Anilkumar 1988, Beninger et al. 1988, Diesel 1989, Johnson 1980). The vas deferens of pinnotherids conforms to other brachyurans in being lined with glandular epithelia. The secretions supposedly contribute to spermatophore formation and seminal plasma (e.g. Hartnoll 1975, Siméo et al. 2009). We found at least two types of secretions in the vas deferens, while Erkan et al. 2009 only observed one type in *Eriphia verucosa*. Again, Simeó et al. (2009) found three different types of secretions, two of them involved in the formation of spermatophores.

Conspicuously, the medial vas deferens of the studied pinnotherids was highly enlarged compared to other brachyurans (see Grobben 1878, Ryan 1967a, Beninger et al. 1988, Martins Garcia and Feitosa Silva 2006, Castilho et al. 2008), And especially, the appendices or appendices of the distal vas deferens, which produce and store seminal plasma, were noticeable.

Secretion of Seminal Plasma

Brachyurans generally secrete large quantities of seminal plasma (Hartnoll 1975, Subramoniam 1993). Usually, the secretion only occurs in the continuous tubes of the medial and distal vas deferens (Grobben 1878, Cronin 1947, George 1963, Ryan 1967a, Hinsch and Walker 1974, Castilho et al. 2008, Erkan et al. 2009, Santos et al. 2009), but not in special appendices as in the studied pinnotherids. However, in the portunid *Callinectes sapidus* (Johnson 1980) and in the spider crabs *Maja brachydactyla* (see Simeó et al. 2009) and *Chionoecetes opilio* (Beninger et al. 1988), the distal part of vas deferens possesses diverticula that produce and store seminal plasma. These 'secretory accessory glands' (Simeó et al. 2009) consist of small diverticula of the distal vas deferens and occur along most of its length, which contrasts with our results. The distal vas deferens of the studied pinnotherids holds appendices that are less numerous, but form large sac-like structures. They are originated at a restricted section of the distal vas deferens. In pinnotherids, the appendices occupy a good part of the male cephalothorax ventrally and also slightly extend into the pleon, which is so far exceptional among brachyurans. However, Martins Garcia and Feitosa Silva (2006) observed similar appendices in the distal vas deferens of the mangrove crab *Goniopsis cruentata*, which are considerably smaller than in the studied pinnotherids, but appear at the same place.

The exact function of the secretion within the appendices of the distal vas deferens remains elusive. However, the advantage of special eversions in the distal vas deferens is obvious. Certainly, the secretion of seminal plasma increases with the extension of the secretory surface. In addition to this, the space for storage of seminal plasma enlarges. In the studied pinnotherids, the quantity of secretion and the room for storage is vast.

The seminal plasma is heterogene among and within species (Spalding 1942, Hinsch and Walker 1974, Jeyalectumie and Subramoniam 1987, 1991). For example, the seminal plasma of *Callinectes sapidus* is rather homogenous (Johnson 1980). Again, in the pinnotherid *Pinnixa* sp., the secretions are combined of vesicles embedded in a heterogeneous matrix, just like in the investigated pinnotherids (Krol et la. 1992). In Simeó et al. (2009), the seminal plasma also appears similar to our findings.

The function of seminal plasma is not yet entirely understood, but its heterogeneity within species already suggests that it assumes several functions. One obvious reason for seminal plasma is the immotility of brachyuran sperm. Thus, spermatophores are diluted in a fluid matrix for the transfer. Several biochemical studies were conducted for the portunid *Scylla*

serrata. Ezhilarasi and Subramoniam (1982) already supposed that seminal plasma serves as nutrition for the metabolism and storage of spermatozoa in the male vas deferens and in the female spermatheca. They consider seminal plasma as particularly important in crabs with long sperm storage inside the female spermathecae. Jeyalectumie and Subramoniam (1991) conducted a biochemical study on the seminal secretion in *S. serrata* that revealed its role in the anaerobic metabolism of sperm inside spermathecae. Furthermore, an antibacterial activity of seminal secretion was found in *S. serrata* by Jayasankar and Subramoniam (1999).

All in all, the secretions are supposed to play a role in the storage and preservation of sperm(atophores) inside the male vas deferens, but also in the female spermatheca (Jeyalectumie and Subramoniam 1991, Beninger et al. 1993, Anilkumar et al. 1996, Jensen et al. 1996, Jayasankar and Subramoniam 1999), where sperm received from copulation is stored until ovulation. Inside the spermatheca, male seminal plasma mixes with products of the pleopod tegumental glands (PTGs) and with female secretions produced by glandular epithelia of the spermathecal wall. The mingling of male and female substances complicates biochemical approaches and comprehension on function. In the females of the European species, the secretory processes are more complex and efficient than in other brachyurans (Becker et al. 2011). That is why, the reproductive system is even more expanded inside the body compared to their males and other brachyurans.

Conclusions

Male pinnotherids are partly free-living and only occasionally found inside the host with the female. They are good pelagic swimmers by paddling with their walking legs (Hartnoll 1972). They presumably wander around in the ocean and visit numerous hosts in search for females to mate with. A special pairing or mating season was not observed in studies on population dynamics (see chap. 2). The vas deferens did not show seasonality according to its size or the presence of spermatophores and seminal plasma - even though samples from summer and winter were used for the present study. In contrast, several crabs were shown to have cyclic gonad maturation and presence of gametes synchronized with the season (e.g. Hinsch 1988a, Minagawa et al. 1993, Peres de Souza and Feitosa Silva 2009). The male pinnotherids seem to be ready to mate all year round, plus the quantity of sexual products and the room for their storage is vast (see above). Besides, pinnotherids have a sexual dimorphism. The adult female is in most cases considerably larger than the adult male and has a very broad pleon filled with ovary (see chap. 4). Thus, the large amount of male exual products may be complementary to the large spermathecae and the high reproductive output of the female.

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Overall Discussion

Review on Taxonomy

Three pinnotherid species were recorded for the European coasts according to the redescription (chap. 3, Becker and Türkay 2010): *Nepinnotheres pinnotheres* (Linnaeus, 1758), *Pinnotheres pisum* (Linnaeus, 1767), and *Pinnotheres pectunculi* Hesse, 1872. Characters that separated these species were the males' first gonopods (G1s) and their pleons. Apart from that, chelipeds were an important character, in particular owing to the consistency of characters in both sexes and among the different morphotypes of the female. Chelipeds differed in general shape, number and arrangement of teeth on the cutting edge of the claw, and in the bearing of setae.

N. pinnotheres (Linnaeus, 1767) and *P. pisum* (Linnaeus, 1758) are easily distinguished while *P. pectunculi* Hesse, 1872 is very similar to *P. pisum*. *Pinnotheres ascidicola* Hesse, 1872 and *Pinnotheres marioni* Gourret, 1888, formerly described as living exclusively in ascidians are junior synonyms of *N. pinnotheres* according to our morphological analysis (chap. 3, Becker and Türkay 2010). These two species entirely conform to *N. pinnotheres* from the Mediterranean pen shell, *Pinna nobilis* - except for size and slight variation of color. Their original authors did not thoroughly compare *P. ascidicola* and *P. marioni* with the earlier described *N. pinnotheres.* They probably expected a certain host-specifity and thence concluded that specimens in sea squirts must be distinct from species described from bivalves. Moreover, the fact that *N. pinnotheres* from ascidians is smaller than *N. pinnotheres* in *P. nobilis* (chap. 3, Becker and Türkay 2010) might have contributed to the introduction of the respective synonyms as well.

Host-Range

The results of the fieldwork conducted for the present study (chap. 2), together with the taxonomic results (chap. 3, Becker and Türkay 2010) provide information on the host-ecology of the European species. In the following, we discuss the hosts-range in broad outline and a number of ecological factors that might specifically determine the host-range of the European species in comparsion to other members of the family Pinnotheridae De Haan, 1933.

N. pinnotheres, P. pisum, and *Pinnotheres pectunculi* are not host-specific (chap. 2, 3). They rather have a certain range of hosts, summarized in table 6.1. This is also the case in other Western Atlantic pinnotherid species such as *Tumidotheres maculatus* (Say, 1818).

Table 6.1. Investigated host-range of the European species. *Nepinnotheres pinnotheres* was not found in the North Sea, while *Pinnotheres pectunculi* only occurred in the Northeast Atlantic (Brittany, France) (common names from: http://www.marinespecies.org).

	Nepinnotheres pinnotheres	***Pinnotheres pisum***	***Pinnotheres pectunculi***
North Sea		*Modiolus modiolus* (Linnaeus, 1758) horse mussel *Mytilus edulis* Linnaeus, 1758 blue mussel *Mactra stultorum* (Linnaeus, 1758) trough-shell *Spisula solida* (Linnaeus, 1758) thick trough-shell *Spisula elliptica* (Brown, 1827) elliptic trough-shell *Gari fervensis* (Gmelin, 1791) *Donax vittatus* (da Costa, 1778) banded wedge-shell	
Northeast Atlantic	*Ascidia mentula* Müller, 1776	*Mytilus edulis* Linnaeus, 1758 blue mussel *M. galloprovincialis* Lamarck, 1819 Mediter. mussel *Spisula solida* (Linnaeus, 1758) thick trough-shell	*Glycymeris glycymeris* (Linnaeus, 1758) dog cockle *Venus verrucosa* Linnaeus, 1758 warty venus *Venus casina* (Linnaeus, 1758) *Clausinella fasciata* (da Costa, 1778)
Mediterranean	*Pinna nobilis* Linnaeus, 1758 pen shell *Ascidia mentula* Müller, 1776 *? Ascidia virginea* Muller, 1776 *Halocynthia papillosa* (Linnaeus, 1767) *Microcosmos spp.* Heller, 1877 *? Phallusia mammilata* (Cuvier, 1815)	*Pinna nobilis* Linnaeus, 1758 pen shell *Ostrea edulis* Linnaeus, 1758 edible oyster *M. galloprovincialis* Lamarck, 1819 Mediter. mussel	
	In ascidians and in the bivalve *P. nobilis*	**In *P. nobilis* and many other bivalve species**	**In bivalves: *G. glycymeris* and venerids**

Figure 6.1. Selection of bivalves investigated from the North Sea (original size). Hosts of *Pinnotheres pisum* in red letters. Photographs: S. Tränkner, Senckenberg.

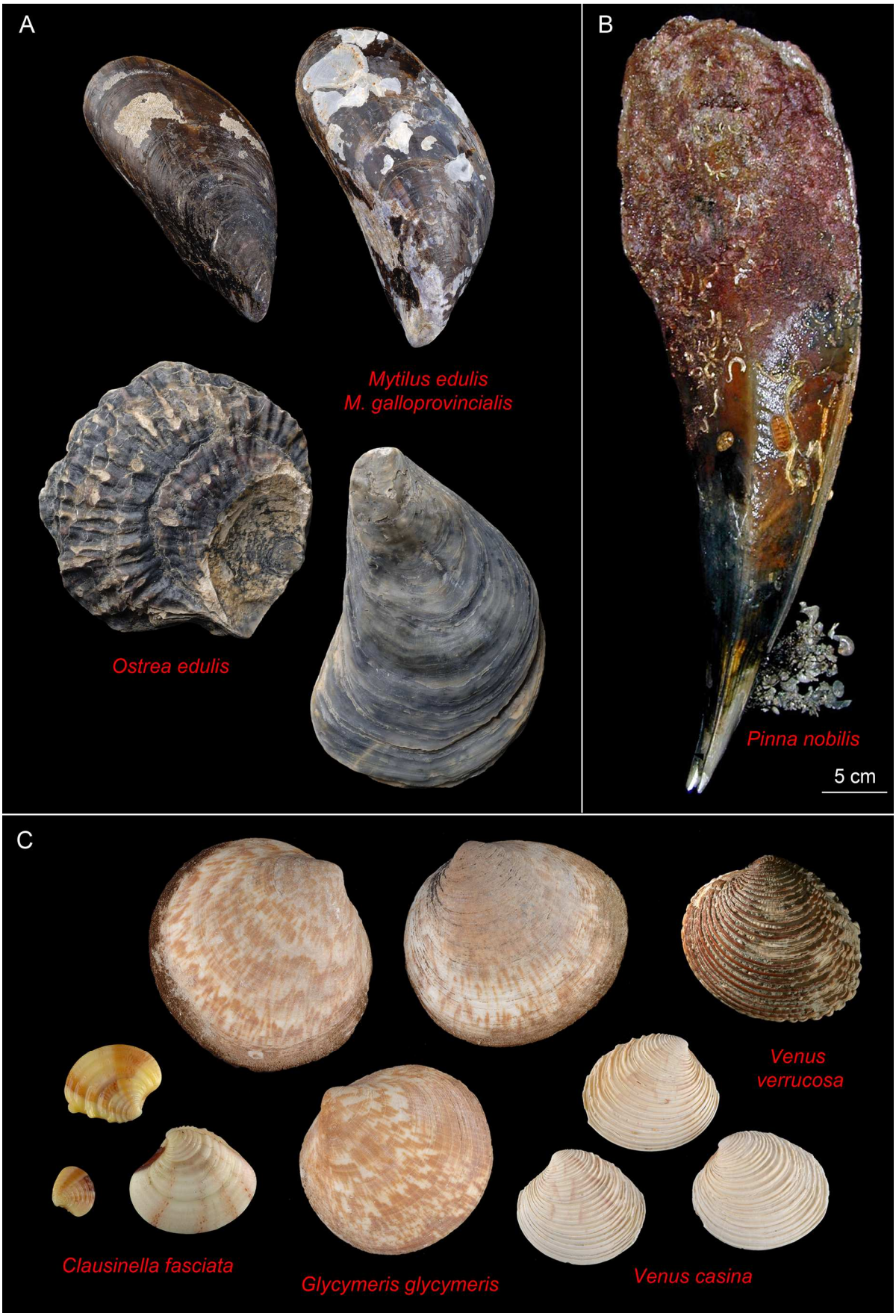

Figure 6.2. Bivalve hosts from the Northeast Atlantic and the Mediterranean (original size, except B). (**A**) Hosts of *Pinnotheres pisum* in the Mediterranen. (**B**) *Nepinntheres pinnotheres* and *Pinnotheres pisum* infest the Mediterranean pen shell. (**C**) Hosts of *Pinnotheres pectunculi*. Photographs: S. Tränkner, Senckenberg (A), most of (C); C. Becker (B); *Venus verrucosa* (C) H. Hillewart, VLIZ, Belgium, source: http://de.academic.ru.

The host-range includes not only bivalves (McDermott 1962a) but also gastropods (Williams and McDermott 2004). *Zaops ostreum* (Say, 1817) from the same region inhabits bivalves too, as well as the tubes of sessile polychaetes (Bezerra et al. 2006). Figure 6.1 and 6.2 show a selection of the investigated and infested bivalve hosts.

Nepinnotheres pinnotheres (Linnaeus, 1758) was found in the Mediterranean pen shell *Pinna nobilis* and in different solitaire ascidian species (tab. 6.1, fig. 6.3). Specimens from sea squirts were generally smaller than the ones from *Pinna nobilis* (chap. 3, Becker and Türkay 2010). The correlation of pea crab and host-size was not recognized by the original authors of *Pinnotheres ascidicola* Hesse, 1872 and *Pinnotheres marioni* Gourret, 1888. This relationship was demonstrated later in several pinnotherid species by Houghton (1963), Pearce (1964), Seed (1969), Pregenzer (1978), and Palmer (1995). For instance, *Zaops ostreum* (Say, 1817) fairly common on the American Atlantic coast, shows direct correlation between its own size and host dimensions (McDermott 1962a). *Tumidotheres maculatus* (Say, 1818), another species of the Western Atlantic, has several very differently sized hosts. Kane and Farley (2006) demonstrated that specimens from the large pen shell *Atrina rigida* are clearly larger than those from the smaller bay scallop *Argopecten irradians*. This trend was present in females but not among the partly free-living males (Kane and Farley 2006).

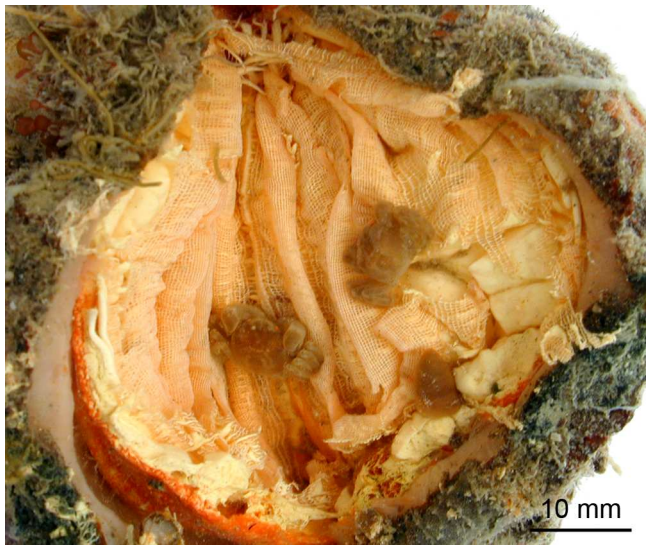

Figure 6.3. Pair of *Nepinnotheres pinnotheres* inside the gill gut of *Microcosmos* (dissected).

Next to differences in size, a certain variety of coloration is present in *N. pinnotheres*. Specimens from sea squirts were generally a little bit darker than pea crabs living in *P. nobilis*. Nevertheless, even among pea crabs from distinct ascidian species, color differed: specimens from *Halocynthia papillosa* and *Microcosmus* spp. were brownish (fig. 6.3) and

slightly darker than those from *Ascidia mentula*. During our fieldwork, *N. pinnotheres* was not found in *Phallusia mammilata* (chap. 2). However, colleagues reported findings, and records for *N. pinnotheres* in *P. mammilata* are also mentioned in the literature (Pesta 1918, Schmitt et al. 1973). Besides, some of the material from the crustacean collection of Senckenberg is labelled as originated from *P. mammilata* (chap. 3, Becker and Türkay 2010). Again, T.G. Honegger (Zoological Institute, University Zürich, Switzerland), pointed out that pea crabs were apparent to him in *Ascidia mentula* but not in *Phallusia mammilata* during his year long research on ascidians (T.G. Honegger, pers. com). Further samplings would be needed to come to a final statement on whether *P. mammilata* is a (regular) host to *N. pinnotheres*. The same applies for *Ascidia virginea*, which was labeled as host in two specimens investigated from the Senckenberg collection (chap. 3, Becker and Türkay 2010). Yet, it was not found to be infested according to our collections (chap. 2).
Next to its ascidian hosts, *N. pinnotheres* was found in just one bivalve species, namely *Pinna nobilis* - even though further bivalves are listed as hosts in the literature (Schmitt et al. 1973, Ingle 1983). Since the present study is based on a high number of specimens from various locations, we exclude the incidence of *N. pinnotheres* in bivalves, except for *P. nobilis* (chap. 2; chap. 3, Becker and Türkay 2010). The records of *N. pinnotheres* in other bivalve species are presumbly misidentifications, probably due to the former taxonomic confusion among European pinnotherids.

Pinnotheres pisum (Linnaeus, 1767) was found in various bivalve species during our fieldwork, but never in ascidians (chap. 2, tab. 6.1). This again, contrasts with the literature where ascidian hosts are listed (Lévi 1951, Schmitt et al. 1973). Taking into account the large number of samples from different locations our conclusions are based on, the incidence of *P. pisum* in ascidians can be excluded for sure. Besides *P. nobilis*, *P. pisum* occurs in commercially important bivalves, such as oysters and blue mussels, also relatively small species are infested, for instance, *Donax vittatus* (chap. 2, fig. 6.2).

For *Pinnotheres pectunculi* Hesse, 1872 formerly only known from the bivalve *Glycymeris glycymeris*, novel bivalve hosts were recorded during our fieldwork. While *P. pectunculi* shows high infestation rates in *G. glycymeris* (chap. 2), it was only occasionally found in the other, smaller bivalve species – interestingly enough all from the family Veneridae (tab. 6.1). The known distribution of *P. pectunculi* was formerly restricted to the Northern Atlantic coast of France around its type locality Roscoff/Brittany (Bourdon 1965, d'Udekem d'Acoz, 1988). Recent collections by C. d'Udekem d'Acoz have demonstrated that it has a wider distribution

around the coast of Brittany (chap. 3, Becker and Türkay 2010). The occurrence of *P. pectunculi* along the Northeast Atlantic coast south to its type-locality Roscoff needs further investigation by taking the novel host records into account.

The Mediterranean Pen Shell

The only overlap in the host-range of *Pinnotheres pisum* and *Nepinnotheres pinnotheres* is the pen shell *Pinna nobilis* (fig. 6.2), which was the most frequented host (chap. 2). During the fieldwork for the present study, most of the Mediterranenan pen shells have been occupied by either *N. pinnotheres* or *P. pisum*. *P. nobilis* was, however, not purposefully collected during fieldwork, because it is an endangered species and under strict protection according to the European Council Directive 92/43/EEC (Annex IV) and the national laws of most Mediterranean countries (Katsanevakis 2007). Nevertheless, it was obtained in low numbers as by-catch from fishermen (fig. 6.4). *Pinna nobilis* is endemic to the Mediterranean. It is not only the largest Mediterranean shell, but also one of the largest bivalves worldwide. It attains lengths up to 120 cm and has a life span of 20 years or more (Galinou-Mitsoudi et al. 2006).
In the past, pen shells were overfished to make use of its byssus as 'sea-silk' for ropes and textiles (Maeder and Halbeisen 2001), for eating, and to utilize its shell as decoration (Voultsidadou et al. 2010). Today, *P. nobilis* is mainly affected by nearshore bottom trawl fishery and due to the decline of its habitat, the *Posidonia*-sea grass meadows. Hughes et al. (2009) pointed out that the decline of sea grasses and consequently of *Pinna* might result in a threat for the associated fauna as the prawn *Pontonia pinnophylax*, which is a symbiont of the Mediterranean pen shell too. Since *N. pinnotheres* and *P. pisum* also infest other hosts, an ongoing decline of *P. nobilis* might not pose an immediate threat to these species. However, if host-choice is lineage specific (as suggested for *Pinnotheres novaezelandiae* Filhol, 1885; see Stevens 1990a), genetic depauperation within the two species is likely to occur.

Figure 6.4. *Pinnotheres pisum* ♂ in *Pinna nobilis*.

Host-Size

The reasons for pinnotherids' host suitability and preference for certain species are not yet understood. An obvious criterion is host-size: some hosts offer larger shelter than others. A preference for larger hosts is present within one host species (Haines et al. 1994, Hsueh 2003) but also among different hosts, which is demonstrated by the results of the present study (chap. 2). Accordingly, the largest host *Pinna nobilis* shows the highest infestation rates for *N. pinnotheres* and *P. pisum*. Moreover, *P. pectunculi* seems to prefer *Glycymeris glycymeris* rather than its smaller bivalve hosts (chap. 2). The importance of shelter space is standing to reason since female pinnotherids can grow bigger in larger hosts (as discussed above). In turn, bigger body sizes provide more space for ovaries, which results in a higher reproductive output (Hines 1992; chap. 4, Becker et al. 2011). Larger hosts also offer greater food resources. *P. pisum*, for example, can infest the bivalve *Donax vittatus* that only measures a few centimeters (fig. 6.1) and the giant *P. nobilis*. In comparison of these two hosts, it is obvious that the situation for the pea crab is strikingly different concerning the supply of food filtered by the host gills and accumulated in mucous strings. Hence, it is not surprising that *P. nobilis* is such a highly infested host (chap. 2). We also expect the damages caused by pinnotherids to be less harmful in such a large bivalve than in smaller hosts.

Factors of Host-Choice

Despite the large shelter space and the vast food supply in *Pinna nobilis*, we never found more than one pair of the same species within its mantle cavity. The incidence of *P. pisum* inside a host excludes the entry of *N. pinnotheres* and vice versa (chap. 2). Navarte and Saiz (2004) demonstrated that the infestation with one gravid female forecloses the intrusion of other females of the same species. Nevertheless, one host can hold several males together with one female (of one species) (Silas and Agarswami 1967, Sun et al. 2005). In *Arcotheres sinensis* (Shen, 1932), infesting *Mytilus galloprovincialis* in the Yellow Sea of China, one to six males were recorded from one mussel (Sun et al. 2005). This phenomenon generally goes alongside with high infestation rates in a bivalve population (Silas and Agarswami 1967). During the present study, this was observed once only in *Mytilus edulis* cultured on the West Atlantic coast of France. The infestation rate of these mussels bought from a fish market bordered 100 %. Once again, only one female per host was found, however, with one or two males. The infesting *Pinnotheres pisum*-specimens were obtained in December and seemed to be in their mating season. Many of the females were in the copulatory hard stage and in some

of the males, pleons were deflexed and their copulatory appendages uncovered. Regretfully, copulation was not observed.

A number of ecological factors obviously play a role in host-choice too, for instance the abundance and distribution of hosts. The significance of the depth from where hosts were collected has been thoroughly studied for *Pinnotheres pisum* inside *Mytilus edulis*, which is a typical and - in some habitats - highly infested host. Yet, the incidence of *P. pisum* inside *Mytilus*-beds in the tidal zone is a rare exception (M. Türkay, pers. obs.). Houghton (1963) and Haines (1994) demonstrated that infestation with *P. pisum* highly increases from intertidal to subtidal. This applies to other species, such as *Tumidotheres maculatus* (Say, 1818) (Kruczynski 1974) and *Pinnotheres novaezelandiae* Filhol, 1885 as well (Jones 1977). On the other hand, *Arcotheres* cf. *placunae* (Hornell and Southwell, 1909) was found at low tide, infesting the bivalve *Amiantis umbonella*, partially dug in the sediment in the Persiuan Gulf of Iran (Saeedi and Ardalan 2010). The occurrence of pinnotherids in the intertidal may depend on the climatic zone of their distribution. In temperate zones, such as the North Sea, variations of temperature are very extreme in the intertidal and may pose a problem to pinnotherids, whereas in tropical zones, the intertidal might be more suitable, because the temperature hardly fluctuates. In the mole crab *Upogebia* sp., a relationship between the habitat use and the climatic zone of distribution was demonstrated and revealed that the mole crabs only inhabit the intertidal of tropical and subtropical zones with relatively constant conditions in temperature, while they were not found in the intertidal of temperate zones (K. Sakai, pers. com).

In contrast to dense host aggregations, e.g. mussel beds, host-distribution can also be patchy as in the pen shell *Pinna nobilis* and in some other bivalves or ascidians. In addition, the abundance of pea crabs inside the hosts appears to be extremely low in some habitats. For example, during our fieldwork in Crete (Greece), only one single specimen of *Nepinnotheres pinnotheres* was found, although large numbers of sea squirts from the same sample site were collected. On the Dogger Bank of the North Sea, the distribution of hosts was patchy too and their infestation with *Pinnotheres pisum* so rare (chap. 2) that it is hard to figure how the pea crabs find their conspecifics in such habitats at all.

Host Recognition and Entry

For a successful completion of their life cycle, pinnotherids at first have to find and intrude a host. How a pea crab manages to enter a specific host is therefore of high interest and

contributes to understanding factors of host-choice. The entry behaviour has never been observed in the case of the European species, however, Eidemiller (1969) witnessed *Tumidotheres maculatus* (Say, 1818) intruding the bay scallop *Argopecten irradians concentricus*. The entry, as described by Eidemiller (1969), starts when the pea crab's legs touch the mantle of the scallop. As a reaction, the mantle gapes apart and opens fully, then closes abruptly but not completely. The crab mostly enters in this very instance. The gaping of the bivalve's mantle is actually a curling of the outer, more sensitive fringe of the mantle towards the point of stimulus (Eidemiller 1969). Only if the pea crab enters successfully, the scallop violently opens and closes its valves as long as the crab is on the mantle tissue. If the crab moves away from there, the scallop's reaction ceases (Eidemiller 1969). The scallop apparently reacts to the tactile stimuli, since the crab's touch evokes the response during which the crab is able to quickly crawl in. Touchless crabs (with disabled setae) were unable to enter, whereas blinded crabs successfully invaded hosts (Eidemiller 1969).

The intrusion of the host was also observed in *Pinnixa tumida* Stimpson, 1858 symbiotic to the holothurian *Paracaudina chilensis* (Takeda et al. 1997). The pea crab started the entry by touching the sea cucumber's tail with chelipeds and walking legs. The touch resulted in a widening of the host's anus, so that the endosymbiont could slowly crawl in (Takeda et al. 1997). Specimens of *Pinnixa* were observed to actually fight over a host if two crabs arrived at the holothurian tail at the same time (Takeda et al. 1997).

In both portrayed cases of host entry, the pinnotherids induce a reaction of the host by tactile stimulus, which facilitates the intrusion. The entry of a host still seems to be a critical event, since males of *Pinnotheres pisum* (which enter hosts repeatedly) were observed to often lack distal articles of walking legs (pers. obs.), probably, because they got squashed within the bivalve shells while trying to enter. Next to the already discussed factors of host-size, small hosts might also complicate the intrusion of a pea crab and, thus, contribute to the low infestation rates we observed in the small bivalve species (chap. 2).

Whether host recognition has a genetic basis or is a learned phenomenon was the matter of studies on New Zealand pea crabs (Stevens 1990b). *Pinnotheres atrinicola* Page, 1983 is considered host-specific to the fan mussel *Atrina zelandica*, whereas *Pinnotheres novaezelandiae* Filhol, 1885 is said to be a host-generalist. In behavioural experiments, it was not possible to induce a change in host recognition by conditioning crabs to novel hosts. Specimens of *P. novaezelandidae* extracted from *Mytilus edulis* were also, but less, attracted to *Perna canaliculus*, which is in its host-range too. These results suggest that populations of *P. novaezelandidae* from different bivalve species represent biologically discrete units with

different host recognition systems (Stevens 1990b). This is supported by a genetic differentiation between host races (Stevens 1990a).

Again, a study by Derby and Atema (1980) rather supports a plastic "chemical search image"-concept. They studied induced host odour attraction in *Tumidotheres maculatus* (Say, 1818) which is a host-generalist. In experiments with subadult posthard and adult crabs extracted from *Mytilus edulis*, the host odour induced movements towards the location of its source (Derby and Atema 1980). Odours from other previously recorded host species did not trigger the searching behaviour. However, adult crabs from *M. edulis* could be induced to respond to odour from *A. i. concentricus* too. Derby and Atema (1980) suggest that such specificity in response may be due to olfactory induction to their hosts.

Sastry and Winston Menzel (1962) studied host-choice in *T. maculatus* as well. The experiments conducted by using a circular choice apparatus showed a statistically significant attraction to both of their hosts *A. i. concentricus* and *Atrina rigida*. In addition, crabs removed from *A. i. concentricus* revealed no preference for one host rather than for the other. Sastry and Winston Menzel (1962) concluded that host attraction is due to chemotactic stimuli.

Yeater (1966) demonstrated that host-choice of *T. maculatus* between *A. i. concentricus* and *Atrina rigida* is influenced by temperature. *A. rigida* was preferred below 22°C, scallops at higher temperatures. This might be due to the seasonal distribution of scallops: they disappear when sea grass dies off during colder weather (Yeater 1966, as cited by Eidemiller 1969).

Ambrosio (2008) investigated chemoreception and behaviour in *Tunicotheres moseri* (Rathbun, 1918) inhabiting ascidians. Interestingly, males responded only to non-gravid females, but not to ovigerous females or to males. The pea crabs also reacted to host-generated cues in the water column, but they did not show a preference for one of the offered ascidian host species (Ambrosio 2008).

The conspecific recognition and host-choice were also studied in *Pinnixa chaetopterana* Stimpson, 1860 from the subfamily Pinnothereliinae Alcock, 1900, symbiotic with sessile polychaetes (*Chaetopterus variopedatus*, *Amphitrite ornata*; see Grove and Woodin 1996). Neither sex showed any attraction to one of its hosts alone. Instead, they were significantly attracted to isolated conspecifics. Yet, crabs collected from *Amphitrite* were significantly attracted to *Chaetopterus*-hosts, which contained a couple of congeners. Interestingly, the competing symbiotic crab *Polyonyx gibbesi* (Anomura: Porcellanidae) was avoided by females but not by males. Grove and Woodin (1996) assume that the attraction to conspecific odours increases chances of finding a suitable mate already present inside the host.

Feeding Strategies

Since Orton (1920) already observed the chelipeds as being involved in feeding of pinnotherids, we did not only study them from a taxonomic view (chap. 3, Becker and Türkay 2010) but also in regard to function in feeding. The fine structure of chelipeds was investigated by scanning electron microscopy (SEM). While the chelipeds of *Nepinnotheres pinnotheres* are pilose all over by short plumose setae (fig. 6.5), the chelipeds of *Pinnotheres pisum* and *Pinnotheres pectunculi* possess a comb of setae ventrally on the claw (fig. 6.6). To reveal its function, we conducted behavioural studies in the aquarium (Becker and Türkay, in prep.). We kept adult females of both *Pinnotheres*-species inside their bivalve hosts with one shell removed to allow observation. The dissected bivalves had to be exchanged on a regular basis, because they died about 24 hours after being deprived of one valve.

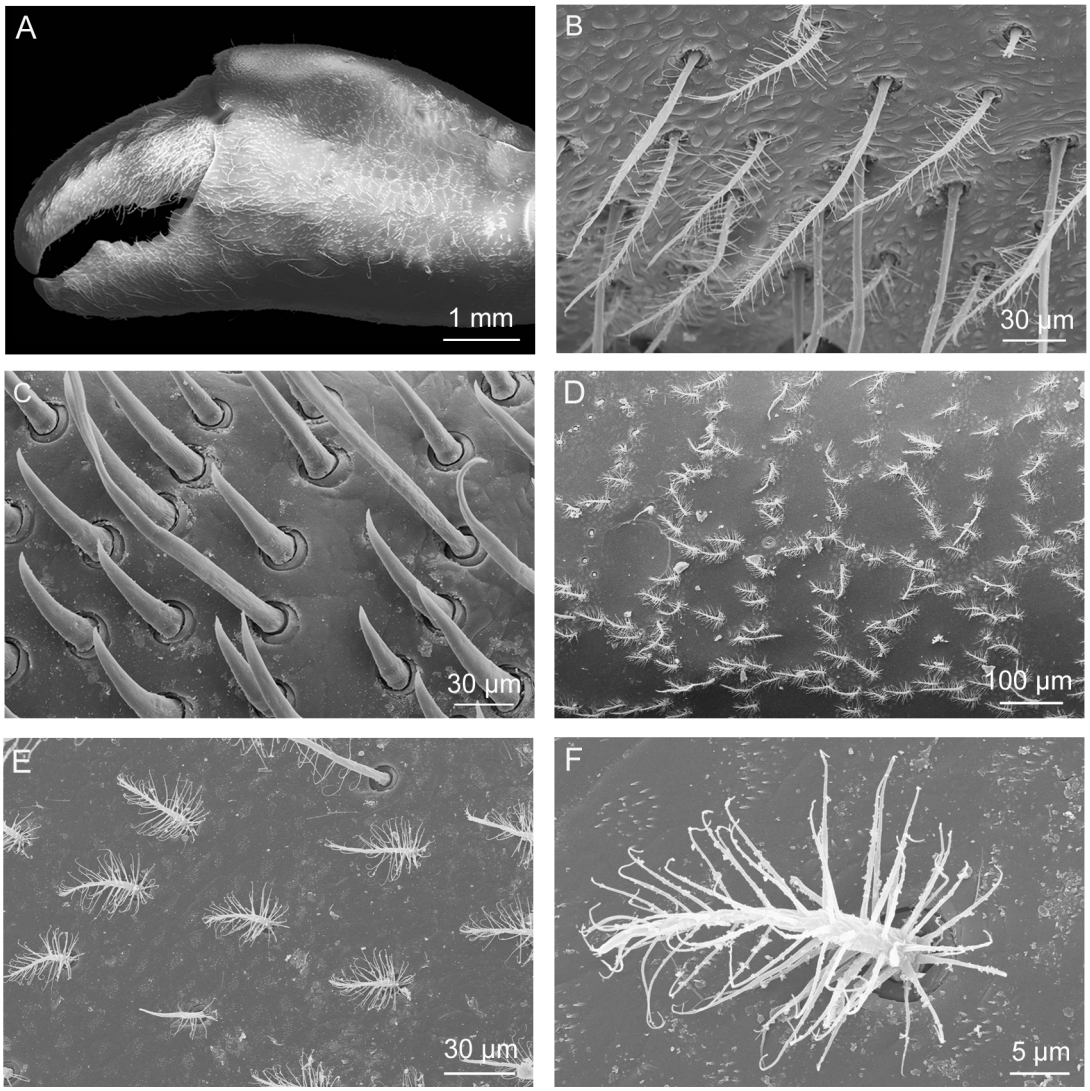

Figure 6.5. Cheliped of adult female *Nepinnotheres pinnotheres* (SEM). (**A**) Claw with fixed and movable finger. (**B**) (**C**) (**D**) The whole surface is setose by different setae types. (**E**) Short plumose setae that cover the whole body. (**F**) Higher magnification of plumose setae showing fine setules.

P. pisum and *P. pectunculi* appeared not to be affected by the handicap of their hosts *Ostrea edulis* and *Glycymeris glycymeris* and feeding was repeatedly observed during the experiments (Becker and Türkay, in prep.). Both species fed by brushing the bivalve gills with the setae comb on the bottom side of the claw. Mucus strings from the gills stick to the pappo-serrate setae (fig. 6.6) and are conveyed towards the mouth opening (fig. 6.7), where the setose third maxillipeds take over the mucus strings (fig. 6.8).

A number of pinnotherids actually possess a setae comb ventrally on the claw similar to that described for the European *Pinnotheres*-species (see Manning 1993a, Campos 1996b, Ahyong and Ng 2007; fig. 6.9), but low attention was paid to this character so far. In other pinnotherid species, such as *Fabia subquadrata* Dana, 1851, walking legs assist feeding (Pearce 1966).

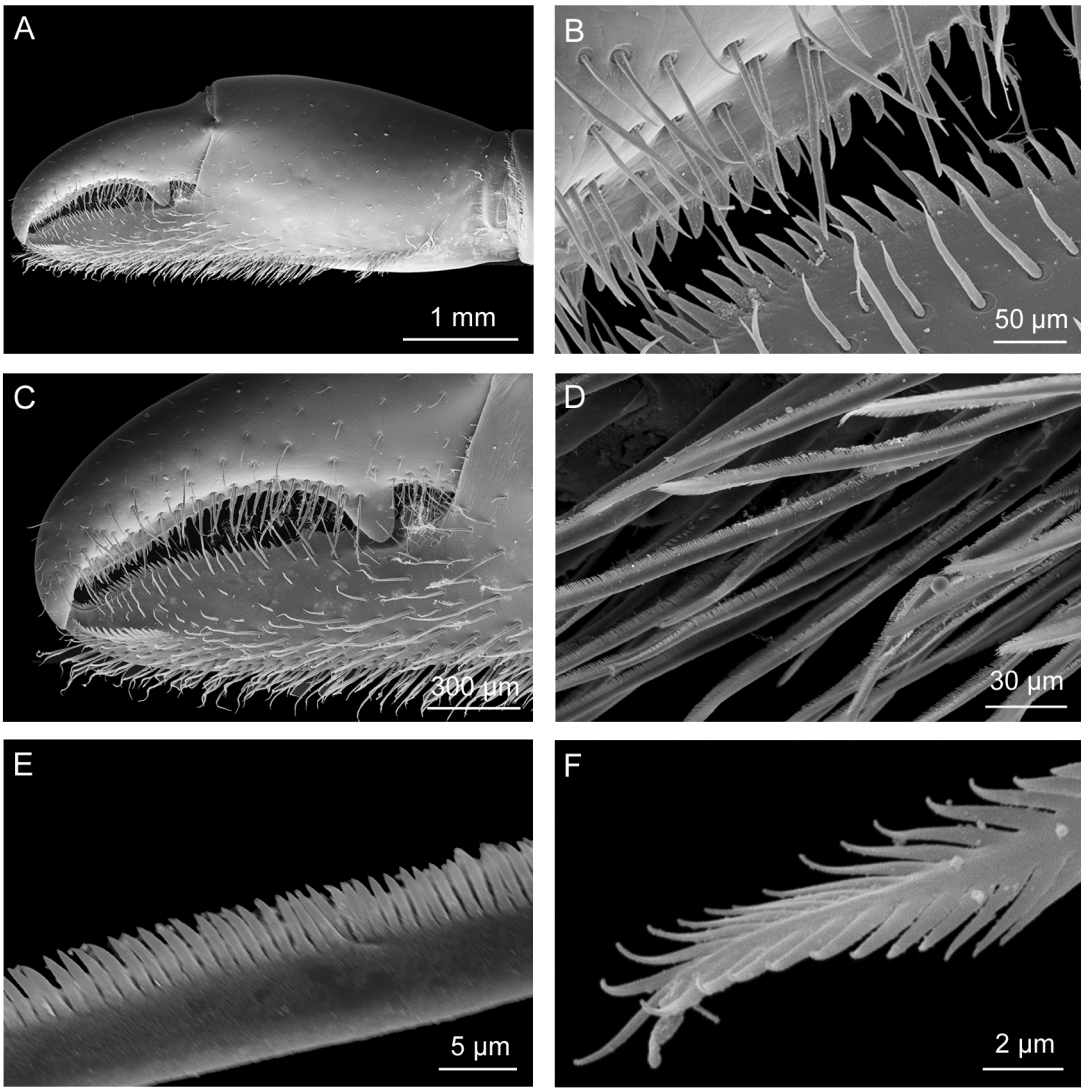

Figure 6.6. Cheliped of adult female *Pinnotheres pisum*. (**A**) Palm of right cheliped showing setae comb. (**B**) Soft denticules and simple setae on cutting edge of claw. (**C**) Fixed (propodus) and movable finger (dactylus) of the claw showing setation. (**D**) Setae comb consisting of long regularly orientated pappo-serrate setae. (**E**) Higher magnification on setulation of setae shaft. (**F**) Distal tip of pappo-serrate setae.

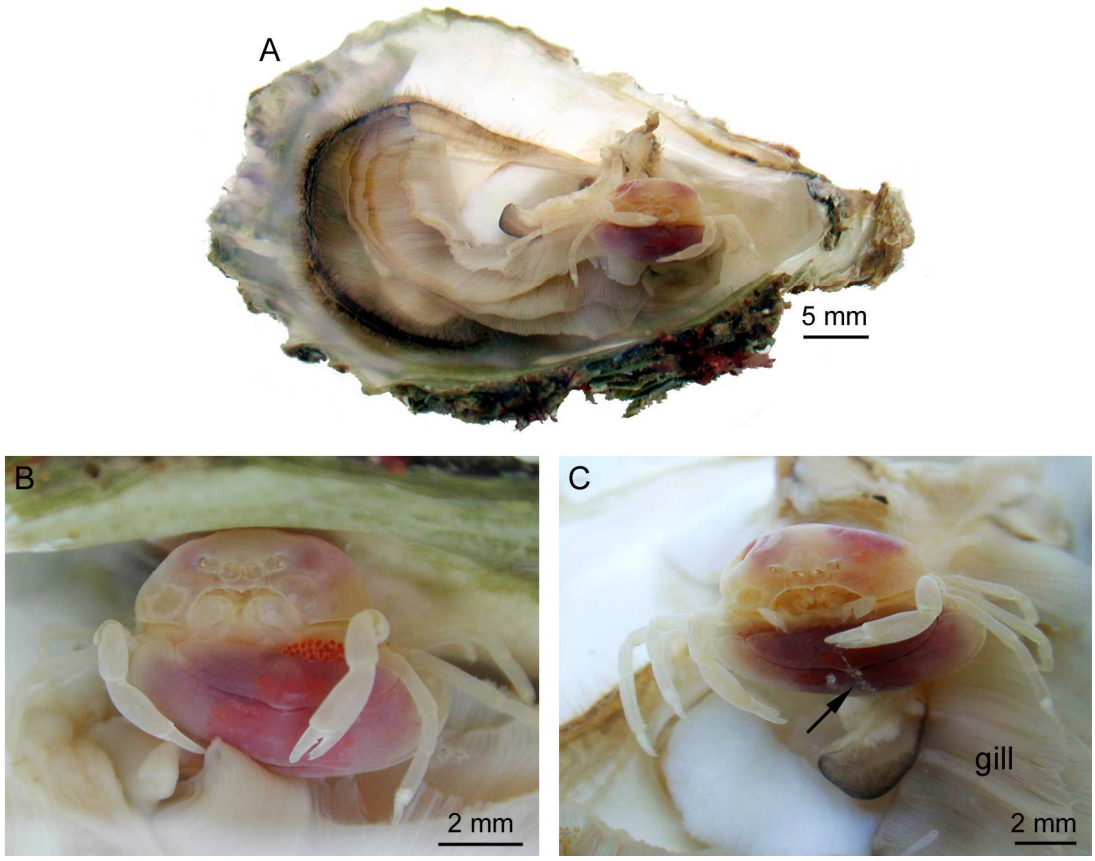

Figure 6.7. Feeding of *Pinnotheres pisum*. (**A**) Adult female of *Pinnotheres pisum* in oyster, *Ostrea edulis*, with right valve removed. (**B**) Chelipeds are oriented ventrally by a distortion of the carpus (**C**) Mucus strings are picked up by setae comb of the claw (black arrow on mucus string).

One pair of walking legs or a single leg can be elongated (Pearce 1966, Campos 1996b) in order to grab the mucus strings (Caine 1975). The relative length of pereiopods and their asymmetry have been important characters among pinnotherids since Bürger's (1895) "Ein Beitrag zur Kenntniss der Pinnotherinen" (see also Gordon 1936, Griffin and Campbell 1969, Campos and Manning 2001, Campos 2002).

The development of asymmetry in the third pair of pereiopods was studied in *Arcotheres alcocki* (Rathbun, 1909) (Watanabe and Henmi 2009). The incidence of left- and right-handed crabs is correlated with their placement on the left or right valve of its bivalve host *Barbatia virescens*, which settles on either shell. Watanabe and Henmi (2007) concluded that the asymmetry develops in dependence of the feeding position and the elongated pereiopod is supposed to pick up the mucus from the bivalve gills.

In *Nepinnotheres pinnotheres* feeding behaviour was not observed during the present study (fig. 6.10). Living specimens were hardly available due to the low infestation rate in ascidians and the strict protection of its sole bivalve host *Pinna nobilis*. Since *N. pinnotheres* lacks the specific setae comb on the claw, feeding must be different from the investigated *Pinnotheres*-species. The chelipeds of *N. pinnotheres* as well as the whole body surface are pilose by short

setae. Furthermore, *N. pinnotheres* has elongated dactyli in the fourth pair of walking legs, characters shared by the very similar genus *Tumidotheres* Campos, 1989. Kruczynski (1975) studied feeding in *Tumidotheres maculatus* (Say, 1818) by marking phytoplankton with radioactive tracers to estimate its food uptake. He compared clawed crabs with clawless crabs with the result that the latter gave no evidence of food uptake. However, clawed crabs could also feed on phytoplankton from Petri dishes by picking planktonic organisms with the chelae from the bottom of the dishes and continuously clean themselves (Kruczynski 1975). In contrast to that, adult females inside a bivalve host, initially grasped mucus with the last pair of walking legs, which possess elongated setose dactyli (Caine 1975). A similar way of feeding is plausible for *N. pinnotheres* that possesses the same characters as *T. maculatus*: elongated dactyli in the fourth pair of walking legs and a general pilosity of the body.

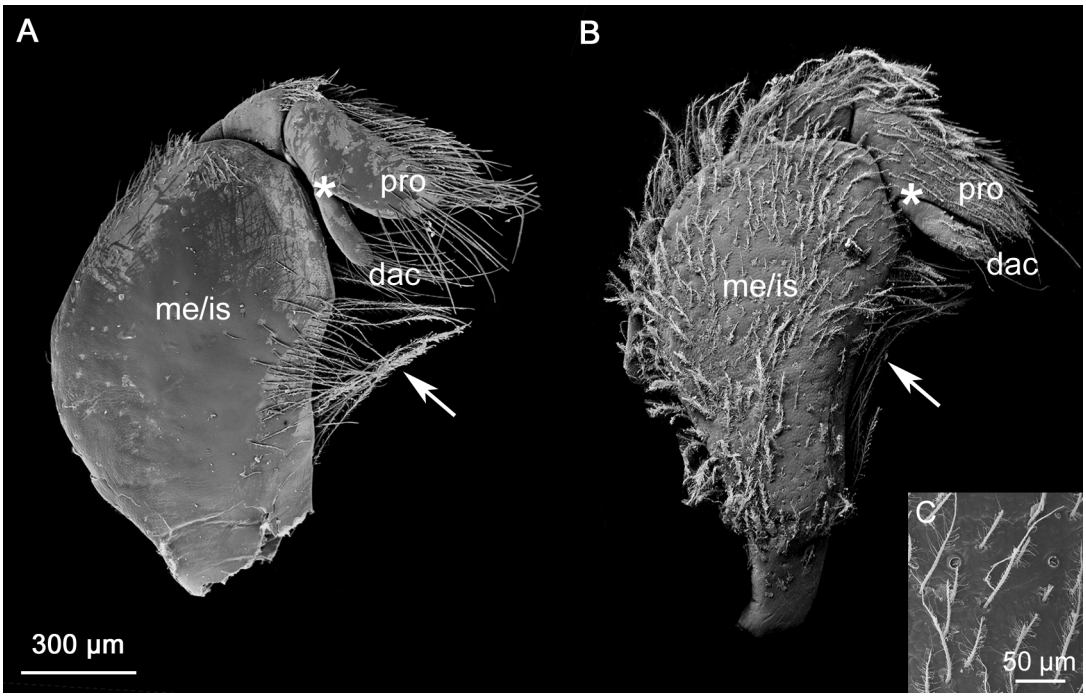

Figure 6.8. Third maxillipeds of *Pinnotheres pisum* and *Nepinnotheres pinnotheres* (SEM). (**A**) *Pinnotheres pisum* (adult female) with smooth merus/ischium-article. (**B**) Pilose third maxilliped of *Nepinnotheres pinnotheres* (adult female). (**C**) Setation of merus/ischium-article in *N. pinnotheres*. White arrows on setose inner margins of merus/ischium-article; white asterisk on insertion of dactylus on propodus. dac = dactylus; me/is = fused merus/ischium; pro = propodus.

An ascidian host may require a different entry strategy from that of a bivalve host. Yet, the hosts are similar in being suspension feeders by filtering organic matter with their gills from the seawater and accumulating food particles in a mucous secretion. However, a difference is still present for the inhabiting pinnotherid in its location inside the host. While the pea crab sits *on* the gills inside a bivalve, they are surrounded by the gills in an ascidian host. According to this, the pilositiy observed in *Nepinnotheres pinnotheres* may be advantageous

for symbionts in ascidians, if the mucus attached to the whole setose body surface is obtained by a constant cleaning as studied in *T. maculatus* (Kruczynski 1975). The pilosity of *N. pinnotheres* actually hampered SEM-observations for the present study. Specimens of *N. pinnotheres* were covered with debris allover, whereas the investigated *Pinnotheres*-species from bivalves were smooth and clean (pers. obs.). Actual observations on food uptake in *N. pinnotheres* could probably be achieved by using endoscopy in future studies.

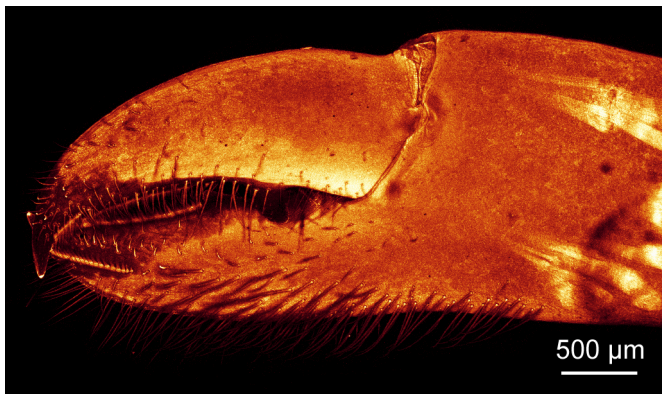

Figure 6.9. Claw of adult female *Arcotheres* cf. *placunae* with seate comb. 3D- projection of clsm-series showing the setae comb.

Not only feeding technique but also the impact on the host has not yet been searched out for *N. pinnotheres* (fig. 6.10). To date, effects on hosts have in the first place been studied for bivalves of commercial interest (chap. 1). Consequences for ascidian hosts are unknown and only sparse information is presented in the literature on the nature of other pinnotherid relations.

Pinnixa tumida from the subfamily Pinnothereliinae Alcock, 1900 lives in the anus of sea cucumber *Paracaudina chilensis*. There, the crab feeds on mucus secreted by the host and suspended food particles (Takeda et al. 1997). This way of feeding speaks for a parasitic relationship.

In the Pinnothereliinae *Pinnixa chaetopterana* Stimpson, 1860 inside the tubes of the polychaete *Chaetopterus variopedatus*, an effect on the pumping activity of the host was observed. Despite that, the pea crab did not influence growth rates of the host (Grove et al. 2000). Again, sand dollars of the genus *Mellita*, harbouring the pinnotherid *Dissodactylus mellitae* (Rathbun, 1900), showed a lower egg production than sand dollars without crabs (George and Boone 2003).

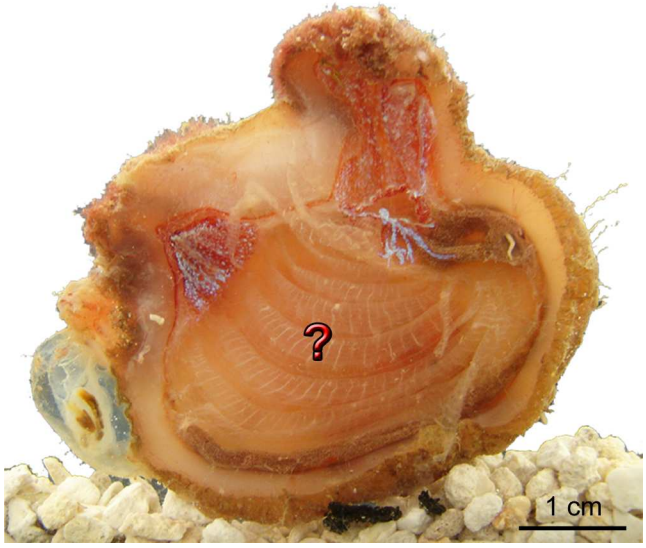

Figure 6.10. Dissected solitaire sea squirt (*Pyura* sp.) showing gills gut. Feeding of pea crabs inside ascidians is unknown.

Larval Morphology

The zoea of *Pinnotheres* and other genera of Pinnotherinae De Haan, 1833 are exceptional in lacking a dorsal spine (Lebour 1928a, 1928b, Atkins 1954, Rice 1975). The larvae of *Pinnotheres pectunculi* were unknown at the start of the present study. The larval morphology is shown in figure 6.11. These larvae were obtained from an ovigerous female inside *Glycymeris glycymeris* (leg. Thomas Wehe, Senckenberg), kept in an aquarium. The larval morphology (zoea 1) is very similar to *P. pisum* through the trilobed telson and the absence of a dorsal spine (Atkins 1954). However, the European *Pinnotheres*-species are distinct since the zoea of *P. pectunculi* has two pairs of lateral spines while *Pinnotheres pisum* only has one pair (Lebour 1928a, 1928b, Atkins 1954, Rice 1975). *Nepinnotheres pinnotheres* possesses lateral and dorsal spines (Lebour 1928a, b) and, thus, rather resembles the typical brachyuran zoea.

Dorsal spines in brachyuran zoea are often seen as antipredatory adaptation (Morgan 1987). The small and spineless zoea of *Zaops ostreum* (Say, 1817) relied on behavioural instead of morphological antipredatory defense in experiments. Attacked by a fish, larvae flexed their abdomen against the body, became motionless, and sank, resembling anorganic matter (Morgan 1987). Long spines actually constitute a disadvantage by being disruptive in the release of larvae from a host. Accordingly, the lack of spines might be an adaptation to the symbiotic way of life (P.F. Clark, pers. com.). However, some symbiotic pinnotherids possess the dorsal spines whereas free-living brachyurans from the families Leucosiidae and Hymenosomatidae lack the dorsal spine as well.

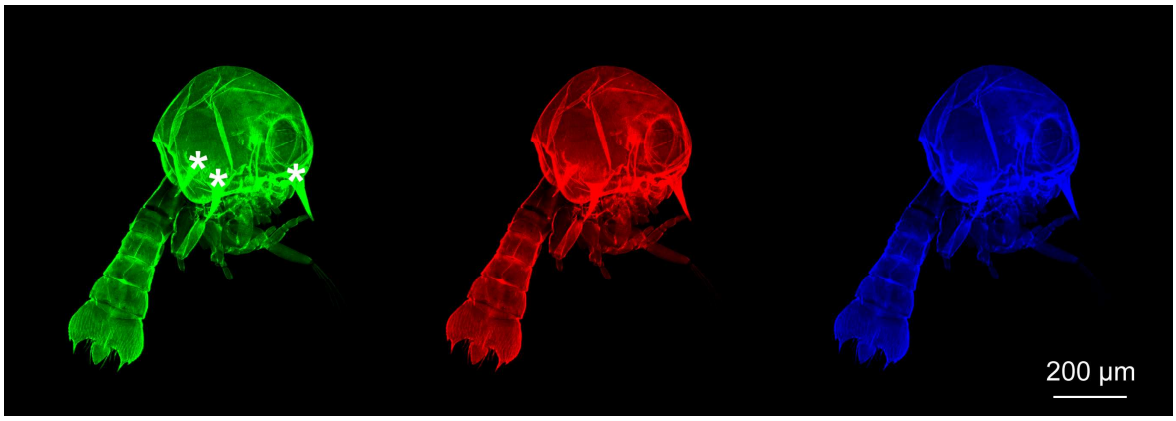

Figure 6.11. Larval morphology (zoea 1) of *Pinnotheres pectunculi*. The same CLSM-projection is shown in three different colors. The telson is trilobed. A dorsal spine is not present but two pairs of lateral spines and one rostral spine. White asterisks on spines in green image.

Larval spines can also promote hovering during larval dispersal. A reduction of spines may therefore facilitate a settlement in close distance to the parental host where larvae are released. This might be beneficial for pinnotherids, which infest hosts that live in aggregations e.g. in mussel beds. Hence, they might settle close to their place of birth in such habitats. Dispersal and gene drift between populations is still possible. Males and juvenile females before metamorphosis are capable to actively swim by paddling with their second and third walking legs, which bear long setose swimming fringes (Hartnoll 1972).

The larval development is generally slightly abbreviated in pinnotherids having only two to four larval stages compared to other brachyurans with five zoea stages. The larval development of *Tunicotheres moseri*, symbiotic to *Ascidia nigra*, is considerably abbreviated, which was supposed to be advantageous in preventing larvae in dispersing too far from the host colony by Goodbody (1960).

While the European species only brood until the larvae hatch, *Tunicotheres moseri* performs a parental care beyond that (Bolaños et al. 2004): the larvae remain under the female's pleon up to the first crab stage. An abbreviated and rapid larval development is also present in *Orthotheres barbatus* with only two larval stages, which develop into first crab stages in just four days (Bolaños et al. 2005).

Male and Female Internal Reproductive Systems

The pinnotherids' reproductive morphology shows characters typical for thoracotreme brachyurans but also features, which are new to date and unique for pinnotherids so far (chapt. 4, Becker et al. 2011; chapt. 5, Becker et al., subm.).

The internal reproductive structures of both sexes show a great expanse inside the body,

especially in the female (chap. 4, Becker et al. 2011). In the European species and in several other genera (e.g. *Tumidotheres* Campos 1989, *Zaops* Rathbun, 1900, *Arcotheres* Manning, 1993a, *Orthotheres* Sakai, 1969; pers. obs.), ovaries extend into the broad pleon. This feature is unique among brachyurans so far. In viviparous Hymensomatidae McLay, 1838, however, the pleon has developed into a brood pouch where offspring develop. A close relationship between pinnotherids and hymenosomatids was formerly assumed (Alcock 1900, Lucas 1980), but is very unlikely according to the present state of knowledge (Guinot and Richer de Forges 1997).

The presence of gonads in the pleon amongst pinnotherids has not been the subject of studies yet. While ovaries inside the pleon are generally visible in species of the subfamily Pinnotherinae De Haan, 1933 due to the transparency of the integument. In the stronger calcified Pinnothereliinae Alcock, 1900, ovaries are not visible and their observation requires the dissection of specimens or histological studies.

The investigated pinnotherids exhibit two prominent glandular epithelia in the spermatheca. A holocrine multi-layered epithelium is located at the connection of the oviduct to the spermatheca. Further, a highly active mono-layered epithelium lines the dorsal sperm storage area. In past studies, mostly holocrine epithelia were found in the dorsal part of spermathecae of brachyuran crabs (Ryan 1967b, Johnson 1980, Jensen et al. 1996). Figure 6.12 shows the distribution and location of secretory epithelia among the Heterotremata and the Thoracotremata investigated to date. The apocrine epithelium has only been described for thoracotremes so far, namely for pinnotherids and ocypodids (fig. 6.12, Lautenschlager et al. 2010). The absence of the apocrine glandular epithelium among heterotreme spermatheca indicates that it is an autapomorphy of Thoracotremata, which has to be confirmed in future studies.

In the studied pinnotherid males, the vasa deferentia are enlarged in comparison to other brachyurans, and appendices of the distal vas deferens extend into the pleon (chap. 5, Becker et al., subm.). Such appendices have only been recorded in the mangrove crab *Goniopsis cruentata* to date Martins Garcia and Feitosa Silva (2006). In *G. cruentata*, these appendices are only small diverticula while they are very expanded in the studied pinnotherids and fill a good part of the ventral cephalothorax (chap. 5, Becker et al., subm.). In constrast to the vasa deferentia, the appendices do not hold spermatozoa, but seminal plasma, the matrix, in which spermatophores are transferred to the female during copulation. Other decapod crustaceans produce vast amounts of seminal plasma as well (Adiyodi and Anilkumar 1988). However, these are not secreted in special appendices, but by the secretory medial vas deferens (Adiyodi

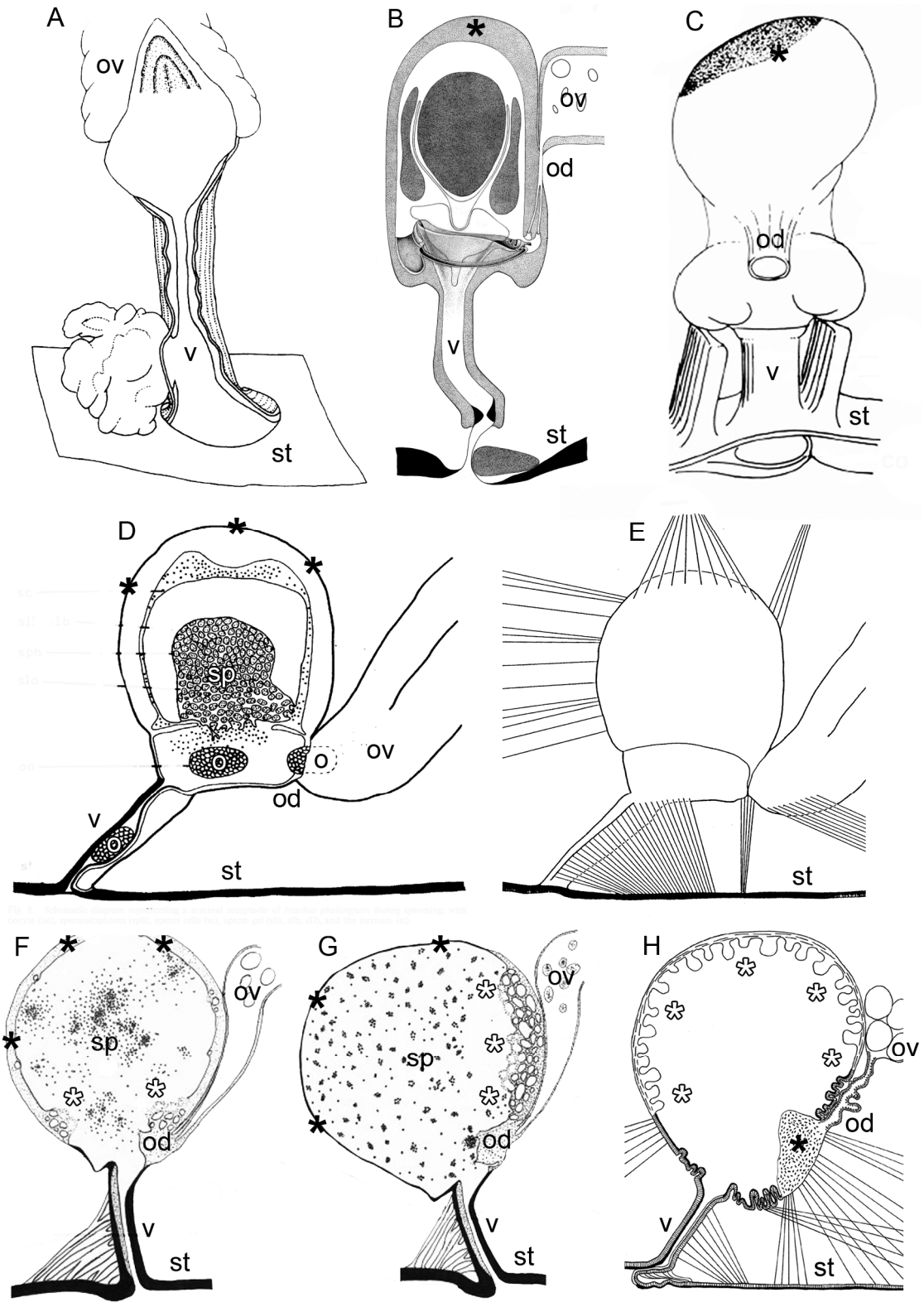

Figure 6.12. Spermathecae of heterotreme (A-E) and thoracotreme crabs. (F-H) with reference to secretory epithelia. (**A**) *Metacarcinus magister* (after Jensen et al. 1996) (**B**) *Potamon* spp. (after Brandis et al. 1999) (**C**) *Chionoecetes opilio* (after Beninger et al. 1988) (**D**) *Inachus phalangium* during ovulation (after Diesel 1989) (**E**) *I. phalangium*, musculature shown (after Diesel 1989) (**F**) *Uca tangeri* (after Lautenschlager et al. 2010) (**E**) *Uca ecuadoriensis*, *Uca* cf. *forcipata* (after Lautenschlager et al. 2010). Black asterisks on multi-layered holocrine glandular epithelium. White asterisks on apocrine glandular epithelium; bu = bulbus; o = oocyte; od = oviduct; ov = ovary; sp = sperm; st = sternum; v = vagina.

and Anilkumar 1988, Beninger et al. 1988, Diesel 1989, Johnson 1980) or in small diverticula originating from the distal vas deferens (Siméo et al. 2009).

Male Copulatory System

While first gonopods (G1s) are very specific and constant characters on species-level, second gonopods (G2s) are less divers and often rather characteristic for higher brachyuran groups. The G2s of the Thoracotremata are uniformly short. In the investigated pinnotherids, a small appendix of the G2 was observed in SEM-investigations (chap. 5, Becker et al., subm.). Shen (1935) has studied the development of pleopods from young to adult crab stages and demonstrated that gonopods are endopodites, while exopodites - present in early developing male stages - become subsequently reduced. In the G2s of the investigated pinnotherids, the reduction of the exopodite is obviously not complete in adult males (chap. 5, Becker et al., subm.), which was previously recorded by Atkins (1959). The remaining exopodite is small and short among the European pinnotherid species, however, in *Arcotheres* cf. *placunae* (Hornell and Southwell, 1909) from the Persian Gulf of Iran, long exopodites were present in the G2 of adult males (fig. 6.13, Naderloo and Becker, in prep.).

In taxonomic studies of "Podotremata" Guinot, 1977 and Heterotremata Guinot, 1977, the G2s are generally described. That is not the case among the Thoracotremata Guinot, 1977 so far due to the small sizes and uniform appearances of their G2s upon first sight. Exopdites in gonopods of adult brachyuran crabs are only known for pinnotherids to date. This stresses the importance to examine and describe the G2s of further pinnotherid species in systematic studies, but also in regard to their function. Based on our histological examination the G2 of the studied pinnotherids revealed a specific interaction with the G1 in sealing the ejaculatory canal to the outside.

Moreover, an interaction with the penis is supposed by the morphological results (chap. 5, Becker et al., subm.). The small rudimentary exopodite we found in the European species might not play a significant role whereas the exopodite observed in *A*. cf. *placunae* should also be considered as being functional elements for copulation.

Morphological Methods

Compared to traditional drawings used in taxonomy (chap. 3, Becker and Türkay 2010), observations with scanning electron microscopy (SEM) are essential to characterize and describe setae types (Abele 1971). For our taxonomic and ecological studies, setae types

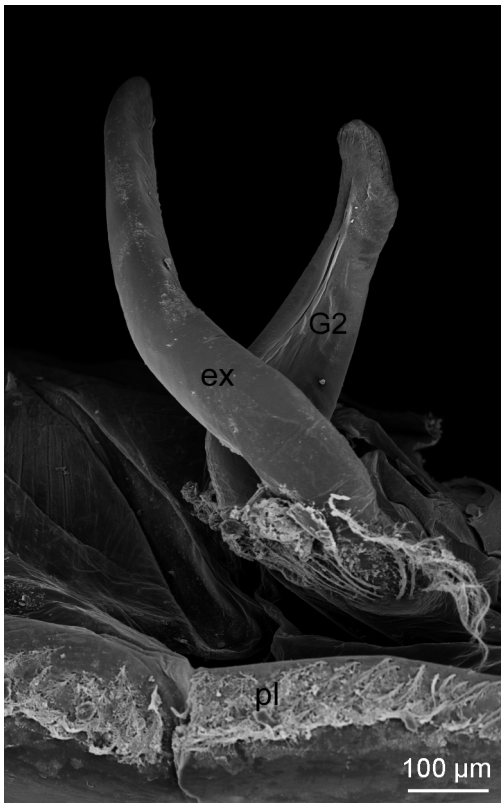

Figure 6.13. Second gonopod of *Arcotheres* cf. *placunae* (SEM). A long exopodite is still present in adult males. ex = exopodite; G2 = second gonopod; pl = pleon

provided important informations (chap. 3, Becker and Türkay 2010; chap. 5, Becker et al., subm.; chap. 6). The histology of the male and female reproductive systems allowed functional conclusions due to the observation of musculature, cuticle, and secretory epithelia.

Observations on the rank of cells and components by transmission electron microscopy (TEM) were essential to demonstrate secretory processes of epithelia (chap. 4, Becker et al. 2011; chap. 5, Becker et al., subm.)

Confocal laser scanning microscopy (CLSM) benefits from the autofluorescence of cuticle exposed to lasers. Serial laser scans throughout the whole object can be studied as single sections, while merged CLSM scan series provide 3-dimensional information on objects. With this method, surface structures of very small arthropods, their larvae or body parts can be observed (Michels 2007, Michels and Büntzow 2010) as well as inner cuticle structures, like cavities and canals, as shown in the gonopods (chap. 5, Becker et al., subm.). Fresh tissue can be treated with antibody-staining to reveal histology and cell morphology (Wanninger 2007). Fresh material was not available during the CLSM-studies conducted for the present study but the investigated material showed a certain autofluorescence giving signal for musculature in old (e.g. formaline-fixed) material as well (chap. 5, Becker et al., subm.).

A capital benefit of CLSM compared to histology, SEM, and TEM is the possibility to apply this method on material without further manipulation or preparation. Samples are simply embedded in glycerine but not sputter-coated as in SEM, or dissected as for histology and ultrastructure (TEM). This is fundamental for the examination of rare material and type species that have to be preserved. For the description of whole specimens, drawings - as prepared in traditional taxonomic studies - are indispensable since crabs - even most of the rather small pinnotherids - are too large for CLSM-studies. Only very small and planar objects (specimens or body parts) are suitable. There, the CLSM technique represents a valuable alternative to other traditional methods. We applied CLSM in particular on the gonopods of the European pinnotherids, but we also tested this method for small bodyparts of pinnotherids (fig. 6.9), larvae (fig. 6.11) and characters of other groups of interest (fig. 6.14)

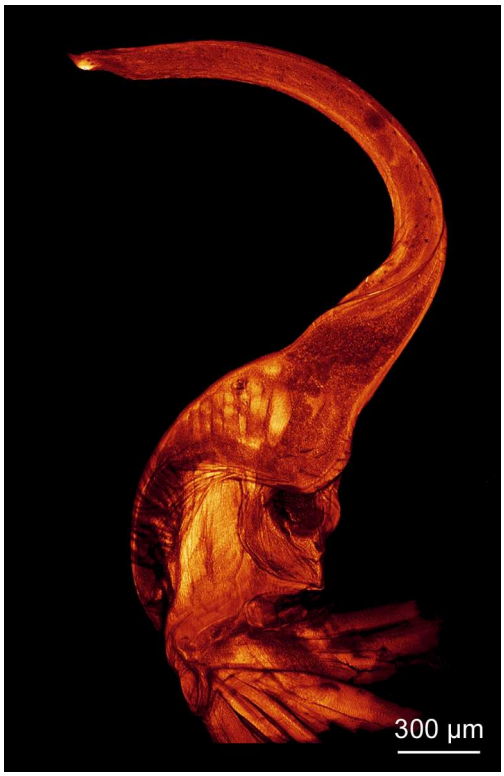

Figure 6.14. Maximum projection of CLSM-scan series. First gonopod of the false spider crab *Hymenosoma orbiculare*.

The Generic Status of the European Species

Our ecological data and the taxonomic study confirm the classification of the European pinnotherid species in two distinct genera (fig. 6.15). *Pinnotheres pisum* (Linneaus 1767) and *Pinnotheres pectunculi* Hesse, 1872 are both restricted to bivalves while *Nepinnotheres pinnotheres* (Linneaus, 1758) inhabits sea squirts and the Mediterranean pen shell *Pinna nobilis*. The mode of life is classified parasitic in *Pinnotheres* (Atkins 1926, Huard and

Demeusy 1968, Haines 1994) while host relations of *N. pinnotheres* or of other pinnotherid-ascidian-symbioses have not been studied yet.

The studied *Pinnotheres*-species are uniform in morphology. Both possess the same setae types in the first gonopods (G1s) distally, which differ from the setae observed in the distal G1 of *N. pinnnotheres* (chap. 3, Becker and Türkay 2010, chap. 5, Becker et al., subm.). However, the general shape of the G1 and its bending are characteristic at species level. Furthermore, the chelae of *P. pisum* and *P. pectunculi* are very similar and both bear a specific setae comb, which has an essential function in feeding from the bivalve gills. Feeding behaviour was not observed in *N. pinnotheres*. Specimens of both sexes are pilose all over, but lack the setae comb, thus initial feeding has to occur differently from *Pinnotheres*, probably with the help of the elongated dactyli of the last pair of pereiopods.

The studied genera *Nepinnotheres* Manning, 1993 and *Pinnotheres* Bosc, 1802 are also very distinct in larval morpohology (Lebour 1928a, 1928b, Atkins 1954, Rice 1975). Besides, the sexual dimorphism in adults is stronger in the studied *Pinnotheres*-species than in *N. pinnotheres* (see chap. 3, Becker and Türkay 2010). In particular, the enlargement and decalcification of the carapace is more advanced in adult females of *Pinnotheres* (chap. 3, Becker and Türkay 2010). Chelipeds are stronger in specimens of *N. pinnotheres*, which are also more mobile than *P. pisum* and *P. pectunculi* (pers. obs.). Overall, females after metamorphosis seem to be more adapted to their parasitic life phase in the European *Pinnotheres* than in *N. pinnotheres*.

The Problem *Nepinnotheres* Manning, 1993

The genus *Nepinnotheres* was established by Manning (1993b). *N. pinnotheres* (Linnaeus, 1758) is the type species, the genus refers to. According to our redescription, other species belonging to *Nepinnotheres* Manning, 1993 differ remarkably from *N. pinnotheres* and appear morphologically closer to *Pinnotheres* or related genera (fig. 6.16). For instance, *Viridotheres viridis* (Manning, 1993) from bivalves was initially described as *Nepinnotheres viridis* by Manning (1993b), which is still used in recent publications (see Wirtz 2009). Manning (1996) subsequently corrected his mistake, but created a new genus for *Viridotheres viridis* (Manning, 1993) (new comb., Manning 1996) instead of considering a comparison to *Pinnotheres*, according to their similarity in characters (fig. 6.16, Wirtz 2009).

Raymond B. Manning (1934 – 2000), introduced several new genera within Pinnotheridae De Haan, 1833. Based on the present state of knowledge, some of these genera should probably be reconsidered and summarized into a lower number of groups. In Manning's publications

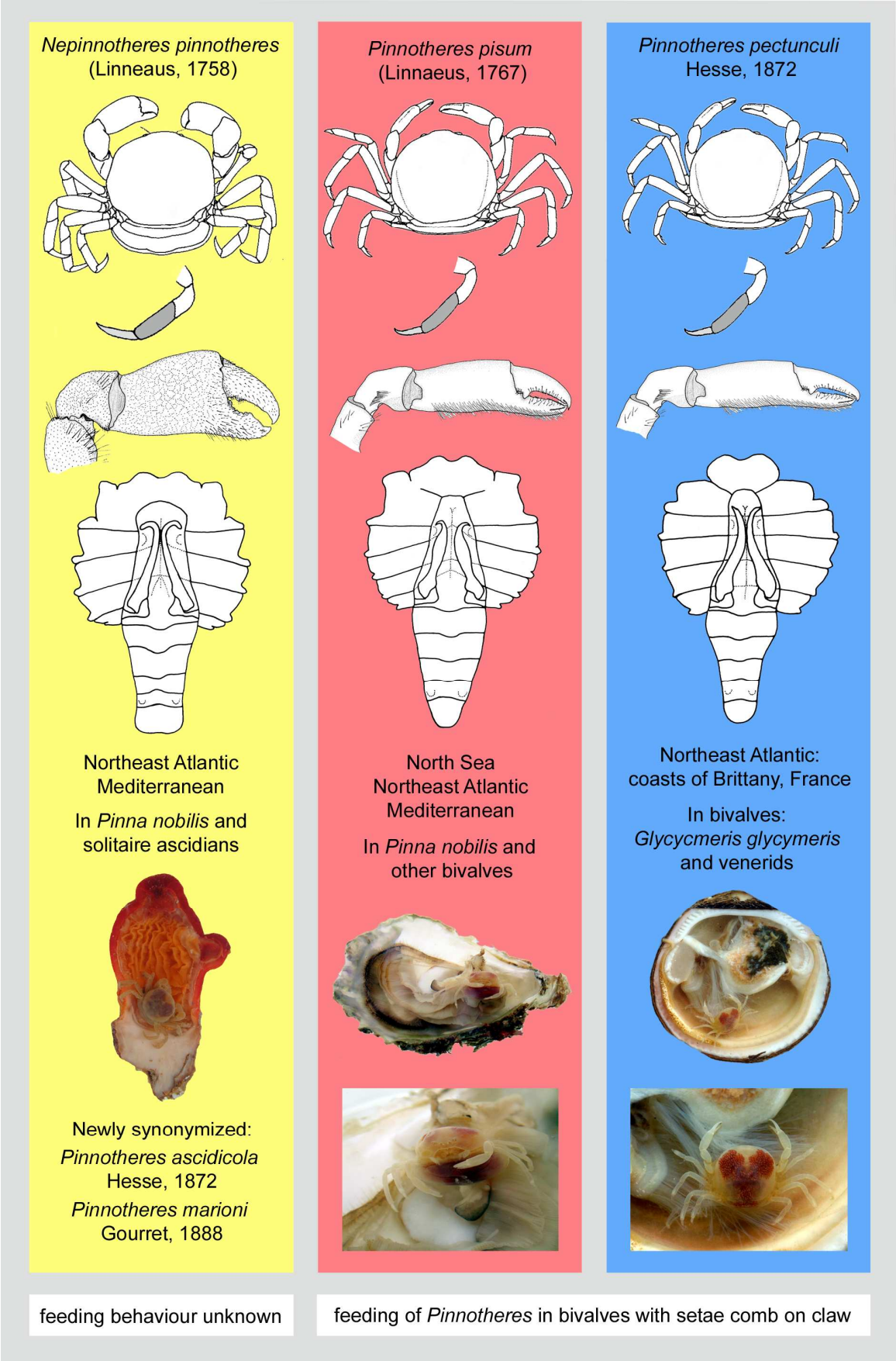

Figure 6.15. Overview on the European species. From top to down: female habitus and relative length of distal articles of fourth pereiopod (P5) (after Gonzales Gurriaran and Mendez 1968), female chelipeds (applies in general for males too), male sterna with first gonopods (pleon opened), distribution, host-range, females in dissected hosts, synonyms (left)/feeding inside the host (middle and right), results on feeding.

Figure 6.16. "*Nepinnotheres*" *viridis* Manning, 1993 inside the bivalve *Pseudochama radians*. This species is now assigned to *Viridotheres* Manning, 1996. Photograph : P. Wirtz, Centro de Ciências do Mar, Universidade do Algarve, Campus de Gambelas, Faro, Portugal ; magnification: ca. 5x.

only females are prevalently included in the descriptions (Manning 1993a, 1996), and walking legs and third maxillipeds are used as main characters (Manning 1993b). Indeed, third maxillipeds are very important for the higher classification since they are an autapomorphic character within Pinnotheridae, with several character states represented among pinnotherid sub-groups (chap. 1). However, to distinguish closely related species, the third maxilliped is a problematic character. In Manning (1993b), the insertion of the dactylus of *N. pinnotheres* and *P. pisum* was compared and categorical differences are shown in the drawings. In contrast to that, we observed the pilosity to be a reliable character, rather than the insertion of the dactylus (fig. 6.8). The position and relative length of dactylus toward the propodus actually slightly varied among sexes, crab-stages and different-sized adults (pers. obs.; chap. 3, Becker and Türkay 2010). The same applies for the relative length of walking legs, respectively their dactyli, which are key character in several genera, such as *Arcotheres* Manning, 1993a and *Viridotheres* Manning, 1996. Strongly elongated pereiopods and asymmetry are not present among juvenile females or males and only fully develop in adult females (Gordon 1936).

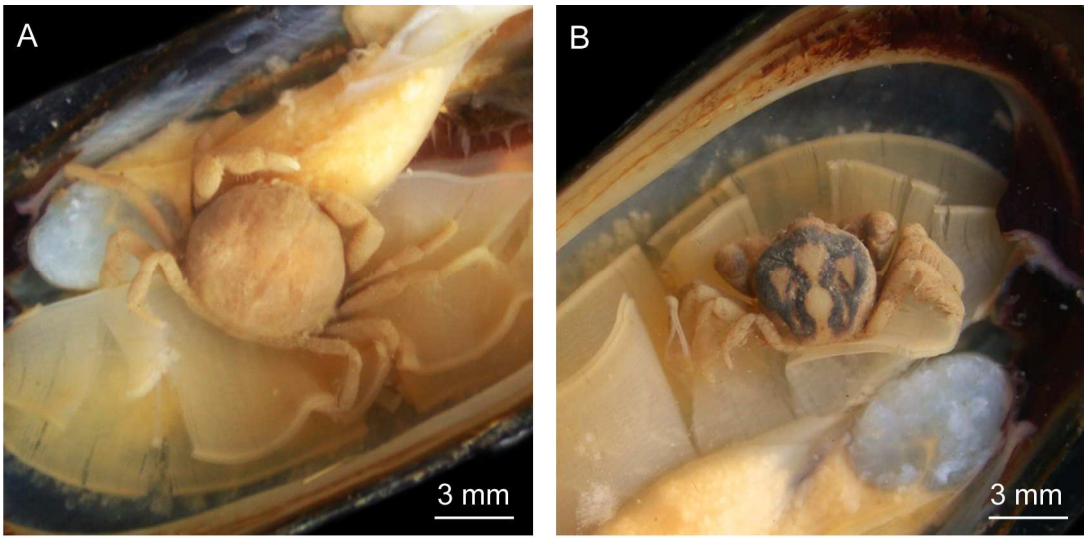

Figure 6.17. *Tumidotheres maculatus* in *Mytilus* sp., bought from local fishery in Montevideo, Uruguay. (**A**) Adult female. (**B**) Male with dark color pattern on carapace.

Nepinnotheres pinnotheres resembles *Tumidotheres maculatus* (Say, 1818) (see fig. 6.17) from the Western Atlantic coast in general body shape and pilosity, form of chelipeds, and most prominently in the distal opening of G1 (pers. obs., Campos 1989; chap. 3, Becker and2010). Thus, *Tumidotheres* Campos, 1989 and *Nepinnotheres* Manning, 1993 might require comparison, reconsideration - and potentially revision.

Systematics of Pinnotheridae - Outlook

Indeed, the present study is based on morphology and the results are therefore exclusively taxonomic. Molecular studies may either confirm our taxonomy or reveal host-races respectively cryptic species. In future studies, it will be particularly interesting to investigate, if there is any genetic separation among populations of *Nepinnotheres pinnotheres* from *Pinna nobilis* and from ascidians. A preliminary molecular study on the European pinnotherids has already been conducted (Becker and Klaus, unpubl. data). The preliminary data set of 16S rRNA gene sequences (51 specimens, 522bp) indicate that *Pinnotheres pisum* and *P. pectunculi* are reciprocal monophyletic lineages with an uncorrected pairwise distance of 3.6 - 7.9% between the two species, while distances within the species did not exceed 2% divergence. Still, the used sequence markers were too conserved to detect any genetic differentiation within the species or host specific lineages, which stresses the need of better resolving markers like microsatelite loci (Becker and Klaus, unpubl. data). Next to better resolving markers (microsatellites), more specimens from different hosts are required, especially from *P. nobilis.* This actually poses a problem due to the decline of *P. nobilis* and its protection by the ICES. To determine the degree of isolation by distance, also different

locations have to be sampled for genetic studies. The incidence of host-related races was investigated in *Pinnotheres novaezelandidae* Filhol, 1885, which inhabits several bivalve species (Stevens 1990b). The results suggest that populations from different bivalves represent biologically discrete units with different host recognition systems (Stevens 1990b). This is also supported by genetic differentiation between host races (Stevens 1990a). Genetic analysis of *Pinnotheres atrinicola* Page, 1983 demonstrated an unusually high degree of structuring between geographic populations, which is atypical for brachyurans and probably maintained by life-history attributes of pea crabs and current movements (Stevens 1991).

To avoid the introduction of confusing synonyms within Pinnotheridae De Haan, 1833, it is essential to describe male *and* female in taxonomic studies. Further, it would be beneficial to display female characters before and after metamorphosis, if they differ from the male. Both may diminish the risk of describing different morphotypes as separate species for their very distinct morphology. In future studies, more attention should be paid to chelipeds, which have not played an important role in the taxonomy of pinnotherids so far. The distribution of cheliped's setae combs used in feeding among pea crabs can be compared with host-ranges to reveal adaptations in feeding morphology to host groups. It is therefore important to preserve pinnotherid specimens together with their host, or at least with proper information on host species. Moreover, museum collections and studies based on their material would benefit from specimens allocated into separate jars according to the infestation (as pair, single female, or male). Several published species descriptions are still based on specimens from unknown host species and/or without knowing the opposite sex (e.g. Bürger 1895, Griffin and Campell 1969, Manning 1993b, Campos 2001, Campos 2009).

Reproduction and Parasitism

The reproductive output of female pinnotherids (Hines 1992) and their reproductive investment (Hartnoll 2006) has already been demonstrated in earlier studies. One of Hines (1992) remarkable results was that the embryonic mass from one spawning is 70 to 90 % of the whole body mass in pinnotherids, compared to an average of 10 % in other brachyuran crab species. Hines (1992) also demonstrated that the production of embryonic mass depends on the space in the cephalothorax, which is available for yolk accumulation. The dominant cephalothorax of female pinnotherids and the broad pleon that holds ovaries can therefore be regarded as an adaptation to produce large numbers of offsprings. As demonstrated in the present study, the enormous reproductive output of pea crabs goes alongside with a high

degree of differentiation of the female spermathecae and of the male internal reproductive structures (chap. 4, Becker et al. 2010; chap. 5, Becker et al., subm.).

Besides the direct metabolic investment into embryonic masses and the costs of secretion, other considerable costs are involved in breeding among crabs (Fernández et al. 2000). Brachyurans, as well as most decapods, perform a certain brood care by carrying the eggs under the female pleon until larvae hatch, instead of broadcasting them directly into the open water as in many other marine invertebrates. Next to the obvious costs of this brood care, like the weight of the embryonic mass that has to be carried and the consequently higher metabolic costs of locomotion, a specific behaviour is accomplished by ovigerous females (Naylor et a. 1997, 1999, Baeza and Fernández 2002). As oxygen is a limiting factor for the development of embryo-batches in aquatic organisms (Naylor et al. 1999), the female ventilates the embryos regularly by active abdominal flapping, which exposes eggs in the center of the batch to water flow (Baeza and Fernández 2002). The high energy costs of female brooding behaviour have been estimated and quantified by Fernández et al. (2000), which confirmed the importance of this factor. In fact, oxygen consumption of brooding females themselves also increases throughout the embryonic development by the accomplished brooding behaviour, which demonstrates that parental care is strongly linked to oxygen provision (Baeza and Fernández 2002). Thus, the costs of reproduction do widely exceed the direct metabolic costs into gonads and embryonic mass.
The intensity and specifity of brooding behaviour vary within crab species, depend on the stage of development in the embryo (Baeza and Fernández 2002), and are linked to water currents in the habitat (Fernández et al. (2000). In the studied pinnotherids, we observed abdominal flapping in ovigerous females and the use of chelipeds for a kind of “sorting” eggs under their broad pleon. The symbiotic way of life inside other marine organisms, which produce a water flux for suspension feeding such as bivalves and ascidians, is advantageous for the provision of oxygen to the embryonic mass.

Another important factor, which should be considered in the pinnotherids’ reproduction, is the general correlation between adult size and brooding. The degree of parental care provided to broods is generally increased in small-sized animals compared to sibling species of larger sizes (Strathmann and Strathmann 1982). This applies only in part for brachyuran crabs (Strathmann and Strathmann 1982) and is harder to assess than in other groups, because all brachyurans are brooders. However, the small-sized groups have developed the most peculiar reproductive strategies among the Brachyura. For instance, the minute Hymensomatidae

MacLeay, 1838 include freshwater forms that are viviparous (Melrose 1975, Lucas 1980). Furthermore, the members of Cryptochiridae Paul'son, 1875, also called gall crabs, are very small brachyurans with females living enclosed in galls induced in madreporian corals (Kropp and Manning 1987). These females have a strange body shape with a large brood chamber formed by the pleon, where offspring develop.

In many parasites - pinnotherids included - life cycle requires that offspring leave the parental host and spend a free-living phase in search for a suitable host. This event is regarded the most critical in the life cycle of a parasite (Bush et al. 2001). An additional problem for parasites – but also for other animals with separate sexes - is the challenge to find a partner of the opposite sex. In the investigated European pinnotherids, mating is supposed to occur inside the host (chap. 4, Becker et al. 2011), which additionally challenges seeking a potential partner to mate with. Other pinnotherid species copulate outside the host. For instance, *Tumidotheres maculatus* (Say, 1818) and *Fabia subquadrata* Dana, 1851, which exhibit an interesting behaviour: males and females have developed a copulatory swarming in the open water during the mating season (Pearce 1964).

A high fecundity among parasites compared to free-living organisms is considered to be one of the most characteristic features of parasites (Whittington 1997) and generally viewed as compensating the losses that are paid tribute to the parasitic way of life (Bush et al. 2001, Tinsley 2004). On the other hand, parasites can actually afford a highly increased investment in reproduction because of the supply of nutrients provided by the host (Bush et al. 2001).

Figure 6.18. Ovigerous female of *Pinnotheres pisum*.

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Europäische Muschelwächter – Taxonomie, Morphologie und Wirtsökologie

Krabben der Familie Pinnotheridae leben auf vielfältige Weise vergesellschaftet mit anderen wirbellosen Meerestieren. Weltweit sind von der Gezeitenzone bis zur Tiefsee über 300 ausschließlich marine Arten beschrieben. Man findet Muschelwächter in den Wohnröhren von Maulwurfskrebsen (Thallassinidae) oder sessilen Borstenwürmern (Polychaeta), in den Körperhöhlen von Seegurken (Holothuroidea), Schnecken (Gastropoden) und zwischen den Stacheln von Seeigeln (Echinoidea). Die europäischen Vertreter leben im Inneren von Muscheln (Bivalvia) und Seescheiden (Ascidiacea).

Die muschelbewohnenden Arten ernähren sich vom Kiemenschleim ihres Wirtes und den darin angereicherten Nahrungspartikeln. Diese Ernährungsweise kann den Stoffwechsel und das Wachstum der Muschel beeinträchtigen oder sogar zu ihrer vorübergehenden Unfruchtbarkeit führen. Die Muschelbewohner sind somit Parasiten, und auch kommerziell genutzte Muschelarten wie Auster oder Miesmuschel werden infiziert und sind weniger „fleischig“ als Muscheln, die keine Parasiten beherbergen. Pinnotheriden gelten deshalb in Fischerei und Aquakultur von Muscheln als Schädlinge, womit ihre Erforschung auch einem wirtschaftlichen Interesse dient.

Während sich die juvenilen Muschelwächter beider Geschlechter noch gleichen – sie besitzen einen harten Panzer (Carapax), sind gute Schwimmer im Freiwasser und halten sich nur zeitweise im Wirt auf –, vollzieht sich beim Weibchen nach seiner Paarung im juvenilen Stadium (präkoxiziös) eine Metamorphose, die in einem ausgeprägten Geschlechts-dimorphismus resultiert. Cephalothorax und Hinterleib (Pleon) wachsen unverhältnismäßig gegenüber den Scheren und Laufbeinen und der Carapax wird durch Dekalzifikation weichhäutig und transparent, sodass man die inneren Organe hindurchsehen kann. Nach der Metamorphose ist das Weibchen stark an den anschließenden rein parasitischen Lebensabschnitt angepasst und verlässt den Wirt von nun an nicht mehr. Das Männchen hingegen bleibt zeitlebens optional freilebend und kann im Laufe seines Lebens eine ganze Reihe von Weibchen im Inneren von Wirten aufsuchen.

Aufgrund ihrer geringen Größe, der verborgenen Lebensweise, dem Geschlechtsdimorphismus und den unterschiedlichen Morphotypen beim Weibchen vor und nach der Metamorphose ist die Taxonomie der Familie Pinnotheridae eine ziemliche Herausforderung. Die Arten *Pinnotheres pisum* und *Nepinnotheres pinnotheres* sind allgemein akzeptiert und weit an den europäischen Küsten verbreitet. Sie können laut Literatur an der relativen Länge der distalen Glieder des letzten Laufbeinpaares unterschieden

werden. Dennoch wurden diese beiden Arten in der Vergangenheit oft verwechselt, was sich in den Museumssammlungen und in der Literatur widerspiegelt. Eine weitere Art, *Pinnotheres pectunculi,* war bislang nur aus der Meermandel *Glycymeris glycymeris* von seiner Typuslokalität in Roscoff (Bretagne, Frankreich) bekannt. Aufgrund der großen Ähnlichkeit mit *Pinnotheres pisum* wurde der Artstatus von *Pinnotheres pectunculi* immer wieder angezweifelt. Noch problematischer sind zwei weitere Arten, die ausschließlich Seescheiden bewohnen sollen: *Pinnotheres ascidicola* aus dem Nordostatlantik und *Pinnotheres marioni* aus dem Mittelmeer. Seit ihrer Erstbeschreibung wurden diese Arten nur selten in der Literatur erwähnt und niemals sorgfältig mit den vorher aus Muscheln beschriebenen Arten verglichen.

In einer aufwändigen Freilandstudie haben wir Pinnotheriden aus zahlreichen Muschel- und Seescheidenarten verschiedener Fundorte an den Küsten des Nordostatlantik, der Nordsee und des Mittelmeeres gesammelt. Mit dem Ziel, standardisierte, vergleichende Beschreibungen der europäischen Arten anzufertigen, wurden die gefundenen Exemplare mit dem bereits in der Senckenberg-Sammlung vorhandenen Material verglichen und auf Merkmale untersucht, die sich vorzugsweise auf beide Geschlechter und die unterschiedlichen Stadien des Weibchens anwenden lassen. Als eindeutige Merkmale für die Unterscheidung der Arten erwiesen sich die männlichen Gonopoden und insbesondere die Scheren, welche eine hohe Konstanz in den verschiedenen Stadien beider Geschlechter aufweisen. Die Mundwerkzeuge gelten in der Systematik von Pinnotheriden als Schlüsselmerkmal, konnten die europäischen Arten aber nur auf Gattungsniveau unterscheiden. Weibchen und Männchen von *Nepinnotheres pinnotheres* und *Pinnotheres pisum* wurden für die vorliegende Studie separat neu beschrieben. Aufgrund unserer Merkmalsanalyse müssen die Arten *Pinnotheres ascidicola* und *Pinnotheres marioni* mit *Nepinnotheres pinnotheres* synonymisiert werden. Die Ascidienbewohner unterscheiden sich in keinem der untersuchten Merkmale von *Nepinnotheres pinnotheres* aus der großen Steckmuschel *Pinna nobilis*. Nur die wirtsabhängige Größe und Färbung zeigten eine gewisse Variabilität. Die Validität der Art *Pinnotheres pectunculi* hat sich bestätigt. Neben einem winzigen zusätzlichen Zahn auf der Schneidekante der Schere, sind die männlichen Gonopoden deutlich unterschiedlich von *Pinnotheres pisum*.

Auf der Basis unserer Feldarbeit konnten das Wirtspektrum der europäischen Arten und ihre Infektionsraten in einzelnen Wirten bestimmt werden. *Nepinnotheres pinnotheres* lebt in Seescheiden und in der großen Steckmuschel *Pinna nobilis*. *Pinnotheres pisum* infiziert verschiedene Muschelarten, einschließlich *Pinna nobilis*. Die im Mittelmeer endemische

Steckmuschel kann bis zu einem Meter groß werden. Sie ist die einzige Art, in der sich das Wirtsspektrum von *Pinnotheres pisum* und *Nepinnotheres pinnotheres* überschneidet, und gleichzeitig der im natürlichen Lebensraum am höchsten frequentierte Wirt mit einer Infektionsrate von fast 85%. In der Freilandzucht von Miesmuscheln wurde eine Überinfektion von fast 100% festgestellt. Hier hielten sich sogar mehrere Männchen – gemeinsam mit nie mehr als einem Weibchen – in einer Muschel auf. Die Infektion des Wirtes durch eine der beiden Arten scheint die andere Art auszuschließen, da sie niemals gemeinsam in einem Wirt gefunden wurden. In Ascidien können die Infektionsraten mit *Nepinnotheres pinnotheres* sehr niedrig sein. So waren zum Beispiel in Seescheiden der Gattung *Micrcosmos* von über 1000 untersuchten Exemplaren nur 3% bewohnt. Für *Pinnotheres pectunculi* wurde der Nachweis drei neuer Wirtsarten aus der Familie der Venusmuscheln erbracht. Bei den *Pinnotheres*-Arten wurde außerdem das Fressverhalten beobachtet. Sie benutzen einen Borstenkamm an der Unterseite der Schere, um den Kiemenschleim mit den darin angereicherten Nahrungspartikeln abzubürsten. Unterschiedliche Strategien der Nahrungsaufnahme und die Wirtsökologie der europäischen Muschelwächter werden ausführlich im Hinblick auf die verfügbare Literatur über andere Pinnotheriden-Arten diskutiert.

Der männliche und weibliche Geschlechtsapparat wurde mit histologischen Methoden, dem Raster– und Transmissionselektronenmikroskop und Methoden der konfokalen Lasermikroskopie untersucht.

Eubrachyuren haben eine innere Befruchtung: paarige Vaginae erweitern sich zu Speicherstrukturen (Spermatheken), welche über Ovidukte mit den Ovarien verbunden sind. Das Sperma des Männchens wird bis zur Eireife in der Spermathek gespeichert. Beim Eisprung werden die Eizellen über den Ovidukt in die Spermathek transportiert, dort befruchtet und gelangen über die Vagina unter das breite Pleon des Weibchens, wo die Embryonen bis zum Schlüpfen der Larven verbleiben. Die Vagina der untersuchten Pinnotheriden ist vom „konkaven Typ“: Flexible Wandanteile der Vagina sind mit Muskulatur versehen und (im Ruhezustand) in starre Wandanteile kollabiert, wodurch das Lumen der Vagina im Querschnitt halbmondförmig verengt ist. Durch eine Kontraktion der Muskulatur entlang der flexiblen Vaginawand wird das Lumen der Vagina zu einem runden Querschnitt erweitert. Die Geschlechtsöffnung ist zusätzlich von einem mobilen Operculum bedeckt. Die konkave Vagina und das mobile Operculum sind charakteristisch für höhere Krabben (Thoracotremata) und zeigen die aktive Rolle des Weibchens bei der Kopulation. Da die Geschlechtsgänge des Weibchens durch Muskulatur kontrolliert werden und nicht

cuticularisiert sind, gehen wir davon aus, dass die Weibchen „hart kopulieren“ anstatt im weichen Zustand unmittelbar nach der Häutung. Vergleichbar mit Parasiten anderer Tiergruppen besitzen Pinnotheriden aufgrund ihrer riesigen Gonaden eine extreme Reproduktionsleistung und hohe Nachkommenzahlen. Das Ovar kann bis 90% der Gesamtkörpermasse ausmachen und erstreckt sich in den Hinterleib (Pleon), was innerhalb der Krabben nur bei Pinnotheriden vorkommt. In der Spermathek der Muschelwächter können morphologisch und funktional zwei Abschnitte unterschieden werden. Im ventralen Bereich findet die Befruchtung statt und es befinden sich die Verbindungen mit der Vagina und dem Ovidukt. Die Spermathekenwand ist hier überwiegend cuticularisiert und wird somit mitgehäutet. Im Mündungsbereich des Ovidukts allerdings befindet sich ein sekretorisches Gewebe, dass die Eizellen bei der Ovulation passieren müssen. Dieses vielzellige Gewebe zeigt einen holokrinen Sekretionsmechanismus, bei dem ganze Zellen in Sekrete umgewandelt werden. Dorsal befindet sich der Hauptspeicherort für die Spermien. Die Spermathekenwand ist hier ein einschichtiges hochsekretorisches Epithel. Der Sekretionsmechanismus ist apokrin, da nur der distale Teil der weit in das Lumen der Spermathek hineinragenden Drüsenzellen beim Abgeben der Sekrete verloren geht. Der basale Teil der sekretorischen Zelle mit dem Zellkern und anderen Zellorganellen bleibt erhalten. Ein vergleichbares, jedoch weniger ausgedehntes Sekretepithel wurde bislang nur für Winkerkrabben der Gattung *Uca* beschrieben. Bei einer Reihe anderer untersuchter Krabbenarten ist der dorsale Teil der Spermathek mit einem mehrschichtigen holokrinen Sekretepithel ausgekleidet.

Der innere männliche Geschlechtsapparat besteht aus paarigen Hoden und langen, verschlungenen Samenleitern. Die Morphologie der Spermien der untersuchten Pinnoteriden entspricht anderer Thorakotremen, unterscheidet sich aber im Detail bei *Nepinnotheres pinnotheres* und *Pinnotheres pisum*. Die Spermatozoen werden im sekretorischen proximalen Vas deferens in Spermatophoren verpackt. Der mediale Vas deferens ist stark erweitert, er speichert Spermatophoren, eingebettet in eine Matrix aus seminalem Plasma. Der distale Vas deferens besitzt Anhänge, die den Cephalothorax ventral fast ausfüllen und sich auch leicht ins Pleon ausdehnen. Große Mengen seminales Plasma werden in diesen Sonderbildungen produziert und gespeichert. Der männliche Kopulationsapparat von Krabben besteht aus paarigen Penes und zwei Paar Hinterleibsbeinen, die im Dienste der Spermienübertragung zu Gonopoden umgewandelt sind. Bei Pinnotheriden überträgt der lange erste Gonopode die Spermien in die weibliche Geschlechtsöffnung. In ihm verläuft der Spermienkanal mit einer proximalen und distalen Öffnung. Der zweite Gonopode ist kurz und keulenförmig. Während

der Paarung sind Penis und zweiter Gonopode in die Basis des röhrenförmigen ersten Gonopoden eingeführt. Der zweite Gonopode ist durch Pumpbewegungen hydraulisch am Transport der männlichen Geschlechtsprodukte zur distalen Öffnung des Spermienkanals beteiligt. Die spezifische Form des zweiten Gonopoden ist stark an seine Funktion bei der Abdichtung des hydraulischen Röhrensystems im ersten Gonopoden angepasst. Längsfaltungen der Cuticula im zweiten Gonopoden greifen dabei genau in eine durch die Röhrenbildung des ersten Gonopoden entstandene Überlappungsnaht. In der Basis des ersten Gonopoden befinden sich Rosettendrüsen, die über Poren ein Sekret in den Spermienkanal abgeben und vermutlich eine Rolle beim Transport des Spermas spielen. Während die ersten Gonopoden von Krabben meistens artspezifisch sind, wurden die zweiten Gonopoden der Thorakotrematen oft als einheitlich betrachtet und nur selten in Artbeschreibungen dargestellt. Im zweiten Gonopoden der untersuchten Pinnotheriden ist ein rudimentärer Exopodit vorhanden und unterscheidet sie diesbezüglich von anderen Krabben, was die Notwendigkeit der Beschreibung zweiter Gonopoden in systematischen Arbeiten zeigt. Die vorliegenden Ergebnisse werden im Vergleich zu den morphologisch und funktional sehr vielfältigen Kopulationssystemen anderer Brachyuren diskutiert und auf ihre systematische Bedeutung hin untersucht. Sowohl im männlichen als auch im weiblichen Geschlechtsapparat der untersuchten Muschelwächterarten fallen die hoch differenzierten sekretorischen Strukturen auf. Die Rolle der Sekrete bei Kopulation, Spermienspeicherung und Ovulation von Krabben wird in der Literatur kontrovers diskutiert. Für einen Teil der Sekrete wurde ein antibakterieller Effekt nachgewiesen. Es gibt aber auch Hinweise darauf, dass die Sekrete von den gespeicherten Spermatozoen metabolisiert werden. Im Allgemeinen wird ihre Funktion in der Erhaltung und Speicherung der Spermien gesehen. In diesem Zusammenhang ist es bemerkenswert, dass die Sekretionsmechanismen bei Muschelwächtern komplexer und möglicherweise effizienter sind als bei den bisher untersuchten Krabbenarten. Die Morphologie der Geschlechtsapparate von Pinnotheriden wird in Bezugnahme auf ihre parasitischen Lebensweise und die hohe Reproduktionsleistung diskutiert.

Durch die präkoxiziöse Paarung des Weibchens müssen die Spermien bei Pinnotheriden über mehrere Häutungen gespeichert werden. Außerdem ist die Wahrscheinlichkeit, potentiellen Sexualpartnern zu begegnen, bei Parasiten gegenüber freilebenden Krabbenarten stark herabgesetzt. Bislang ist völlig unklar, ob das Weibchen seine zahlreichen Bruten mit dem gespeicherten Sperma der ersten Paarung befruchtet oder noch weitere Kopulationen im adulten Stadium stattfinden.

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CURRICULUM VITAE

Professional experience - Senckenberg: Marine Zoology: Crustacea (Prof. Dr. M. Türkay)

Since 2008 GSF-project "Leibniz-Aquanet - networking aquatic invertebrate museum collections"
2006 - 2007 GSF-project LEVAR (CeDaMar): "Levantine Basin biodiversity variability"
2005 - 2006 BMBF-project GBIF "Global Biodiversity Information Facilities"
2005 GSF-project DIVA2 "Diversity of the Atlantic benthos", Co-coordination of sea expedition Meteor 63-1.

Education - Goethe-University, Frankfurt, Germany

Since 2007 Dissertation in Biology: "European pea crabs - taxonomy, morphology, and host-ecology"
2000 - 2004 Main study period certificated grade: very good)
1996 - 2000 Basic study period (certificated grade: very good)

Research Visits, Expeditions, Workshops

Expedition "monitoring of the mega-epifauna of the Dogger Bank", RV HEINCKE, Feb. 9 – 21, 2010.

Deep sea-expedition DIVA3: "Diversity of the Atlantic Benthos", R.V. METEOR 79-1, Montevideo, Uruguay – Ponta Delgada, Azores, Portugal. Jul. 10 – Aug. 23, 2009.

Research visit, University Copenhagen, Denmark, Synthesys-project DK-TAF-4264: "Application of confocal laserscanning microscopy on decapod systematics". Apr. 15 – May 30, 2008 (J.T. Hoeg, A. Wanninger).

Workshop "Analysing methods for molecular data", Feb. 12 – 16, 2007 (A. Klussmann-Kolb, C. Printzen).

Field trip (scuba diving), Crete, Greece. Jan. 16 – 23, 2007.

Deep sea-expedition LEVAR "Levantine Basin biodiversity variability: biology and biogeochemistry of the Eastern Mediterraean Sea", R.V. METEOR 71-2. Dec. 28, 2006 – Jan. 15., 2007.

Workshop "Biodiversity conservation imperative: biological, ethical, and economic perspectives in the Middle East and Europe", University of Teheran, Iran, Dec. 8 – 14, 2006.

Research visit, American University of Beirut (AUB), Libanon,,7. – 21. Nov. 2006, DAAD-project "Establishment of a Middle Eastern biodiversity network" (Dr. M. Bariche).

Co-organisation of field expedition, North Sea, DAAD-project "Middle Eastern biodiversity project: collection management and Natural History Museum curatorship", Sept., 2006 (Dr. F. Krupp).

Workshop on molecular methods, Grunelius-Möllgaard-Laboratory, Department for Molecular Evolution, Senckenberg, June 19 – 23, 2006 (Dr. C. Printzen).

Fieldwork (scuba diving, R.V. BURIN), Northern Adriatic Sea (Mediterranean), Institute Ruđer Bošković, Rovinj, Croatia. Aug. 22 – Sept. 2, 2005, Apr. 3 – 14, 2005, Sept. 2 – 16, 2003.

Expedition: "Long term monitoring of the mega-epifauna of the Dogger Bank", R.V. SENCKENBERG, Aug. 2 – 16, 2003.

Awards & Grants

Award for best poster "The female reproductive system of European Pinnotheridae", International Crustacean Conference (ICC7), China, June 20 – 25, 2010 (page 181).

Award of DAAD-Competition: "Dialogue through Cooperation", 2008.

Award for best oral presentation „Commensal pea crabs in bivalves and sea squirts", Colloquium Crustacea Decapoda Mediterranea (CCDM), Torino, Italy, Sept. 2 – 6, 2008.

Synthesys-grant DK-TAF-4264 "Application of CLSM on decapod systematics", University Copenhagen, Denmark. Apr. 15 – May 30, 2008.

Award for oral presentation "Secret love life of a pea crab". ICIRD, Panama City, Panama, Aug. 6 – 9, 2007.

Travelgrant of the International Society of Invertebrate Reproduction (ISIR) for "1st Congress on Invertebrate Reproduction and Development (ICIRD)", STRI, Panama City, Panama, Aug. 6 – 9, 2007.

Award for best student presentation. National Crustacean-Conference (CrustTag), DZMB, D-Wilhelmshaven, Germany. Feb. 17 – 20, 2005.

Other funds: Hermann Willkomm-Stiftung, Freunde und Förderer of Goethe-University.

Teaching Experience

Since 2006 Teaching and trainig at Senckenberg-School.
Dec. 2008 Lecture "Crustacea", main study course "diversity and phylogeny of animals", Goethe-University.
2003 - 2006 Practical course "diversity and phylogeny of animals" (main study period), Goethe-University.
2001 - 2003 Assistant in practical courses of ecology, taxonomic (Zoology and Botany. Goethe- University.

Museum & Public Relations

Conception, organisation and execution of events „Research live – Frankfurt by the sea" Apr. 2009, „Night of the Museums" Apr. 2009, „One day as a deep sea researcher" June, 2009 (Dr. G. Winter).
Special exhibition „(R)evolutionary", Morphisto & Senckenberg, Frankfurt, Germany, Mar. – Apr., 2009
Television broadcast „Urzeitkrebse", Hessischer Rundfunk (HR), 2006.
Display cabinet for special exhibition „amongst parasites", Senckenberg, 2005 (Dr. B. Herkner).
Conception and public relations for the presentation of the Marine Zoology Department Senckenberg on special event „Hessentage" 2004 – 2006.

Methods

Scientific drawing, histology, scanning and transmission electron microscopy (SEM, TEM), confocal laser scanning microscopy (CLSM), molecular methods. Operating and handling of sampling gear of research vessels. MS Office, Adobe Photoshop & Illustrator, Databases (Access, SeSam).

Language

German mother tongue, English and French fluent in speech and writing, basic Spanish.

Publications

Naderloo R, Becker C. Redescription of *Arcotheres placunae* (Hornell and Southwell, 1909) from the Persian Gulf of Iran (Crustacea, Brachura, Pinnotheirdae) (in prep.).

Becker C, Türkay M. Host ecology of European Pinnotheridae (Crustacea, Brachyura) (in prep.).

Becker C, Türkay M, Storch V. The male inner reproductive system of European Pinnotheridae (Crustacea, Decapoda, Brachyura) (in prep.).

Becker C, Türkay M, Brandis D. The male copulatory system of European Pinnotheridae (Crustacea, Decapoda, Brachyura). (submitted to J Morph).

Türkay M, Becker C, Hendrycks E, Karp E, Schneider M. Mega-Epifauna. Cruise Report Meteor 79/1 – Meteor-Berichte, Universität Hamburg (in press).

Becker C, Brandis D, Storch V. 2011. Morphology of the female reproductive system of European Pinnotheridae (Crustacea, Decapoda, Brachyura). J Morph 272(1):12-26. (in press). DOI: 10.1002/jmor.10884.

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Pea crabs in sea squirts and bivalves (Brachyura, Pinnotheridae) (presentation). International Crustacean Conference (ICC7), Qingdao, China, June 20 – 25, 2010.

The Female reproductive system of European pea crabs (Brachyura, Pinnotheridae) (poster). International Crustacean Conference (ICC7), Qingdao, China, June 20 – 25, 2010 (see opposite page).

Study of the male reproductive system of European pea crabs (Brachyura, Pinnotheridae) (presentation). National Crustacean Conference, Rostock, Germany. Apr. 2 – 5, 2009.

Traditional towards modern morphological methods - taxonomy of pinnotherids. (Crustacea, Brachyura, Pinnotheridae) (poster). World Conference of Marine Biodiversity (WCMB), Valencia, Spain, Nov 11 – 15, 2008.

Morphology and function of the male and female reproductive systems in European pinnotherids (Brachyura) (poster). Advances in Crustacean Phylogenetics (ACP), Rostock, Germany, Oct. 7 – 11, 2008.

The secret sexual life of pea crabs (Crustacea, Brachyura, Pinnotheridae) (presentation). XX. International Congress of Zoology (ICZ), Paris, France, Aug. 26 – 29, 2008.

Taxonomy and morphology of European pea crabs (Brachyura, Pinnotheridae) (presentation). National Crustacean Conference (CrustTag), Frankfurt, Germany, Mar. 15 – 18, 2007.

Pea crabs - feeding and breeding inside a clam (Invited lecture). American University of Beirut (AUB), Lebanon, Nov. 25, 2006

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(check CV for awarded presentations)

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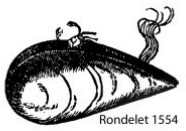
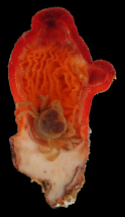


The female reproductive system of European pea crabs (Decapoda, Brachyura, Pinnotheridae)

Introduction

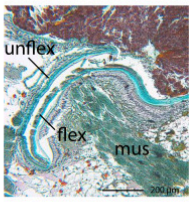
European pinnotherids live commensally inside bivalves or sea squirts (A-F). They show a strong sexual dimorphism (B, C). While adult females never leave their host, focusing on feeding and breeding, the males are optional free-living. (Photos: A and G by S. Tränkner, rest by C. Becker)



D *Nepinnotheres pinnotheres* in a sea squirt

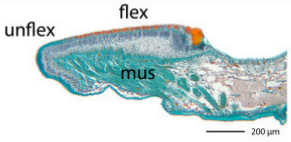
Due to pinnotherids' small size and cryptic way of life, not much is known about their reproductive biology except from the enormous reproductive output. We studied the underlying morphology of the female reproductive system by histology and electron microscopy.

Vagina (**vag**)



The vagina is a cuticular tube with one part of its wall collapsed into the other. The collapsed part is flexible (**flex**) and connected to musculature (**mus**), while the unflexible (**unflex**) part is rigid. With a contraction of this musculature the lumen of the vagina extends.

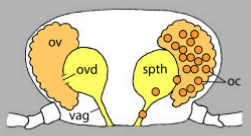
Mobile operculum (**mo**)

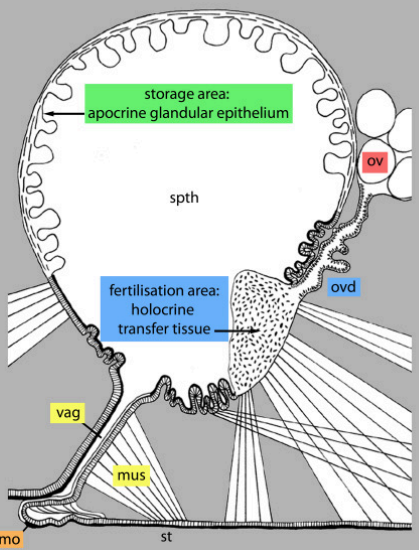


The genital openings are covered by a mobile operculum. Just like in the vagina flexible (**flex**) parts are connected to musculature (**mus**). This cuticle stains red while the unflexible (**unflex**) parts stain green-blue. By muscular contraction, the mobile operculum moves and opens the passage into the vagina.

Overview

Within Eubrachyura, internal fertilisation has developed. Paired vaginas (**vag**) enlarge into storage structures, the so-called spermathecae (**spth**). The ovary (**ov**) is connected to the spermatheca by the oviduct (**ovd**). Sperm is stored, until the oocytes (**oc**) are mature. At ovulation, they are transported into the spermatheca, where the internal fertilisation takes place.



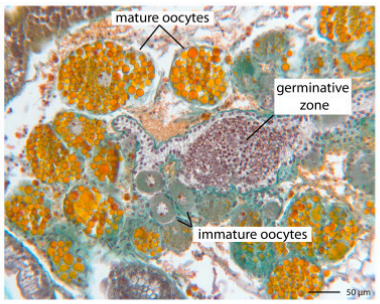


Spermatheca

The spermatheca (**spth**) of the studied pinnotherids can be divided into two distinct areas by function and morphology. The dorsal part is the main sperm **storage area**, which is lined by a highly productive **apocrine glandular epithelium**.
The ventral part, that includes the connection with vagina (**vag**) and oviduct (**ovd**), is lined with cuticle except where the oviduct enters the spermatheca by a **holocrine transfer tissue**.

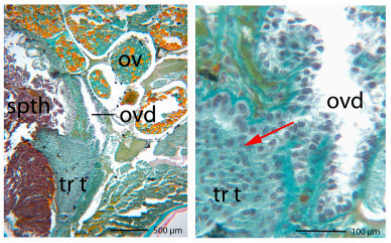
mo mobile operculum
mus musculature
ov ovary
st sternum

Ovary (**ov**)



The ovary of pinnotherids is huge and even extends into the pleon. In every subunit all stages of oocytes are present, which shows a **simultaneous gestation** of several generations. In the **germinative zones** cell division takes place. There are **immature oocytes** present and grown **mature oocytes** full of yolk staining orange.

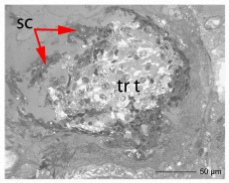
Oviduct (**ovd**) and transfer tissue



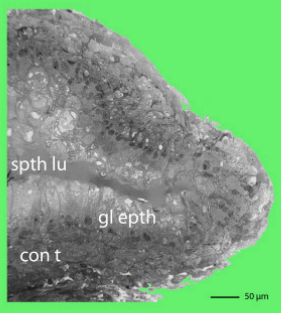
The oviduct (**ovd**) enters the spermatheca (**spth**) by a special transfer tissue (**tr t**). There is no passage present in this tissue, densely packed with nuclei, therefore it is likely that changes take place in this area during ovulation.

ov ovary

Holocrine transfer tissue



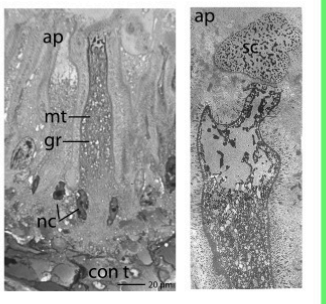
The transfer tissue (**tr t**) carries out holocrine secretion by dissolving whole cells into secretes (**sc**).



section through storage area of spermatheca

Apocrine glandular epithelium

The storage area is lined with a **glandular epithelium** (**gl epth**). It consists of large elongated secretory cells underlined by connective tissue (**con t**). In their base huge, very lobate nuclei (**nc**) are located. The cell body is rich of mitochondria (**mt**) and secretory granules (**gr**), especially apically (**ap**). The secretes (**sc**) are released into the spermatheca lumen (**spth lu**) by dissolving the apical (**ap**) part of the cell, therefore the mechanism is called **apocrine**. The basal part of the cell, where the secretes are produced, remains intact.



secretion process by the apocrine breaking of the glandular cells

Conclusions & Summary

- active role in copulation (muscular control of vagina & mobile operculum)
- outstanding reproductive outputdue to the expanded, very active ovaries
- two ways of secretion in the spermatheca: apocrine glandular epthelium and holocrine transfer tissue (role of secretion in sperm storage and fertilisation...?)

Thanks to Prof. Dr. Michael Türkay