UNIVERSITY OF SOUTHAMPTON

FACULTY OF ENGINEERING, SCIENCE AND MATHEMATICS

School of Ocean and Earth Science

ECOLOGY OF THE INTERTIDAL CRAB *DOTILLA INTERMEDIA* FROM TSUNAMI-IMPACTED BEACHES IN THAILAND

Christopher John Allen

Thesis for the degree of Doctor of Philosophy

April 2010

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ABSTRACT

FACULTY OF ENGINEERING, SCIENCE & MATHEMATICS

SCHOOL OF OCEAN & EARTH SCIENCE

Doctor of Philosophy

ECOLOGY OF THE INTERTIDAL CRAB *DOTILLA INTERMEDIA* FROM TSUNAMI-IMPACTED BEACHES IN THAILAND

Christopher John Allen

Crabs of the genus *Dotilla* are ecologically important members of intertidal sandy shore communities. Exposed sandy shores represent one of the main habitat types along the coast of the Laem Son National Park in Thailand, and *Dotilla* sp. is the dominant macrofaunal species on these beaches, occurring in immense numbers. Despite their importance as a key member of the faunal community on these beaches, little is known about the ecology of *Dotilla* crabs in the Laem Son. Taxonomic investigations identified the *Dotilla* crabs present on the exposed oceanic beaches in the Laem Son to be *D. intermedia*. This represents the first time that *D. intermedia* has been recorded from Thailand. *Dotilla intermedia* inhabits a very well defined zone on the sandy beaches, and the factors underlying these zonation patterns were examined. The gradient of the beach was correlated to the height at which the boundaries of the *Dotilla* zone occurred, with physical factors associated with the beach gradient driving the distribution of *D. intermedia* on the beach. The upper limit of the *Dotilla* zone was controlled by the total water content of the sediment, with *D. intermedia* absent from areas with less than 15% total water content. Tidal influences defined the lower boundary of the *Dotilla* zone, with crabs requiring an area with a minimum exposure time between tidal immersions of 4-5 hours to feed on the sediment. Within the *Dotilla* zone, size segregation was observed; larger crabs occurred higher on the shore, and small crabs lower down. The sandy shores of the Laem Son were heavily impacted by the tsunami of 26th December 2004, which effectively destroyed the populations of *D. intermedia* on the beaches. However, by April 2005 *D. intermedia* was present again on the beaches. A temporal population genetic study was undertaken to investigate the impact of extinction and recolonisation on the genetic variation of a population. Genetic variation in mtDNA markers was found to decrease over time, matching the predictions of mathematical models concerning the effect of bottlenecking events on genetic diversity within populations. The impact of the tsunami on *D. intermedia* is discussed further in light of the ecological and molecular data produced in this thesis.

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1. GENERAL INTRODUCTION

1.1. PROJECT BACKGROUND

Exposed oceanic sandy beaches are one of the major habitat types within the Laem Son National Park in Ranong Province, Thailand. The numerically dominant fauna on these beaches are crabs from the genus *Dotilla*, which occur in immense numbers with records of around 20 million crabs present along a 5 km stretch of beach (GLJ Paterson pers. obs.). Crabs of the genus *Dotilla* are ecologically important members of tropical sandy-shore intertidal communities, and can be found on tropical shores and mudflats from East Africa and the Red Sea eastwards towards Japan where they generally inhabit the mid to low shore of the beach (Alcock 1900b). However, there have been very few studies based in the Laem Son, and no detailed work involving *Dotilla* from the exposed sandy shores in this region. This project employed a range of methods to increase our knowledge of *Dotilla* on the oceanic beaches in the Laem Son, beginning with ascertaining the identification of the *Dotilla* species, before undertaking an investigation into the ecology of the species. Sandy beaches are a very dynamic environment, with faunal zonation patterns hypothesised to be driven by physical factors rather than biological interactions as seen on rocky shores (McLachlan & Jaramillo 1995). The *Dotilla* occupy a very well defined zone on the sandy beaches in the Laem Son, and the examination of the ecology of these crabs offered an opportunity to investigate what physical factors structured the distribution of these crabs on the beach.

The beaches in the Laem Son were heavily impacted by the tsunami of the 26th December 2004. The populations of *Dotilla* were effectively destroyed on these beaches by the impact of the tsunami wave, but by April 2005 the *Dotilla* had returned to the beaches, albeit in lower numbers. The *Dotilla* crabs offered an ideal case study with which to examine the ecological consequences of recolonisation after a major disturbance event, with the tsunami representing an agent of catastrophic change in the soft sediment marine community.

1.2. THE ECOLOGY AND BIOLOGY OF THE GENUS *DOTILLA*

1.2.1. GENUS *DOTILLA* STIMPSON, 1858

The genus *Dotilla* was described by Stimpson in 1858. The genus is found within the family Ocypodidae, in the subfamily Dotillinae (Stimpson 1858), which is also commonly known by the synonym Scopimerinae (Alcock 1900a). The most recent classification of *Dotilla*, along with the only key to the genus, appears in Kemp (1919). The most defining feature of the genus is the formation of the abdomen, which serves to distinguish *Dotilla* from any other crustacean genus. The fourth abdominal somite overlaps the fifth, with a brush of setae at the distal end of the fourth somite (Fig. 1.1).

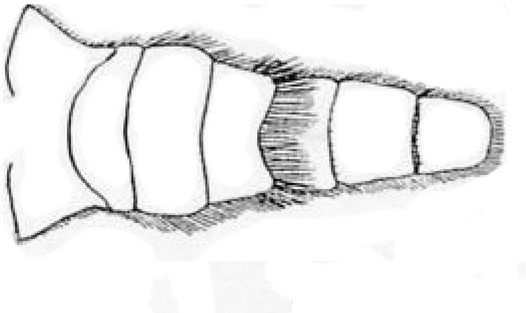


Fig. 1.1. The abdomen of a male *Dotilla wichmanni*, showing the characteristic formation of the *Dotilla* abdomen (from Rathbun 1910). The fourth somite bears a band of setae along the distal margin and overlaps the fifth somite

There are currently eight described species of *Dotilla*:

Dotilla sulcata	(Forskål, 1775)
Dotilla myctiroides	(H. Milne Edwards, 1852)
Dotilla fenestrata	Hilgendorf, 1869
Dotilla intermedia	De Man, 1888
Dotilla wichmanni	De Man, 1892
Dotilla blanfordi	Alcock, 1900
Dotilla malabarica	Nobili, 1903
Dotilla pertinax	Kemp, 1915

There are several characters that have been used to distinguish between the different species of *Dotilla.* The carapace in *Dotilla* is highly sculpted, with each species having a specific pattern (Fig. 1.2). These patterns can be observed even on the smallest individuals, although species identification is harder to confirm on small crabs as the grooves on the carapace are less clear (Vogel 1983). Gordon (1941) illustrated the first male pleopods for all of the *Dotilla* except *D. blanfordi* (Fig. 1.3), whilst Vogel (1984) demonstrated that the structure of the spoon tipped setae located on the inner surface of the largest part of the second maxilliped are also species specific (Fig. 1.4).

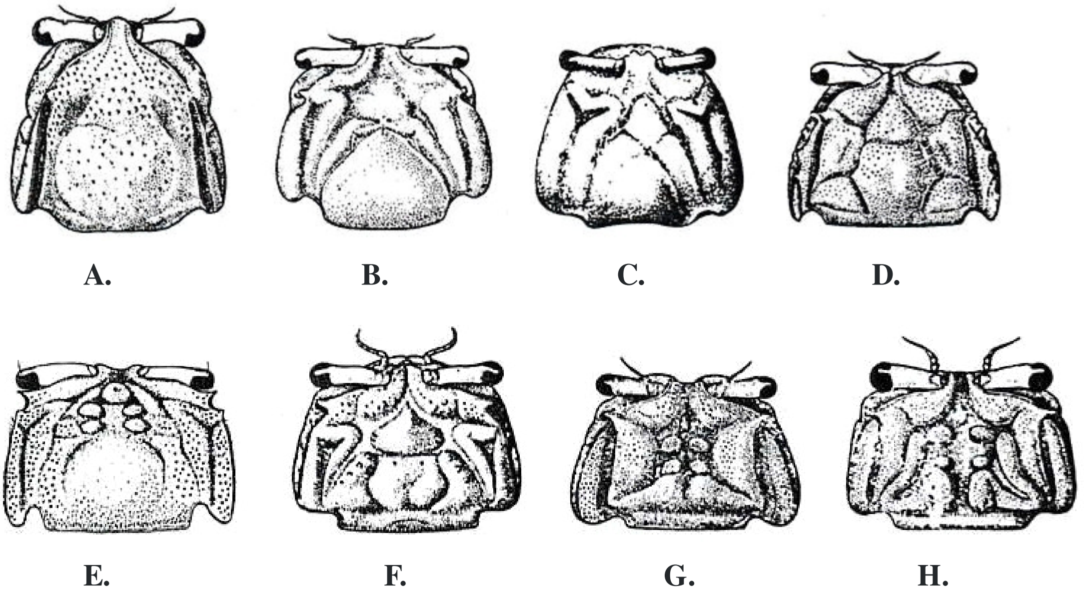


Fig. 1.2. Carapace sculptures of *Dotilla* (from Kemp 1919 and Vogel 1983). A. *D. myctiroides*; B. *D. sulcata*; C. *D. fenestrata*; D. *D. wichmanni*; E. *D. malabarica*; F. *D. pertinax*; G. *D. blanfordi*; H. *D. intermedia*

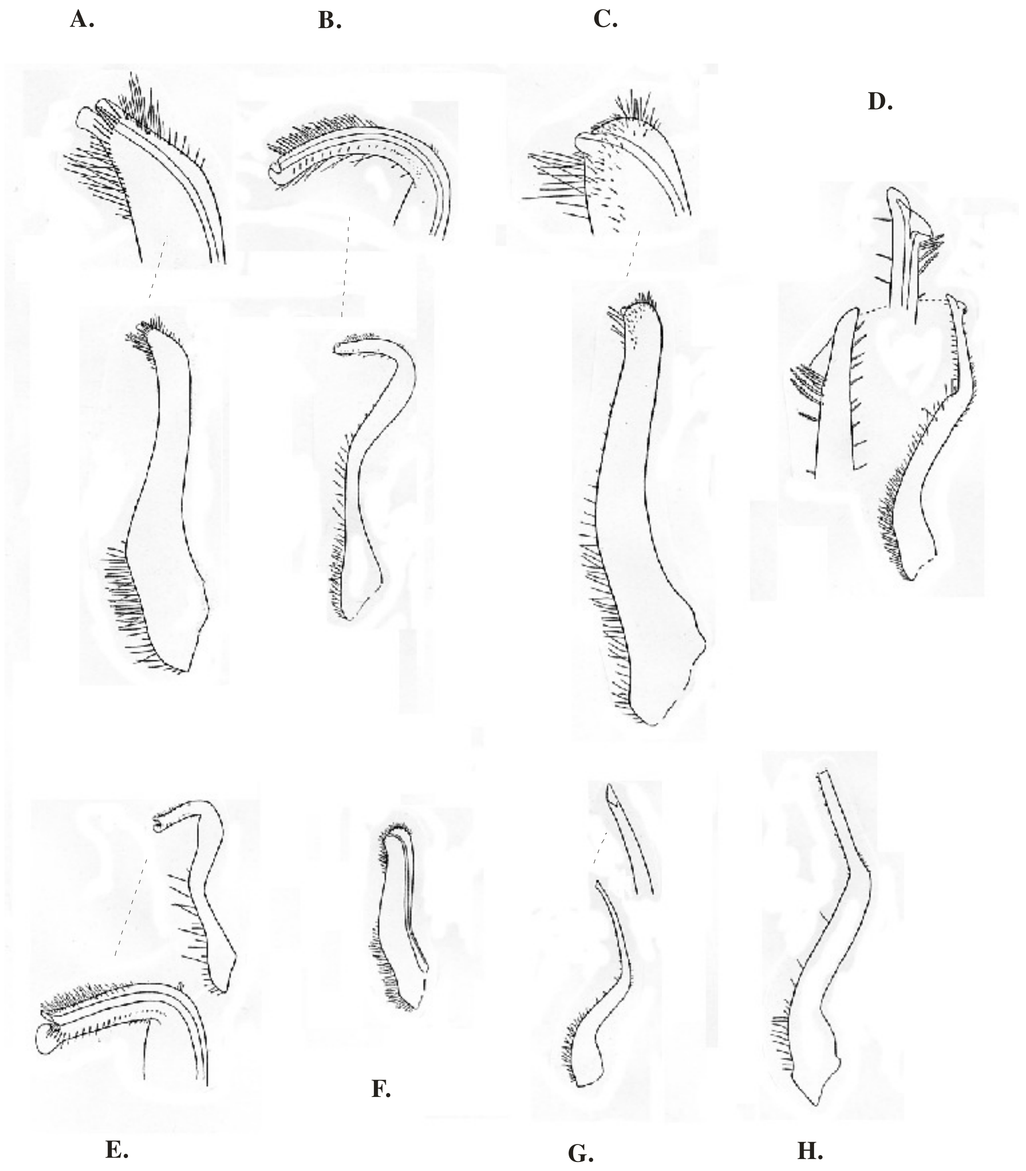


Fig. 1.3. First male pleopods of *Dotilla* (from Gordon 1941). A. *D. wichmanni*; B. *D. fenestrata*; C. *D. myctiroides*; D. *D. malabarica*; E. *D. sulcata*; F. *D. intermedia*; G. *D. pertinax* (young co-type); H. *D. pertinax* (apex missing)

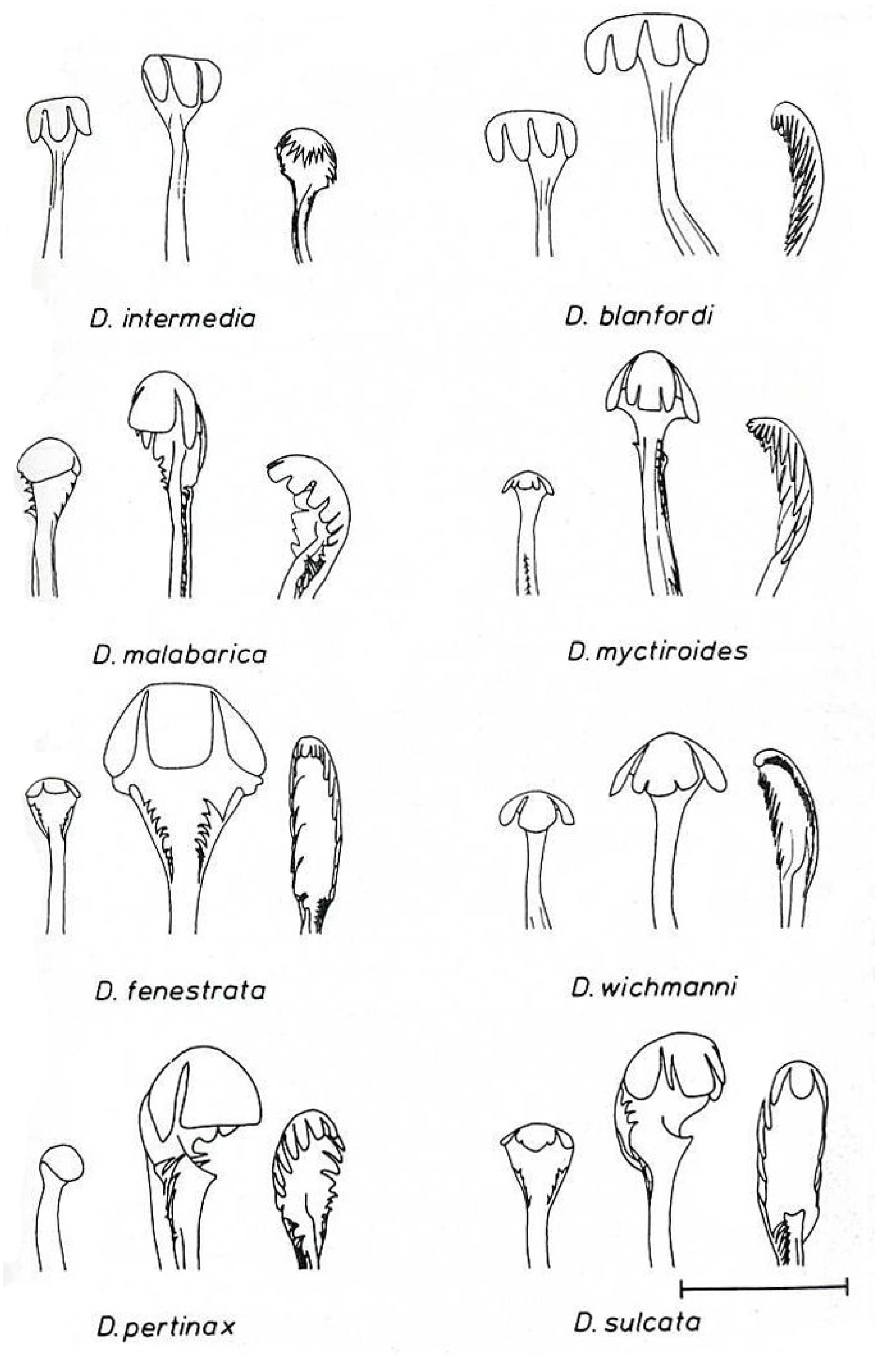


Fig. 1.4. Species-specific shape of the spoon tipped setae in *Dotilla*. Scale 50 µm (from Vogel 1984)

Both sexes in *Dotilla* possess similarly narrow abdomens. Excluding ovigerous females and large male crabs with strongly developed secondary sexual characteristics (e.g. well developed teeth on the cutting edge of the chela), reliable sexual identification of *Dotilla* requires examination of the pleopods, and is very difficult to perform in the field (Kemp 1919; Vogel 1983).

1.2.2. ADAPTATIONS

Dotilla possess various morphological, physiological and behavioural adaptations enabling them to inhabit the intertidal zone (Gherardi & Russo 2001). *Dotilla* adopt an isospatial strategy (i.e. they do not resort to mobility to avoid a phase that is unfavourable for them), but remain within a belt along the sea-land axis, with alternating exposure to air and water (Vannini & Chelazzi 1985). The adaptations that *Dotilla* possess set the intertidal limits for their distribution. As air-breathers they require an area that is regularly uncovered by the tide for a period long enough for them to forage a sufficient ration for metabolic requirements. As surface deposit-feeders they require an area in which the surface food supply is regularly renewed when covered by the tide, and in which the sediment particle size is of an appropriate size for the mouth parts of the crab. *Dotilla* also require areas with sediment types of a suitable particle size and water content for the construction of their burrows (Ansell 1988).

Dotilla demonstrate several adaptations for living in a semi-terrestrial habitat. All species of *Dotilla* possess a large flat membranous disk on the meral segments of their legs. Originally thought to have a function in hearing (Aurivillius 1893), these discs, known as tympana, are respiratory structures used in aerial gas exchange (Maitland 1986). A thinning of the cuticle and epidermis creates a respiratory surface that acts to oxygenate venous blood returning from the leg sinuses. The tympana offset the fact that *Dotilla* do not possess a lung. Preventing gas exchange across the tympana reduces total oxygen consumption by 60% (Maitland 1986). The total surface area of these structures is comparable to that of the branchiostegites in the more typical bimodal breathing crabs that are adapted to semi-terrestrial life (Henry 1994). In the possession of tympana, the Dotillinae differ from all other decapods (Kemp 1919). Three of the *Dotilla* also possess tympana on their thoracic sterna – *D. myctiroides, D. fenestrata* and *D. wichmanni* (Gordon 1941; Vogel 1983).

Specialized water-uptake setae are widespread in the semi-terrestrial crabs of the Grapsidae and Ocypodidae, and act to conduct water into the brachial chamber to aid in osmoregulation (Hartnoll 1973). *Dotilla* possess a group of water-uptake setae that are situated on the distal margin of the fourth abdominal somite (Fig. 1.1). These setae allow for the uptake of interstitial water by lowering the abdomen and pressing the setae into the sand, a behaviour known as 'sponging' (Fielder 1970). The water picked up by the setae passes to a space between the abdomen and sternum through openings formed by the emargination of the proximal part of the fifth abdominal segment. All *Dotilla* have well-defined grooves at the edge of the abdomen that allow for the water to run posteriorly into the postero-median corner of each brachial chamber via a channel beneath the proximal end of the first abdominal segment (Hartnoll 1973).

These physiological and behavioural adaptations allow a *Dotilla* to remain at the surface feeding for long periods using only the water acquired through its specialised setae, avoiding the need to periodically return to a burrow to replenish its water supply, a behaviour that can be observed in the closely related *Scopimera* and *Uca* (Fielder 1970). The specialized setae negate the necessity of *Dotilla* to excavate burrows that descend through the sediment to below the water table, as demonstrated by *D. fenestrata* (Hartnoll 1973). Currently, there is no evidence as to whether the setation of *Dotilla* is more or less efficient as a method of the uptake of capillary water than returning to a burrow to uptake the standing water there. There is also no evidence as to why *Dotilla* have this special setation, in contrast to *Scopimera,* which exploits substantially the same niche as *Dotilla*, but at a generally higher and drier level on the shore (Hartnoll 1973).

1.2.3. FORAGING

Dotilla build their burrows in the area from mean low water level to just above mean sea level. As a result, their habitat is periodically flooded, leading to a continuous renewal of organic content in the uppermost sediment layer. The organic components of the sediment (detritus, algae, bacteria) are the main food of *Dotilla*. They act as filter feeders, extracting their food from the substratum, which requires highly complex feeding apparatus and processes (Vogel 1984).

The chelipeds of *Dotilla* are slightly curved, and are used to scrape wet sand or mud from the sediment onto the lower portions of a groove formed by the outer third maxillipeds. The process of sifting out edible material occurs inside the mouth cavity. The second maxillipeds are of prime importance in the sifting processes. They bear a large number of spoon-tipped setae that were first observed in *Dotilla* crabs by Bigalke (1921) when he studied the feeding mode of *D. fenestrata*. These setae are found in other members of the Ocypodidae, including *Uca* (Crane 1941; Feest 1969) and *Scopimera* (Ono 1965). Crane (1941) first suggested that spoon-tipped setae play an integral role in the feeding of ocypodid crabs. The spoon tipped setae are located on the inner surface of the largest part of the second maxilliped. This surface is composed of the merus and carpus, which together form a functional unit. The propodus and dactylus are completely or almost completely reduced and there is no trace of an exopodite on the maxilliped (Vogel 1984). As previously mentioned, the shape of the spoon-tipped setae is species-specific in within *Dotilla* (Fig. 1.4).

Altevogt (1957) hypothesised that ocypodid crabs could separate food particles from sediment grains by using a floatation mechanism. Miller (1961) confirmed this hypothesis by studying *Uca*, and found that water was actively introduced into the buccal cavity, which served to sort particles for ingestion based on their specific gravity. Heavy, inorganic particles sank to the base of the buccal cavity and thus were rejected, whilst the less dense organic particles remained in suspension for ingestion. After separating the edible material from most of the inorganic particles, the unusable matter is rejected in the form of a small pellet at the upper edge the third maxillipeds. Once this globule has reached a size of approximately 3 mm, it is pinched off by the claw of one of the chelipeds, and passed under the walking legs of the crab (Vogel 1984).

Feeding activity in *Dotilla* is usually centred on the burrow, from which the crabs move to and fro while scraping up and sorting the sand around their burrows (Ansell 1988) (Fig. 1.5). Feeding crabs move sideways along a radius from the burrow, creating a row of pellets in a tangent to the burrow entrance (Zimmer-Faust 1987). If disturbed whilst feeding, the crabs will retreat back into their burrows. Once the perceived danger has passed, the crabs re-emerge and continue to feed along the same trench as before they were disturbed. Crabs continue to feed in the

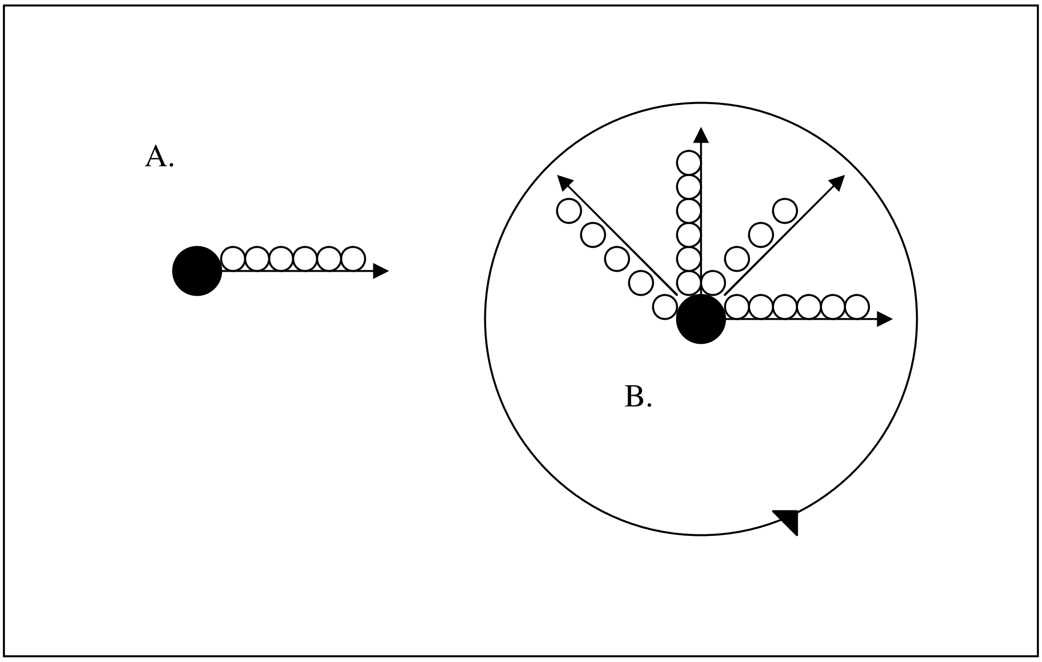


Fig. 1.5. Feeding patterns in *Dotilla*. A. Crabs move sideways from their burrow, laying out a line of pellets in a tangent to the burrow entrance as they feed. B. After moving a set distance from the burrow, subsequent feeding trenches are laid down in a circular pattern around the burrow entrance, feeding always proceeding in one direction.

same direction after a disturbance, even if any signs of their previous activity have been experimentally removed. *Dotilla wichmanni* has been shown to utilise skylight polarization patterns to re-orientate themselves after re-emerging from their burrow. Visual landmarks close to the burrow also play a role in orientation when astronomical cues provide no useful information, such as on overcast days (Luschi *et al.* 1997). *Dotilla* feed in circular patterns around their burrows, a foraging behaviour that is defined by maximising their foraging efficiency in an environment where food is distributed unevenly in patches across the shore, and the importance of the burrow to the crabs. Each of these factors is outlined in more detail below.

The most unpredictable parameter for crabs that feed on surface deposits is the distribution of food over the shore. Food gradients change in a complex way from tide to tide so that crabs cannot efficiently sample them (Gherardi *et al.* 1999). Random foraging has sometimes been assumed to be a suboptimal strategy for obtaining a minimum daily energy ration (e.g. Stanton 1982), although this is not always the case (Zimmerman 1979). In highly complex habitats where resources cannot be assessed from a distance, the time and energy needed to find high-quality patches may become so great that the most efficient response is simply to harvest each patch encountered. Selective grazing can be too inefficient due to sampling costs and the neural investment needed to register food quality. Patches are harvested at random with respect to quality, though it might be expected that movements between patches will not be random in order to minimize travel and to avoid feeding at patches already visited (Zimmer-Faust 1987).

Zimmer-Faust (1987) studied the foraging behaviour of *Scopimera inflata*, and observed that crabs rarely fed in the same place twice. Such orientation behaviour would only be advantageous if the unexploited substrates contain more biomass than those in the area where the crab has already fed. The crabs only removed the top 3mm of sediment as they foraged, so the biomass concentrations between 0-3mm depth and 3-6mm depth were compared, and found to be significantly greater in surface sediments than those from the subsurface. *Dotilla* forage in an identical manner to *Scopimera*, and this study demonstrates that the foraging movements and orientation shown by *Scopimera* and *Dotilla* act to enhance their food intake.

Circular paths optimize net resource yields, but only when the resources are homogeneously distributed (e.g. Covich 1976; Hixon 1980), which, as already mentioned, is an unlikely scenario for deposit feeding crabs to encounter. However, circular paths should also be expected if animals either fail to discriminate among food concentrations above threshold levels, or if a particular place (e.g. a burrow) provides an essential or limiting resource constraining foragers to balance conflicting demands (Zimmer-Faust 1987). Crabs that forage in a circular pattern around their burrows minimize the distance they travel away from a burrow. Burrows provide some species of crab with their only reliable source of water during an ebb tide, a resource that is essential to enable feeding and to resisting desiccation, as well as providing a refuge from predation (Zimmer-Faust 1987). The water uptake setae present on the abdomen of *Dotilla* grant them a degree of independence from burrow water supply, but the burrow is still an important central place as a refuge from predators, and environmental exposure such as heat irradiation and desiccation (Warner 1977).

1.2.4. BURROWING

Two different types of burrowing behaviour have been reported for two species of *Dotilla*: *D. fenestrata* (Hartnoll 1973) and *D. myctiroides* (Tweedie 1950). The first burrow type is a simple open vertical tube structure. Individual crabs dig these burrows in well-drained and firm sand, where the water table falls well below the bottom of the burrow at low tide. The second type of burrow involves the crab making a shallow depression in the sediment. Within this depression the crab turns onto its side, and rotates backward, pushing particles of sand upward and outwards. The semi-liquid pellets coalesce to form a circular wall, to which the crab adds further pellets, creating a dome-like structure, which has been termed the 'igloo' (Tweedie 1950). Igloos are only found in areas where the water drains inadequately, and do not occur in firm sand with a low water table. Igloo construction traps some air inside with the crab, and appears to be an adaptation enabling the construction of an air chamber in semi-fluid sand, where vertical tunnels cannot be supported by the sediment. After an igloo has been formed, the crab continues to scrape sand from the lower surface of the air chamber that it occupies, and subsequently attaches it to the ceiling. Consequently, the crab and the air chamber descend gradually downwards through the sediment (Takeda *et al.* 1996). Individuals have been observed to construct igloos whilst feeding in semi-fluid sand when disturbed or when the advancing tide approaches (Tweedie 1950). Igloos appear to only be used temporarily compared to the vertical burrows built higher up the shore (Tweedie 1950; Hartnoll 1973; Halis & Yaziz 1982).

1.2.5. BIOTURBATION AND IMPACT ON MEIOFAUNA

Dotilla act as efficient bioturbators of the uppermost few millimetres of the sediment, with their feeding and burrowing activities turning over large volumes of sediment (Bradshaw & Scoffin 1999; Litulo *et al.* 2005). The amount of sediment turned over by a population of *D. myctiroides* with an average burrow density of 91 m^{-2} has been estimated to be 540 kg m^{-2} yr^{-1} (0.41 m^{3} m^{-2} yr^{-1}), of which 33% arises from burrowing and 67% from feeding (Bradshaw & Scoffin 1999). *Dotilla sulcata* causes 6 kg of sediment to be turned over per day per square metre of population on a sandy beach in the Red Sea (Fishelson 1983). Nematode assemblage structure is not affected by the foraging activity of *Dotilla fenestrata*, with the crabs not effectively eating the nematodes. Nematode species that are well-adapted to sediment disturbance, and that do not rely on the first 2mm of sediment for food or oxygen uptake, can survive the continuous and widespread bioturbation caused by the crabs. However, the settlement of nematode colonisers that are not adapted to intense bioturbation and predation may be inhibited by the activities of these deposit feeding crabs (Olaffsson & Ndaro 1997).

1.2.6. DROVE FORMATION

The formation of dense aggregations of crabs, known as 'droves' (Crane 1975), is a behaviour that has been observed in several of the genera within the Ocypodidae, with *Dotilla* being no exception (e.g. Tweedie 1950; Zimmer-Faust 1987; Altevogt 1957). For several Ocypodidae species drove formation has been attributed to serve a reproductive function by increasing the opportunity for crabs to encounter potential mates (e.g. *Ilyoplax pusillus* Wada 1978; *Scopimera globosa* Wada 1981; *Uca* spp. Crane 1975). Another suggested reason for drove formation is as a response to dry sediment conditions in burrow areas, forcing crabs to aggregate in an area where the sediment is more moist in order to facilitate feeding (e.g. Ono 1965; Wada 1981). High population densities have also been cited as a potential factor in causing droves to form (e.g. Ono 1965; Wada 1981).

Droving behaviour constitutes an alternative foraging strategy to feeding around the burrow in *Dotilla fenestrata.* Crabs that wandered in a drove had stomach contents richer in both organic and microalgal mass than sedentary crabs (Gherardi *et al*. 2002). These results are similar to observations made on several species of *Uca* (e.g. Crane 1975; Murai *et al.* 1982, 1983) and *Mictyris longidactylus* (Cameron 1966). Wandering within a drove offers several potential advantages to an individual crab. Grouping in a drove offers passive defences against predation by the effects of dilution and confusion (Russo *et al*. 1998). Wandering in a drove increases the likelihood of encountering richer substrates, whilst at the same time reducing the amount of individual time (and energy) spent searching for food. However, such aggregations have the negative effect of forcing conspecifics to share resources and to compete with each other (Gherardi *et al*. 2002).

The main disadvantage associated with wandering behaviour is experienced when the crabs return to the residential sites with the flooding tide. The wandering crabs can only construct igloos as there is insufficient time to construct a vertical burrow before the tide inundates the area, and the sediment is too waterlogged to support an attempt to excavate a new vertical burrow (Gherardi *et al.* 2002). If a wandering crab is large enough, a profitable option is to force a smaller resident crab out of its burrow, and to steal this refuge for its own use (Gherardi *et al.* 1999).

1.2.7. REPRODUCTION AND GROWTH

There have been very few studies into the reproduction and breeding of *Dotilla.* Berried females were found all year round in a population of *D. myctiroides* from a West Malaysian beach, with two main periods of reproductive activity, from November to February and May to July. Laboratory observations indicated that the hatching of eggs and the release of larvae did not appear to be associated with the lunar cycle (Halis & Yaziz 1982). *Dotilla fenestrata* also shows continuous reproduction throughout the year, with peaks in the number of ovigerous females occurring in February, April and August (Litulo *et al.* 2005).

Sexual asymmetry has been recorded in *Dotilla*, with the male crabs reaching larger average sizes than the female crabs (Halis & Yaziz 1982; Litulo *et al.* 2005). This is not an uncommon phenomenon in brachyuran crabs (e.g. Spivak *et al.* 1996; Henmi 2000). Sexual dimorphism can develop from females concentrating a part of their energetic budget into ovary development, which may lead to lower somatic growth when compared to males. In addition, larger body sizes furnish male crabs with an advantage in intra-specific fights and increases the chance of obtaining females for copulation (Schöne 1968; Christy & Salmon 1984; Dray & Paula 1998).

The life span of *Dotilla myctiroides* has been estimated by using growth rates, with male crabs taking approximately 13 months to attain their maximum size from juvenile stage I, and females requiring approximately 10 months (Halis & Yaziz 1982). However, the majority of individuals never attain their maximum size, suggesting that the average life span of the crabs must be less than these figures, a situation already previously noted from other tropical crustaceans and molluscs (Ansell *et al.* 1972; McLusky *et al.* 1975).

1.3. SANDY BEACH ZONATION

1.3.1. ZONATION ON ROCKY AND SANDY SHORES

The zonation of fauna is a distinctive feature on intertidal shores. Although the zonation of rocky shore species is considered to be maintained both by physical factors and by biological interactions (Menge & Sutherland 1976, Underwood & Denley 1984), faunal zones are defined on a biological basis (Lewis 1964). In contrast to rocky shores, the zonation patterns on sandy beaches are not particularly visible. This is probably a consequence of the dynamic beach environment combined with the general invisibility of the fauna in the sand, and the fact that sandy beach faunal populations regularly shift their position on the shore over time. The role of biological factors in structuring beach communities is uncertain, and beach fauna are able to escape from physical stress by burial in the sand. Zonation is thus expected to be less sharply defined and more variable in exposed sandy beaches than most other intertidal environments (McLachlan & Jaramillo 1995).

1.3.2. SANDY SHORE ZONATION SCHEMES

Several different generalised zonation schemes have been proposed for sandy beaches. The most simple zonation scheme suggests that the shore can be split into two regions, consisting of a zone of air-breathers at and above the drift line (i.e. the limit of the high tide swash), and a zone of water-breathers below this (Mortensen 1921, McLachlan & Brown 2006). Due to its simplicity, this zonation scheme is widely applicable and there is no disputing its validity. However, few authors have adopted this scheme, and it would be more satisfying to have a more complete subdivision of the shore (McLachlan & Jaramillo 1995).

There are two other general zonation schemes for sandy beaches. The first is based on biological parameters, with the beach split into three regions, each defined by the presence of characteristic crustaceans (Dahl 1952):

- Subterrestrial fringe - talitrid amphipods in cool regions, ocypodid crabs in warm areas
- Midlittoral zone - cirolanid isopods
- Sublittoral fringe - variety of animals, including haustoriid, phoxocephalid and oedicerotid amphipods in cool regions, hippid crabs and other decapods in warmer regions.

The second zonation scheme is based on physical parameters, with the beach split into four zones defined by changes in sand moisture content (Salvat 1964):

- Drying zone - dry sand above normal high tide mark, only wetted by spray
- Retention zone - reached by all tides but loses capillary water as the tide recedes, so sediment remains damp but not wet during low tide period.
- Resurgence zone - experiences discharge of water during ebb tide, characterised by interstitial water flow in and out of the sediment with the tide.
- Saturation zone - sand is permanently saturated with water, limited interstitial water flow occurs.

The position of the zones defined by these schemes is only representative of the shore during low tide. As the fauna are mobile, the boundaries of the zones are not expected to have sharp boundaries (McLachlan 1983). The two schemes of zonation generally coincide, with Dahl's subterrestrial fringe and midlittoral zones corresponding to Salvat's drying and retention zones (see Fig. 1.6), showing a relationship between physical and biological zones on sandy beaches. Considering that exposed sandy beaches are one of the most physically controlled of all marine ecosystems, it is not surprising that there is an underlying physical basis for any scheme of animal distribution in such systems (McLachlan 1990).

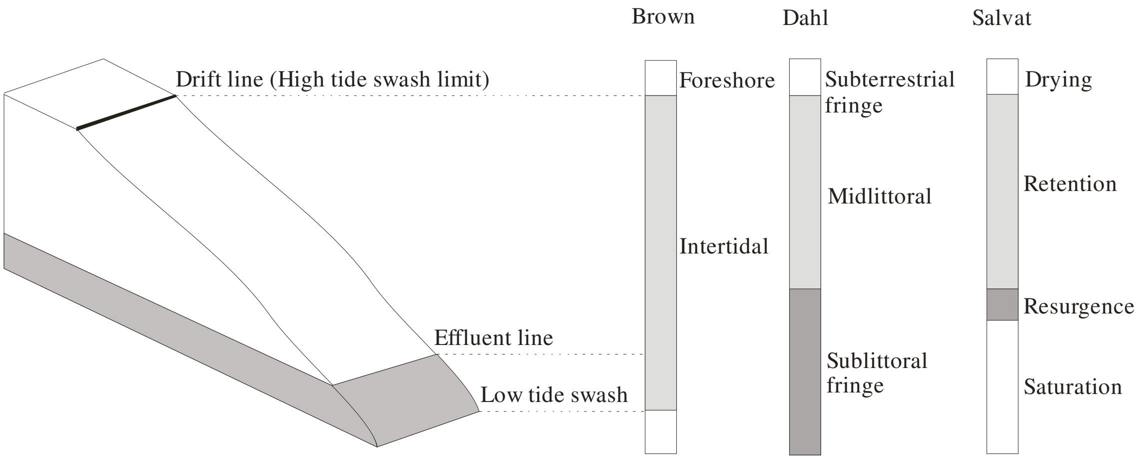


Fig. 1.6. Three schemes of macrofaunal zonation on sandy beaches

McLachlan & Jaramillo (1995) proposed another general zonation scheme based on a combination of both Dahl (1952) and Salvat's (1964) work. They identified three zones based on the presence of characteristic fauna:

- Supralittoral zone - air-breathing crustaceans situated at and above the driftline, containing talitrid amphipds, oniscid isopods and ocypodid crabs.
- Littoral zone - occurring from the drift line down to damp sand at or just above the effluent line, characterised by true intertidal taxa such as cirolanid isopods, spionid polychaetes and opheliids.
- Sublittoral zone - an upward extension of the surf zone with its upper edge on the lower or mid-intertidal near the effluent line, with hippid crabs, donacid bivalves and haustoriid amphipods being characteristic organisms within this zone.

1.3.3. TEMPORAL VARIABILITY

Zonation as recorded by the researcher reflects faunal distribution only at the time of the investigation. These zones are modified in the short term by tidal migrations and in the medium to long term by responses to seasons, storms, and beach accretion/erosion cycles. The movements and migration of fauna on sandy beaches are important for the survival of the organisms in such a harsh environment, resulting in zonation patterns that are variable in both time and space (McLachlan & Jaramillo 1995).

The most important rhythmic activities are tidal migrations, which occur in many mobile species inhabiting open sandy beaches. In crustaceans these migrations are a result of endogenous rhythmic behaviour, whilst in molluscs they tend to be direct responses to physical cues. Tidal activity rhythms have been widely observed in intertidal organisms (reviewed by Palmer 1973). Tidal migratory behaviour allows organisms to remain within an optimal zone on the shore. Some organisms like the hippid crab *Emerita analoga* try to a maintain position within the swash zone where feeding conditions are optimal, and where marine and terrestrial predators have limited access (Fusaro 1980; McLachlan & Hesp 1984). In supralittoral organisms tidal migration takes the form of emergence at low tide to avoid immersion. Zonation patterns blur towards high tide as many species move up the shore and zones compress (McLachlan & Brown 2006). Semi-lunar rhythms are coupled to spring-neap cycles and are found in both molluscs and crustaceans (McLachlan 1987).

Intertidal organisms have been shown to respond to changes in hydrostatic pressure associated with on-shore tides (e.g. Enright 1962; Morgan 1965). The cumacean *Dimorphostylis asiatica* inhabits the sublittoral region and emerges at night, displaying a clear rhythmic pattern synchronised with both tidal and day-night cycles (Akiyama & Yoshida 1990). Experimental studies on *Uca* have shown crabs moving from a tidal-based activity rhythm to a circardian pattern when monitored under constant illumination (Barnwell 1966). The tidal rhythm of *Carcinus maenus* can be switched off by maintaining individuals in non-tidal aquaria for four weeks (Naylor 1960). Conversely, *C. maenus* from a non-tidal dock can be induced to display a tidal activity pattern by chilling individuals (Naylor 1963). Ontogenetic differences have been recorded in the circadian activity rhythm of the amphipod *Orchestoidea tuberculata*, with juveniles displaying a different peak in activity to adults, which is suggested to avoid intraspecific predation (Kennedy *et al.* 2000).

Longer-term temporal changes in zonation have been recorded at both the community and population level. Some of the most extreme changes in zonation are associated with the monsoon in India. During this stormy and erosive period, much of the fauna disappears from the beaches (Ansell *et al.* 1972). Although less dramatic than the Indian monsoon, seasonal changes have also been recorded in other communities. In temperate regions, seasonality is typically shown by a higher abundance of intertidal macrofauna in summer than during winter (e.g. Leber 1982;

Degraer *et al.* 1999). In tropical areas, seasonality is more related to disturbance due to rainfall or storms (McLachlan & Brown 2006).

1.3.4. INTRASPECIFIC ZONATION BY SIZE

Many species of sandy-beach macrofauna have been shown to demonstrate zonation by size class. Intraspecific variation by size has been widely recorded in molluscs. In *Donax trunculus* on the French Atlantic coast, the smallest individuals are found highest on the shore, whilst the largest are located near low water spring tide (Ansell & Lagardère 1980). Both *Mesodesma mactroides* from Brazil (Bergonci & Thomé 2008) and *Donax denticulatus* in Jamaica (Wade 1967) have a similar intraspecific distribution, with adults confined to the lower saturated shore and juveniles found in unsaturated sand higher up the beach. Size class zonation has also been shown to occur in the isopods *Excirolana braziliensis* (Glynn *et al.* 1975) and *E. armata* (de Alava & Defeo 1991). Populations of *Hippa pacifica* in Hawaii have small individuals in the upper part of the swash zone and larger individuals in the lower part, possibly resulting from competitive dominance by large individuals during feeding (Haley 1982). The dominant intraspecific competitors for space or food tend to occupy those beach levels with optimal environmental conditions, whilst small individuals may be displaced towards suboptimal areas on one or both extremes of a species distribution range (de Alava & Defeo 1991; Kennedy *et al.* 2000). Sexual asymmetry in individual sizes can lead to differential zonation patterns between sexes (Defeo & McLachlan 2005).

1.3.5. RECRUITMENT

On rocky shores, larval recruitment for sessile species is critical for establishing and maintaining a given area. However, the mobility of the sandy shore fauna lessens the importance of larval recruitment for zone maintenance, although recruitment has a significant role in the initial establishment of a population on a beach (Brown 1983). Most of the studies concerning the larval dispersal of sandy beach fauna have been restricted to sheltered shores (e.g. Woodin 1976, 1986). Little is currently known about the long-term recruitment variability in populations of exposed sandy beach macrofauna (Defeo 1996). There may be some biotic interactions involved in settlement success, for example filter feeders removing settling larvae (McLachlan & Brown 2006). Woodin (1976) suggested that discrete densely populated patches of infaunal invertebrates maintain their integrity by preventing the recruitment of larvae from other species. The exact method of the adult-larvae interaction depends on the functional group of the adult (e.g. bioturbators suffocate the larvae, whilst suspension feeders remove them from the water column). Some work has been done on how larvae select the physical environment on which to settle. For example *Donax serra* spat has a strong tendency to settle in areas of fine sand in the surf zone (Lastra & McLachlan 1996), whilst the megalopa larvae of *Emerita* sp. settle in greater numbers near large adult populations (Dugan & Hubbard 1996). Several sandy shore species explore the substratum before settling, and depending on the sedimentary characteristics of a beach (including the organic content), larvae may delay metamorphosis until suitable conditions are found (Brown 1983). Efford (1970) proposed four hypotheses to explain how the dispersal mechanisms and settlement behaviour of sandy beach macrofauna lead to the restocking of adult populations, based on his work with *Emerita analoga*, which are outlined below. The first two hypothesise how larvae can settle back onto their beaches of origin, whilst the last two hypothesise how beaches can be restocked from populations on other beaches.

- The counter current hypothesis – larvae remain near the coastline of their origin as a result of coastal currents and counter-currents running parallel to the shore
- The gyre hypothesis – a gyre regularly brings larvae back to shore
- The nursery area hypothesis – larvae drift offshore and spend the rest of their larval lives in offshore nursery areas. Larvae found in the plankton many miles offshore are destined to die or populate new beaches, but not those beaches that are from their original coastline.
- The rearing current hypothesis – species with restricted spawning areas produce planktonic eggs and larvae that are dispersed to another restricted but suitable area for settlement and recruitment.

1.3.6. COMPETITION

Competition on sandy shores is less evident than on rocky shores. Compared to rocky shores, space does not appear to be a limiting factor on sandy beaches. The three-dimensional nature of sandy beach substratum allows species to burrow into it, thereby minimising competition by interference. The crushing and undercutting of individuals common on rocky shores is therefore unlikely to occur on sandy beaches. Considering the mobility of the sandy beach fauna, the overgrowth interactions that are the primary cause of competition-based mortality in hard-substrate communities are therefore difficult to envisage (Peterson 1979). Individuals that can interact are usually able move away from each other, reducing the amount of potential competitive interactions. Although food can be a patchy resource on sandy shores, it is unlikely to be limited for filter and deposit feeders that are opportunistic by nature compared to rocky-shore grazers (McLachlan & Brown 2006).

Despite these considerations, some competitive interactions have been demonstrated for several species on sandy shores. Species are more numerous and often more specialised and less opportunistic in sheltered conditions compared to the fauna of high-energy shores, which increases the potential for competition on sheltered beaches (McLachlan & Brown 2006). Negative correlations between the horizontal distributions of the haustoriid amphipods *Acanthohaustorius millsi* and *Haustorius canadensis* have been demonstrated on a sheltered beach in Maine, supported by laboratory coexistence experiments (Croker & Hatfield 1980). The distributions of haustoriid amphipods on Texas beaches have also been hypothesised to be driven by biological competition (Shelton & Robertson 1981). On very sheltered shores tube-building polychaetes have been shown to inhibit deposit feeders (Woodin 1977). Within dense assemblages of deposit feeding polychaetes, large burrowers have been shown to negatively affect small surface deposit feeders, which in turn ingest the larvae of the former (Wilson 1981).

There seems to be little evidence to date to indicate the importance of competitive interactions as an influencing factor in zonation on exposed beaches, with data being mainly circumstantial or correlational rather than experimental (McLachlan & Jaramillo 1995). For example, a negative relationship has been shown between densities of *Donax hanleyanus* and *Emerita brasiliensis* (Cardoso & Veloso 2003), whilst mass mortality in *Mesodesma donacium* (related to an increase in seawater temperature during an El Niño event) led to an increase in the densities of *Donax peruvianus* and *Emerita analoga* (Arntz *et al.* 1987). Both these studies suggest competition between the species involved, but there have been no experimental data to support these hypotheses.

1.3.7. PREDATION

There are three main groups of predators that feed on sandy beaches (Defeo & McLachlan 2005):

- birds, arachnids and insects from land
- fishes from the sea
- resident invertebrates, notably crabs and gastropods

Predators on rocky shores are considered to potentially hold competing populations below the threshold at which competition becomes an important factor, leading to increased diversity (Branch 1984). In contrast, all experimental tests of predator exclusion on sandy shores have resulted in an increased or unaltered level of diversity, and an increase in the abundance and density of individual species (e.g. Dauer *et al.* 1982; Bottom 1984; Quammen 1984; Frid & James 1988; Wilson 1991). These studies support the contention that competition is less important on sandy beaches than on rocky shores (McLachlan & Brown 2006). The limited effect of predation on competition on exposed beaches can only increase the evenness of a community by reducing the abundance of dominant species, without changing species richness (McLachlan & Jaramillo 1995). Caging experiments on a sand flat have shown that apart from reducing the mean size of individuals, large carnivorous fish and birds had little effect on the benthos. The exclusion of smaller predators (such as crabs, shrimp, and juvenile fish) led to significant increases in the population sizes of meiofauna, small macrofauna and the juveniles of larger macrofauna (Reise 1985). Whilst predation may have impacts on sheltered shores and sand flats (Reise 1985; Peterson 1991), no such effects have yet been demonstrated on open exposed beaches (McLachlan & Brown 2006). Predation effects are assumed to be greatest in benign habitats, and decrease as the harshness of the habitat increases (Connell 1975).

Some of the temporal variations in zonation patterns on sandy beaches can be explained by predation (Defeo & McLachlan 2005). As prey items change their zonation patterns, predators follow in a synchronised migration (Ansell *et al.* 1999). Microhabitats could be actively selected by prey to avoid predation, or conversely by predators searching for food concentrations (Takahashi & Kawaguchi 1998).

1.3.8. ENVIRONMENTAL FACTORS

The zonation of species may be related to the gradient of environmental variables across the intertidal region. Interspecific differences in preference and tolerance to temperature, moisture, and air exposure may act to drive zonation patterns (Brown 1983). However, most intertidal animals have tolerance levels to natural variables that exceed those necessary for survival in their given habitat, meaning that zonation does not necessarily reflect the tolerances of species (Newell 1979). Some exceptions do occur, for example ocypodid crabs may experience environmental conditions close to their lethal limits (Brown 1983; Fishelson 1983). Intertidal species tend to be more tolerant of environmental extremes than those species that occupy subtidal habitats (McLachlan & Brown 2006). For example, the intertidal mole crab *Emerita talpoida* can survive salinity changes better than the more subtidal *Emertia analoga* (Bursey 1978). The latitudinal distributions and tidal level of species of *Bullia* and *Donax* are related to their thermal tolerance (Ansell 1983; Brown 1983), in addition to correlating well to their respective resistances to desiccation (McLachlan & Brown 2006).

Air tolerance is unlikely to affect zonation in an environment where exposure is routinely escaped by burrowing into the sand, although the distribution of species on the upper shore may be influenced by moisture zones in the sand and tolerances to desiccation (McLachlan & Jaramillo 1995). The ability of crustaceans to utilise the upper intertidal and supratidal levels of sandy beaches must relate to their adaptations to avoid desiccation, including behavioural adaptations such as burrowing to deep moist layers and adopting nocturnal activities (McLachlan & Jaramillo 1995).

1.3.9. BEACH MORPHODYNAMICS

There is an emerging field in sandy shore ecology examining how beach morphodynamic models influence macroscale community patterns. Exposed sandy beaches can be divided into three main morphodynamic types, based on wave height, wave period and sediment grain size - reflective, dissipative and intermediate (Short 1996). Reflective beaches are characterised by coarse sand, short swash periods, steep slopes, high substrate penetrability, low organic matter and low sediment water content. Dissipative beaches have fine sand, flat slopes, low substrate penetrability and high water content. Intermediate beaches fall between the two extremes of reflective and dissipative beaches. A general trend has been demonstrated whereby species diversity, total abundance and biomass increases from reflective to dissipative beaches (reviewed in McLachlan & Dorvlo 2005).

Several hypotheses have been proposed to explain these patterns in macrobenthic communities. McLachlan (1990) adapted the Autoecological Hypothesis (Noy-Meir 1979), stating that the communities of physically controlled environments such as sandy beaches are structured by the independent responses of individual species to the physical environment, and that biological interactions are minimal. The Swash Exclusion Hypothesis (SEH, McLachlan *et al.* 1993) proposes that the increasingly harsh swash climate and coarseness of sands towards the reflective end of the beach morphodynamic spectrum leads to the exclusion of species. There are several studies that support the SEH, demonstrating a strong relationship between community parameters and physical factors (e.g. McLachlan 1990; Defeo *et al.* 1992; McLachlan *et al.* 1993; Jaramillo *et al.* 2000). The Habitat Harshness Hypothesis (HHH, Defeo *et al.* 2001, 2003) extends the predictions of the SEH, postulating that the harsh environment of reflective beaches forces organisms to divert more energy towards maintenance, resulting in lower fecundity, growth, mass and survival. The HHH has been tested for abundance (e.g. McLachlan 1990; Jaramillo *et al.* 2000), fecundity, growth and survival (Brazeiro 2005; Celentano & Defeo 2006), burrowing rates (e.g. Nel *et al.* 1999, 2001) and reproductive strategy (e.g. Delgado & Defeo 2006, 2007). Most of these studies have been based on sublittoral species such as *Emertia* or *Donax*, and there has been very little work examining how beach morphodynamic state influences littoral species like *Dotilla* crabs.

1.4. POPULATION GENETICS

1.4.1. POPULATION GENETIC STRUCTURE

Population genetics is the quantitative study of the frequency of alleles and genotypes in a population. Mechanisms that change allele frequencies and stratify genetic diversity at the population level are responsible for the formation of new phylogenetic lineages and eventually new species, driving the process of evolution (Mayr 1963). By investigating how different factors result in the partitioning of population genetic structure we can gain an insight into the evolutionary process.

In the absence of selection, the genetic structure of a population is determined by the amount of gene flow between demes (i.e. spatially discrete interbreeding groups of organisms with definable genetic characters), relative to the stochastic rate of mutation and genetic drift within demes (Slatkin 1987). Genetic drift and mutation leads to divergence between demes, whilst gene flow acts to mix the distribution of alleles, reducing genetic diversity between demes. Populations can attain equilibrium where genetic drift and mutation are balanced by the opposing effect of gene flow, which is related to the number of individuals in the population contributing to the next generation (Ne) and the fraction of migrants per generation (m) (Wright 1931).

1.4.2. EXTINCTION AND RECOLONISATION

One measure of the genetic variation in a population is the expected heterozygosity per locus. In a population of constant size, where mutation and genetic drift are balanced, the expected heterozygosity for a neutral locus can be expressed as $4\ Nv/(4\ Nv + 1)$, where N is effective population size, and v the mutation rate per locus per generation (Kimura 1968). Changes in N alter the expected average heterozygosity per locus. Throughout a population's evolutionary history there will often be drastic changes in the population size, which will alter the genetic variability within the population. If population size suddenly decreases, the average heterozygosity per locus is expected to decrease due to the loss of low frequency rare alleles, whilst an increase in population size is matched eventually by an increase in the expected average heterozygosity due to occurence of new mutations in the population (Wright

1931). But what happens to the genetic variation of a population if a sudden decrease in population size is then followed by a rapid increase in population size, as would be characterised by an extinction and recolonisation event?

Mayr (1963) predicted that a dramatic decrease in population size followed by an increase would result in the substantial loss of genetic variability within the population. Nei *et al.* (1975) were the first to mathematically model the problem. They showed that average heterozygosity declined rapidly in early generations after a bottleneck event, reaching a certain minimum level, before slowly increasing again. The changes in the genetic variation of the population were influenced not only by the size of the bottleneck, but also by the rate of subsequent population growth. Higher population growth rates led to a lower reduction in average heterozygosity even if the magnitude of the bottlenecking event was very high. Further theoretical models have elaborated on this model (e.g. Chakraborty & Nei 1977; Sirkkomma 1983; Maruyama & Fuerst 1985), allowing predictions on the loss of genetic variability in populations after a bottleneck event. However, there have been few empirical studies with which to test these predictions, examining how the genetic variability of a natural population changes with time after a bottleneck event.

1.4.3. CRUSTACEAN POPULATION GENETICS

Mitochondrial DNA (mtDNA) provides a particularly useful single-locus markers for detecting genetic structure among populations. mtDNA is maternally inherited, and therefore has an effective population size only one quarter that of the nuclear genome (Birky *et al.* 1989). Consequently, mtDNA approaches genetic equilibrium rapidly, and the amount of divergence among demes due to genetic drift can be up to four times greater than a nuclear loci (Birky *et al.* 1989). The use of mtDNA genes, such as the large subunit (16S) ribosomal DNA and cytochrome *c* oxidase subunit I (COI), has been widespread in molecular studies of crustacean genetics at both the population and species level (e.g. Sarver *et al.* 1998; Schubart *et al.* 2000; Petersen 2007; Cook *et al.* 2008; Palero *et al.* 2008). Another mtDNA marker frequently used in crustacean population genetic studies is the mtDNA control region (e.g. Tolley *et al*. 2005; Maggio *et al*. 2009).

A major criticism of using mtDNA markers is that the genes are linked and inherited as a single unit. Also, 16S (and COI to a lesser degree) can be too conserved in some crustacean species to act as good a intraspecific marker (Bucklin *et al.* 1992). Efforts have been made to identify highly variable nuclear DNA markers in crustaceans for population level molecular studies. There has been some success, with high genetic variation seen in elongation factor-1α intron sequences amongst populations of penaeid shrimp (Duda & Palumbi 1999; France *et al.* 1999). Chu *et al.* (2001) investigated the potential use of the first internal transcribed spacer (ITS-1) of ribosomal DNA as a marker for studying both interspecific and intraspecific genetic variation in crustaceans. They found that ITS-1 was highly divergent among different crustacean groups including barnacles, spiny lobsters and mitten crabs, and that there were extensive variations within populations, indicating that ITS-1 could be a suitable marker for molecular systematic studies at both population and species level.

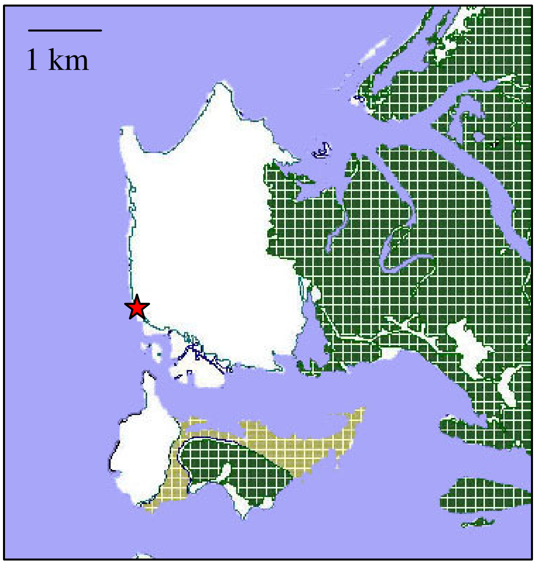
1.5. STUDY AREA

This study was based in the Laem Son National Park, in Ranong Province, south-west Thailand. The coastline of the Laem Son is bounded by the Andaman Sea. The Andaman Sea is part of the Indian Ocean, in the south-east of the Bay of Bengal, situated between approximately 6° to 14° N latitude, and 93° to 99° E longitude, with Thailand to the west and Burma to the north. The Andaman and Nicobar islands mark the western boundary of the water body. The stretch of coastline on the Eastern boundary of the Andaman Sea is characterised by mangrove forests, small estuaries with patchy sea grass beds, and long exposed oceanic sandy beaches. Poorly developed fringing reefs can also be found around islands and rocky outcrops (Chansang & Poovachiranon 1994).

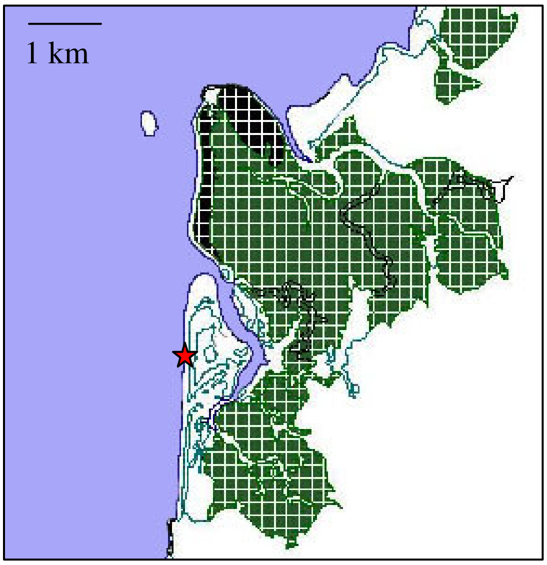
There are two seasons that can be distinguished in this geographical region. Between May and October the southwest monsoon season prevails, characterised by heavy rainfall and strong westerly onshore winds, creating moderate to high wave action throughout this period. From November to April the northeast monsoon, or dry season, dominates. There is little rainfall, limited wind, and waves have reduced intensity, frequency and height. The Andaman Sea coastline has a semidiurnal tide with an amplitude that varies between 1.1 and 3.2 m, with a mean of about 2.2 m (Chansang & Poovachiranon 1994).

Two exposed oceanic sandy beaches in the Laem Son were examined during the study: Prapas beach (9º21’57 N 98º23’41 E) and Bang Ben (9º36’11 N 98º27’56 E) (Fig. 1.7). Both beaches were of an intermediate morphodynamic state, and consisted of moderately sorted fine sand. Bang Ben was flatter and wider than Prapas beach, with Bang Ben having a beach slope of approximately 2.26 %, and Prapas beach having a slope of approximately 3.69 %. The area above the shore was fringed by beach forest at both locations. The macrofaunal assemblages on these beaches were characterised by the presence of the crabs *Scopimera* sp. and *Ocypode* sp. on the upper shore. Just below *Scopimera* there was a well-defined *Dotilla* zone that ran from approximately mean high water neap to the mean low water neap. On the low shore there were sporadic patches of the polychaete *Diopatra* sp. and the callianasid shrimp *Neocallichirus indicus*. Prapas beach was bounded by a rocky headland to the south, and the Kampuan estuary to the north. To the south of the area sampled on

Bang Ben was a tidal estuary. In the estuarine areas to the north and south of Prapas and Bang Ben respectively, sediment particle size was much finer, with *Uca* sp. and *Dotilla myctiroides* found instead of the *Dotilla* sp. that inhabited the oceanic beaches.



A. Bang Ben (9º36'11 N 98º27'56 E)



B. Prapas beach (9º21'57 N 98º23'41 E)

Fig. 1.7. Map of Thailand (from www.cia.gov/library/publications/the-world-factbook/geos/th), with the area of the Laem Son National Park marked by a red box. Two smaller maps show the study beaches; A. Bang Ben, B. Prapas beach. Red stars signify approximate sampling areas, whilst green hatched areas represent mangrove forests (Maps adapted from Kendall *et al.* 2006).

Both Prapas beach and Bang Ben were heavily impacted by the tsunami of 26th December 2004. A study by Kendall *et al.* (2006) demonstrated that the tsunami caused a large change in the sediment granulometry of the sandy beaches within the Laem Son National Park, with a marked increase in the coarse sediment fraction observed post-tsunami in April 2005 compared to 2000. The tsunami also led to an accumulation of sediment onshore and an overall steepening of the beaches (Fig. 1.8). There appeared to be partial re-establishment of faunal populations on the sandy beaches. Lower densities of fauna were recorded in April 2005 than in 2000, with individuals possessing smaller body sizes, suggesting recent post-tsunami recolonisation.

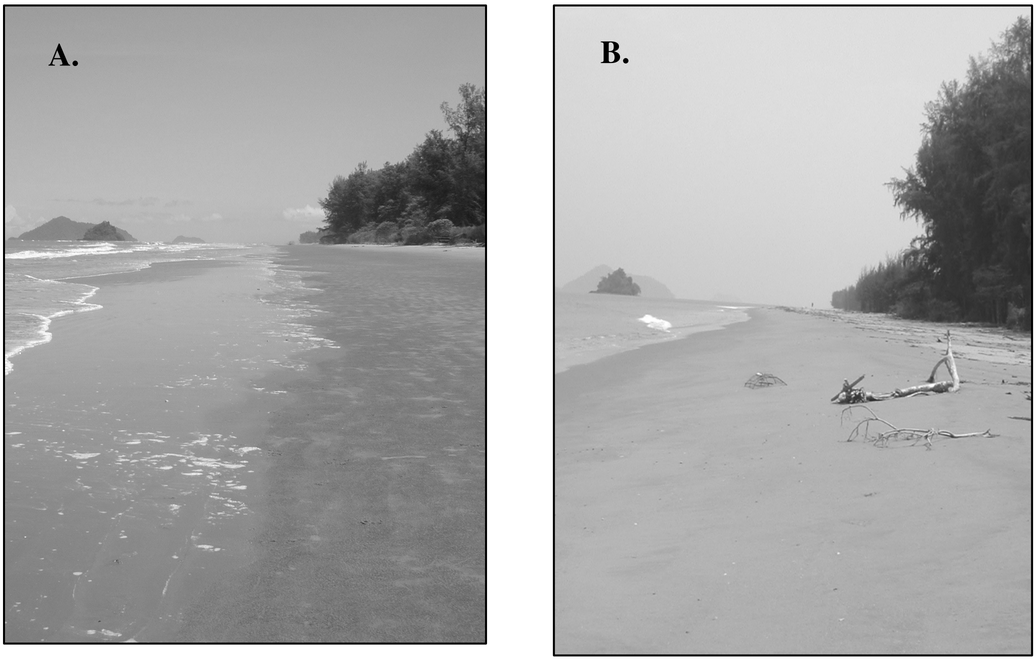


Fig. 1.8. Prapas beach. A. pre-tsunami in 2000; B. post-tsunami in April 2005 (photos taken from Kendall *et al*. 2006)

1.6. PROJECT AIMS AND OUTLINE

- **What was the identity of the *Dotilla* species present on the exposed oceanic sandy beaches of the Laem Son National Park?**

  i. The *Dotilla* in the Laem Son National Park have not been previously studied. Morphological taxonomy was used to identify the species of *Dotilla* that were present on the exposed oceanic sandy beaches that are a major habitat type in this area.
  ii. A small molecular phylogeny was also constructed using specimens from the study beaches and the other *Dotilla* species known from Thailand in order to confirm the morphological diagnosis that the unknown *Dotilla* from the Laem Son was neither of the species of *Dotilla* previously recorded from Thailand.
  iii. The key to the genus *Dotilla* (Kemp 1919) is very old, and is based on using the carapace sculpturing of *Dotilla* as a defining character. Whilst examining museum collection material to confirm the identity of *Dotilla* collected in Thailand, the existing key to the genus was tested, and potential alternative diagnostic characters were investigated. The shape of the first male pleopod is typically used as an identifying character in most modern decapod taxonomic studies. Although the first male pleopods have already been figured for the majority of *Dotilla* species (Gordon 1941), the figures lack detail and *D. blanfordi* was omitted, so the opportunity was taken to refigure this character for the whole genus. (Chapter *2*).

- **What can be determined about the ecology of the *Dotilla* species on the exposed sandy beaches in the Laem Son?**

  i. *Dotilla* are an important species that have a large impact on their environment and can be found over a wide geographical area. The distribution patterns of *Dotilla* on the sandy beaches in the Laem Son were closely examined. *Dotilla* inhabit a very well defined zone on the shore, and the examination of what factors are responsible for driving the observed distribution of *Dotilla* provides a good case study for investigating how sandy shore zonation patterns are established and maintained.

ii. An understanding of the ecology of *Dotilla* in the Laem Son National Park would provide a useful context in which information on their population genetic structure can be placed, so the population size structure and the sex ratio of *Dotilla* was also examined (Chapter *3*).

- **What was the effect of the tsunami on the population genetic structure of the *Dotilla* species from the exposed sandy beaches in the Laem Son?**

i. A temporal molecular study was based on a *Dotilla* population from Prapas beach, which was heavily impacted by the tsunami. This study provided a data set with which to test the predictions of Nei *et al.*'s (1975) mathematical model concerning the impacts of bottleneck events on the genetic variability of populations.

ii. Comparisons between the genetic structures of *Dotilla* populations from different beaches (i.e. Prapas beach and Bang Ben) was also undertaken to examine the geographical genetic variability between spatially separate populations (Chapter *4*).

2. THE IDENTIFICATION OF *DOTILLA* FROM THE LAEM SON

2.1. INTRODUCTION

Crabs assigned to *Dotilla* are distinguished by their abdominal morphology, where the fourth somite overlaps the fifth and possesses a band of setae along its distal margin (Kemp 1919). They can be found on sandy beaches throughout the Indo-Pacific, the Red Sea and on the East coast of Africa (Halis & Yaziz 1982). Only two species of *Dotilla* have been reported from Thailand: *Dotilla myctiroides* has been widely recorded, whilst *D. wichmanni* has only been reported on the Eastern coast (P. Ng pers. comm.).

Two species of *Dotilla* were collected in the Laem Son National Park. One species was found in estuarine and sheltered mangrove areas where the sediment was fairly fine. The distinctive carapace shape and long chelipeds identified this species as *D. myctiroides.* The other species was present in large numbers on the exposed oceanic sandy beaches. Although initially thought to be *D. wichmanni*, this diagnosis was uncertain. These beaches were heavily impacted by the tsunami, and it was important to establish the identity of this *Dotilla* species to complement investigations into the effect of this event on their populations. Therefore a thorough morphological examination of the unknown *Dotilla* species was undertaken.

The only key to *Dotilla* is that of Kemp (1919) (see Appendix I), which relies on examination of the distinct carapace sculpture of *Dotilla* to distinguish between the species. Whilst confirming the identity of the *Dotilla* crabs found in the Laem Son, the strength of the key was examined, and any alternative diagnostic characteristics besides carapace sculpture were considered.

The first male pleopods are often used as diagnostic characters for the identification of decapod crustaceans. Although the first male pleopods have been illustrated for most of the *Dotilla* species by Gordon (1941), the figures lack detail and *D. blanfordi* was omitted. Therefore particular attention was paid on illustrating and describing the first male pleopods of all the species of *Dotilla*.

To compliment the morphological identification of the unknown *Dotilla* species, a small molecular phylogeny was constructed to genetically compare the unknown *Dotilla* species to the two other *Dotilla* species that have been previously recorded from Thailand. Specimens of *D. myctiroides* and *D. wichmanni* were collected from the Laem Son and from Singapore respectively to use for this molecular phylogeny.

There has yet to be a phylogeny for the genus *Dotilla* published, so an attempt was made to create a preliminary morphological phylogeny for this genus. The relationships between the species of *Dotilla* suggested by the phylogeny were then examined in relation to their biogeographical distribution.

2.2. METHODS

A number of specimens of the unidentified *Dotilla* crab were collected from Bang Ben and Prapas beach and preserved in 100% ethanol. The key to *Dotilla* by Kemp (1919) was used as a first reference point for identification. Morphological drawings were made with the aid of a drawing tube and compared to figures in the literature. Specimens of *Dotilla* deposited in the collections of several museums were also examined, described, figured, and compared to the unknown species of the genus. The collections of the following museums were examined: the Natural History Museum, London (NHM); the National University, Singapore (NUS); the National Museum of Natural History, Leiden, Netherlands (RMNH); and the Zoological Museum, Amsterdam (ZMA). Descriptions were based on diagrammatic representations of crabs from Shen (1931) (see Appendix II). The first male pleopods were dissected for all *Dotilla* species and mounted in Polyvinyl Lactophenol.

A molecular phylogeny was constructed for the mitochondrial large ribosomal subunit (16S) using specimens collected in the field and from data on GenBank (see Table 2.1). 16S was chosen as a marker as it has been widely used for phylogenetic studies within the Ocypodidae (e.g. Kitaura *et al.* 1998, 2002; Kitaura & Wada 2006), and there were already nucleotide sequences for *Dotilla wichmanni* and *D. sulcata* on GenBank with which to compare sequences from field collected specimens. *Scopimera* sp. from Prapas beach was also collected to provide an outgroup for the phylogeny. On collection specimens were immediately preserved in 100% ethanol. Total genomic DNA was extracted from muscle tissue taken from the chelipeds and pereiopods (see Chapter *4.2.2.*). The polymerase chain reaction (PCR) and sequencing reactions were undertaken on the extracted DNA for 16S (see Chapter *4.2.3*). After sequence alignment, a neighbour joining tree was constructed using MEGA ver. 4 (Tamura *et al*. 2007). Bootstrap values were generated for the NJ tree within MEGA using 1000 replicates. A Bayesian inference tree was also constructed using Mr. Bayes ver. 3.1.2 (Ronquist & Huelsenbeck 2003). In Mr Bayes, the evolutionary model was set as the General Time Reversible (GTR) model (Tavaré 1986) with gamma-distributed rate variation across sites and a proportion of invariable sites. A search was run for 200,000 generations, with trees sampled every 10 generations (the first 500 tress were discarded as the burn in).

Table 2.1. List of species examined for a molecular phylogeny including collection date, locality and GenBank accession numbers (where applicable).

Species	**Location collected**	**Accession number**
Dotilla myctiroides	13/11/06 Kampuan estuary, Laem Son, Thailand	
Dotilla sulcata		AM180688
Dotilla wichmanni	24/11/06 Sembawang, Singapore	
Dotilla wichmanni		AB002126
Unknown *Dotilla* sp.	11/11/06 Prapas beach, Laem Son, Thailand	
Unknown *Dotilla* sp.	20/11/06 Bang Ben, Laem Son, Thailand	
Scopimera sp.	11/11/06 Prapas beach, Laem Son, Thailand	

A morphological phylogeny of *Dotilla* based on 22 morphological characters was attempted (Table 2.2). A species of *Scopimera, S. bitympana* Shen, 1930, was selected as an outgroup for the phylogeny. The characters selected for use in the phylogeny were those that were the most informative in distinguishing between the species of *Dotilla*. Therefore the characters focused on the differences between the carapace sculpture, first male pleopod and the spoon-tipped setae on the second maxilliped. The character states were only assessed on adult specimens of *Dotilla*. Although some characters such as the carapace sculpture are identical between large and small crabs, other characters like the first male pleopod can only be assessed in mature individuals. Therefore the phylogeny may not reflect any potential shifts in morphology associated with ontogenetic changes.

Table 2.2. A list of characters used to construct a morphological phylogeny of *Dotilla*

1. Spoon-tipped setae on merus of second maxilliped bowl shaped (0) or not (1).
2. Spoon-tipped setae on merus of second maxilliped bowl without (0) or with (1) a thumb-like projection beneath the bowl.
3. Proximal edge of the bowl of the spoon-tipped setae on the merus of the second maxilliped without (0) or with (1) serration.
4. Carapace as long as it is broad (0) or broader than it is long (1).
5. Conspicuous grooves absent (0) or present (1) on carapace.
6. Transverse groove parallel to posterior margin of carapace absent (0), incomplete (1) or complete (2).
7. Gastric region of carapace without (0) or with (1) five tubercles.
8. Gastric region of carapace without (0) or with (1) raised triangular shape.
9. Grooves parallel to lateral margin of carapace without (0) or with (1) anterior bifurcation.
10. Groove running from front to posterior of carapace absent (0) or present (1).
11. Complete raised area on the posterior of carapace absent (0) or present (1).
12. First male pleopod slightly (0) or very (1) sinuous.
13. Curve in distal portion of first male pleopod absent (0), at ~40º (1) or 90º (2).
14. Tip of first male pleopod sparsely (0) or numerously (1) furnished with setae.
15. Setae on tip of first male pleopod unbranched (0) or both plumose and unbranched (1).
16. Tip of first male pleopod blunt (0) or projecting (1).
17. First male pleopod without (0) or with (1) bifid tip.
18. Sternal somites without (0) or with (1) tympana.
19. Dorsal surface of merus chelipeds without (0) or with (1) spine.
20. Tympana on 4th ambulatory leg occupies none (0), a half to two thirds (1) or almost all (3) of merus surface area.
21. Dactylus of 4th ambulatory leg the same (0), two thirds (1), one and a half (2) or twice (3) propodus length.
22. Setal band on 4th abdominal somite entire (0) or two separate tufts (1).

A character matrix was created (Table 2.3), and a maximum parsimony phylogeny constructed using PHYLIP v. 3.69 (Felsenstein 1989). Character scoring for *Scopimera bitympana* was generated from description of the species by Shen (1930, 1932). The character matrix was run through “Seqboot” to generate bootstrapping values using 1000 replicates. A phylogeny was then estimated using “Pars”. “Pars” carries out the Wagner parsimony method with the following assumptions:

- Ancestral states are unknown.

- Different characters evolve independently.
- Different lineages evolve independently.
- Changes to all other states are equally probable.
- These changes are a priori improbable over the evolutionary time spans involved in the differentiation of the group in question.
- Other kinds of evolutionary event such as retention of polymorphism are far less probable than these state changes.
- Rates of evolution in different lineages are sufficiently low that two changes in a long segment of the tree are far less probable than one change in a short segment.

Scopimera bitympana was set as an outgroup, and a consensus tree was created using “Consense”. “Consense” carries out a family of consensus tree methods called the M_l methods (Margush & McMorris 1981). These include strict consensus and majority rule consensus.

Table 2.3. Character data matrix used in the analysis of a morphological phylogeny of the genus *Dotilla*. *Scopimera bitympana* was set as an outgroup. ? = missing data, x = unscorable/ inapplicable conditions

Species	**Character Number**
	0000000001 1111111112 22
	1234567890 1234567890 12
Dotilla blanfordi	1001121001 0001011001 31
Dotilla fenestrata	0111100110 1120101111 00
Dotilla intermedia	1001121001 0001000001 30
Dotilla malabarica	0011101010 1110111000 31
Dotilla myctiroides	0000000000 1001000102 00
Dotilla pertinax	0111120110 0110010002 11
Dotilla sulcata	0111100110 1020101011 00
Dotilla wichmanni	0001110110 1101011101 20
Scopimera bitympana	???10000x0 0??0000002 0x

(Abbreviations used in species descriptions: CW, carapace width; juv., juveniles; ovig., ovigerous; NHM, the Natural History Museum; NUS, National University of Singapore; RMNH, the National Museum of Natural History, Leiden, Netherlands; ZMA, the Zoological Museum, Amsterdam)

2.3. RESULTS

2.3.1. SYSTEMATIC ACCOUNT

Dotilla intermedia De Man, 1888

Dotilla intermedia De Man 1888: 135, pl. ix, Figs 4-6; Kemp 1919: 331, Fig. 9f, 10; Gordon 1941: 137, Fig. 11c; Vogel 1984: 225, Figs 2a, 4.

Dotilla clepsydrodactylus Alcock 1900a: 367, pl. lxii, Fig. 2, 2a; Kemp 1915: 226.

Material Examined

Prapas beach and Bang Ben, Laem Son National Park, Thailand; 12 ♂ (CW 2.4 - 4.9 mm), 2 ♀ ovig. (CW 3.6 - 3.7 mm), 10 ♀ (CW 2.4 - 4.4 mm); Mergui Archipelago; 2 ♂ syntypes (CW 3.5 - 3.9 mm), 1 ♀ syntype (CW 3.7 mm) (ZMA 102540); Burma, Andaman Sea, Mergui Archipelago, Sullivan Island, 1880; 1 ♂ syntype (CW 3.4 mm), 1 ♀ syntype (CW 3.2 mm) (RMNH 27); Chaudipar, Balasore, Orissa, India; 6 ♂ (CW 5.4 - 6.4 mm) (NHM 1919.11.1.95-100); Mergui; 4 ♂ (CW 3.6 - 3.8 mm), 2 ♀ (CW 3.1 - 3.6 mm) (NHM 86.52)

Description

Carapace (Figs 2.1, 2.2): Broader than long, strongly sculptured with a deep mid-dorsal groove running from front to posterior margin. Gastric region marked by five small lobules broadly forming five pointed star pattern. Single groove runs obliquely from side of mesogastric region to antero-lateral angle. Two grooves run obliquely from side of cardiac region to postero-lateral angle. Posterior two-thirds of carapace bears distinct grooves that do not bifurcate anteriorly and run parallel to lateral margin. Complete transverse groove present close to posterior margin. Two parallel grooves run from posterior groove to edge of gastric region, marking lateral edge of cardio-intestinal region.

Second maxilliped (Fig. 2.3): Spoon-tipped setae located on merus not bowl-shaped, but resemble a three-tined fork that is curved at distal end. Peduncle of setae single stalked, with deep groove along axis.

Chela (Figs 2.4b, 2.5b): Specimens from India (NHM 1919.11.1.95-100; NHM 86.52; RMNH 27; ZMA 102540) show distinct tooth on mid-point of cutting edge on both fingers, forming hour-glass shape when chela is closed. In specimens from Laem Son only dactylus bears a distinct tooth. Finger of propodus serrated on proximal half of cutting edge, granular ridge runs back from apex along propodus and onto palm for a short distance. Similar granular ridge runs along dactylus. Fingers at least 1.5 times length of propodus palm.

Fourth ambulatory leg (Figs 2.4a, 2.5a): Dactylus is ca. twice length of propodus. Tympana present on both sides of merus. Tympanum on dorsal surface of merus occupies ca. third to half of total area of merus.

Sternum: First somite possesses a transverse ridge running either side of abdominal trough. Sternal tympana absent.

Abdomen (Fig. 2.30d): Comprises seven separate moveable somites. Distal margin of fourth somite shallowly emarginate and overlaps fifth somite. Fourth somite bears a single band of setae that extends along whole width of distal margin. Male and female abdomens are similar, not v-shaped and u-shaped respectively.

First male pleopod (Figs 2.6, 2.7): Slightly sinuous appendage, ca. uniform in diameter throughout, becoming slender towards tip. Tip of pleopod blunt with numerous unbranched setae. Proximal part has a fringe of plumose setae on outer edge. Dorsal surface bears a line of plumose setae near to outer margin from proximal end almost to tip.

Remarks

Kemp (1919) reported that males of *Dotilla intermedia* display two distinct dimorphic types, that he termed ‘high’ and ‘low’. The above description is of the ‘high’ male form. The ‘low’ male differs in the shape of the first male pleopod, the chela and in form of the first somite. The first pleopod of the ‘low’ male is more slender, longer and more sinuous than the ‘high’ form, with the distal end bent at 90° to the main stalk of the pleopod. The tip of the ‘low’ pleopod does not bear conspicuous setae. The chela of the ‘low’ male only has a tooth on the moveable finger, which is positioned closer to the palm than in the ‘high’ male. The first somite of the ‘low’ male has two triangular teeth that extend beyond the anterior limit of the abdominal trough (see Kemp 1919, Fig. 10).

Distribution

Dotilla intermedia appears to be restricted to the Bay of Bengal, ranging from near Madras in India, and along the coast through to Maungmagan and the Mergui Islands in Burma.

Discussion

The specimens collected from Prapas beach and Bang Ben in the Laem Son National park were identified as *Dotilla intermedia* using the key in Kemp (1919). This diagnosis was supported by examination of figures and descriptions of *D. intermedia* from the literature. The carapace sculpture of the collected specimens closely agreed with the figures of *D. intermedia* in Kemp (1919). The shape of the first male pleopod agrees with figures of *D. intermedia* in Kemp (1919) and Gordon (1941), whilst the spoon-tipped setae are identical those described for *D. intermedia* in Vogel (1984). Comparisons to museum collection material showed a strong resemblance, confirming the diagnosis of the species from the Laem Son National Park to be *D. intermedia*.

There are two morphologically distinct male forms in *Dotilla intermedia* (Kemp 1919; see remarks on *D. intermedia* above). The specimens collected from the Laem Son displayed a transverse ridge on the first abdominal sternum, along with a short and robust pleopod. Both of these features are typical of the 'high' male form. However, the chelae of the 'high' male form possess a large tooth on the cutting edge of both fingers. The Laem Son specimens only had a tooth on the dactylus. Kemp (1919) mentioned that the dimorphic characters of the sternum and chela are not always well developed on small crabs. All specimens examined from the Laem Son were less than 4 mm in carapace width, suggesting that the 'high' male chela character may not have fully developed yet. Kemp (1919) stated that the difference in shape of the first male pleopod remains constant between the two morphs, which provides support to the Laem Son specimens being the 'high' form of *D. intermedia*.

The significance of the dimorphism in male *Dotilla intermedia* is unknown. In the form of the carapace the two morphs resemble each other exactly. The 'low' form has only been found at the same localities as the 'high' form, leading Kemp (1919) to suggest that they are incontestably the same species. Kemp (1919) hypothesised that it would be improbable that more than one type of sexual appendage could be employed in the sexual process, and suggested that the 'low' males do not breed. Only the ‘high’ form was observed in the Laem Son National Park.

The identification of these specimens represents the first time *Dotilla intermedia* has been reported from Thailand. Further investigations were made on beaches to the south of the Laem Son National Park to Phuket. None of the beaches examined were found to be inhabited by *D. intermedia.* The size of the largest specimens of *D. intermedia* (~4.5 mm *CW*) found in the Laem Son were smaller than the largest size seen in the museum material examined and reported in the literature (~6–7 mm *CW*). The Laem Son National Park may represent the south-eastern most limit of the range of *D. intermedia* around the Bay of Bengal. The small size of the specimens encountered in the Laem Son could reflect that they are at the edge of their niche. Further investigations along the western coast of Thailand are needed to confirm whether the Laem Son National Park does indeed mark the boundary of the distribution of *D. intermedia.*

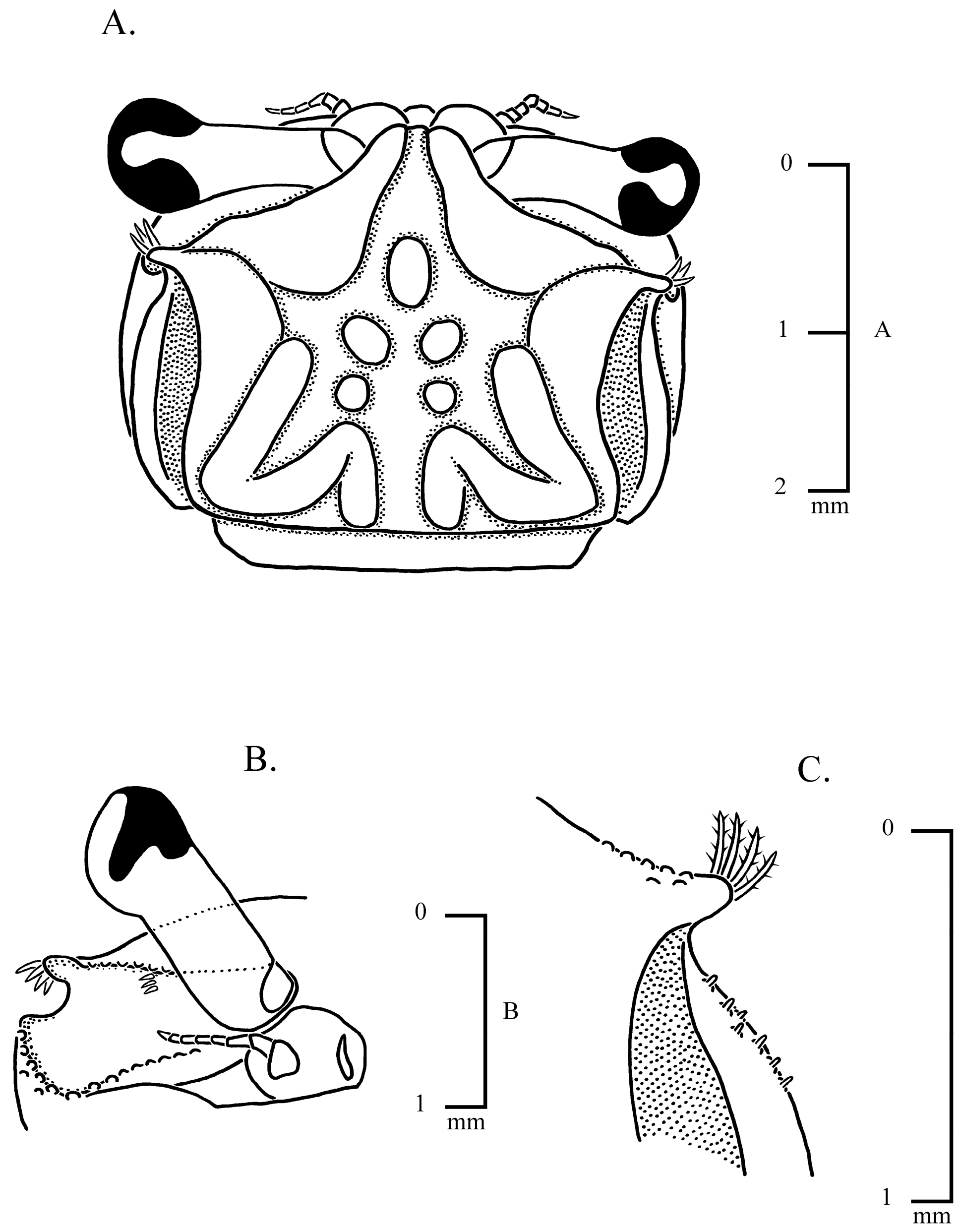


Fig. 2.1. *Dotilla intermedia* Laem Son National Park. A. Carapace, dorsal view; B. Front, anterior view; C. Right antero-lateral margin, dorsal view

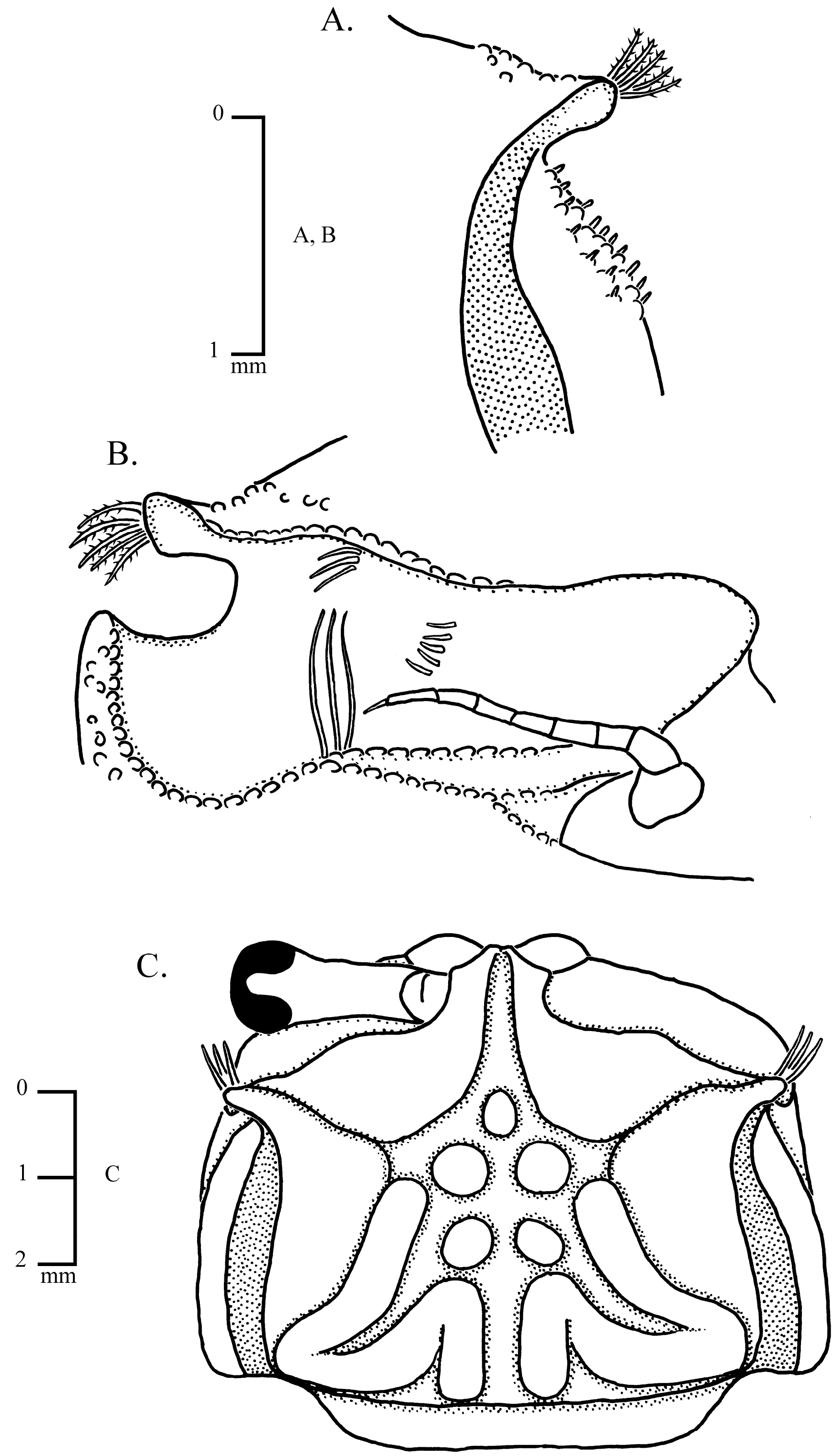


Fig. 2.2. *Dotilla intermedia* (from India; NHM 1919.11.1.95-100). A. Right antero-lateral angle, dorsal view; B. Front, anterior view (eye-stalk missing); C. Carapace, dorsal view (right eye-stalk missing)

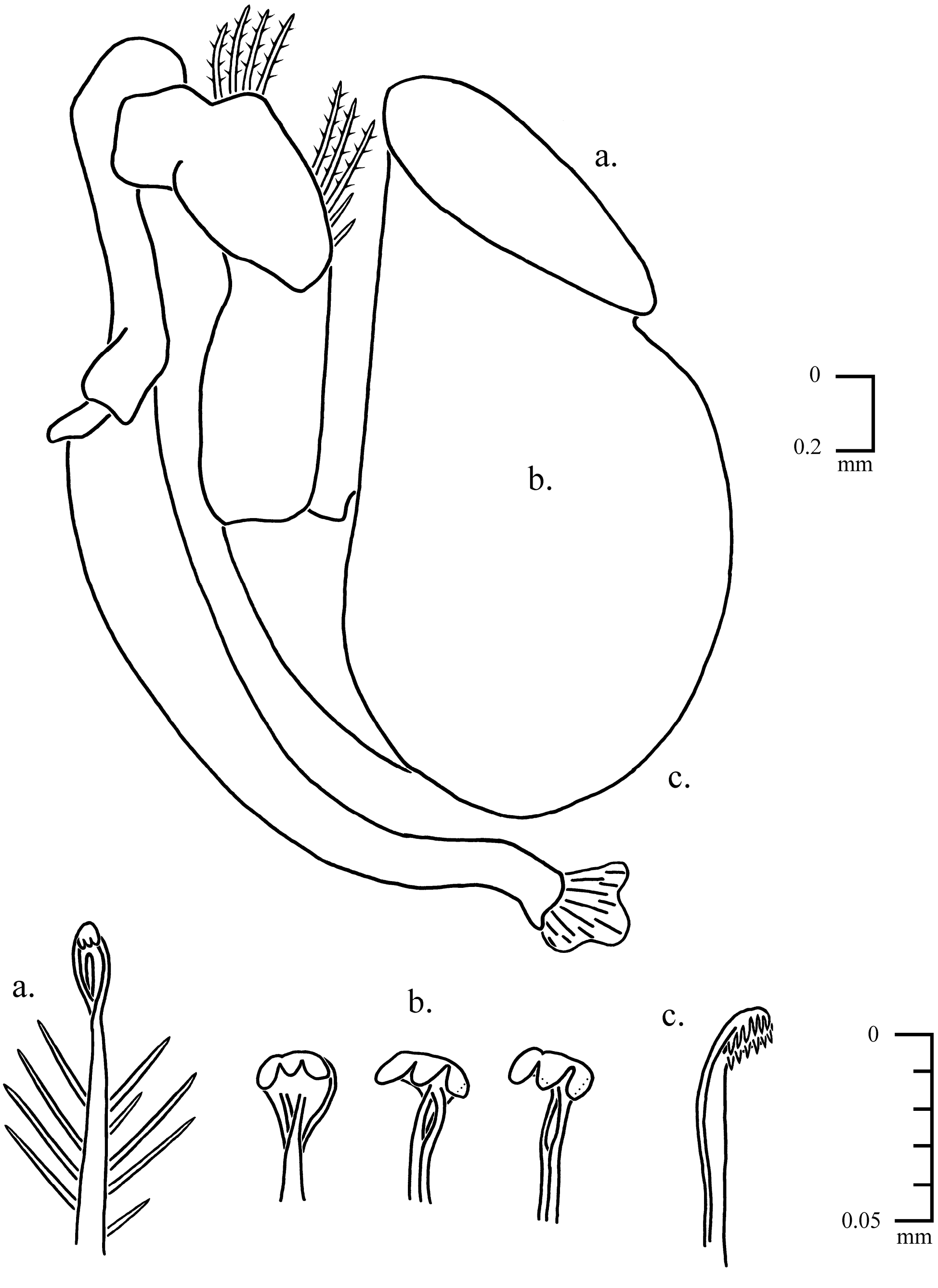


Fig. 2.3. *Dotilla intermedia* Laem Son National Park. Second maxilliped and spoon-tipped setae found on three regions of the maxilliped

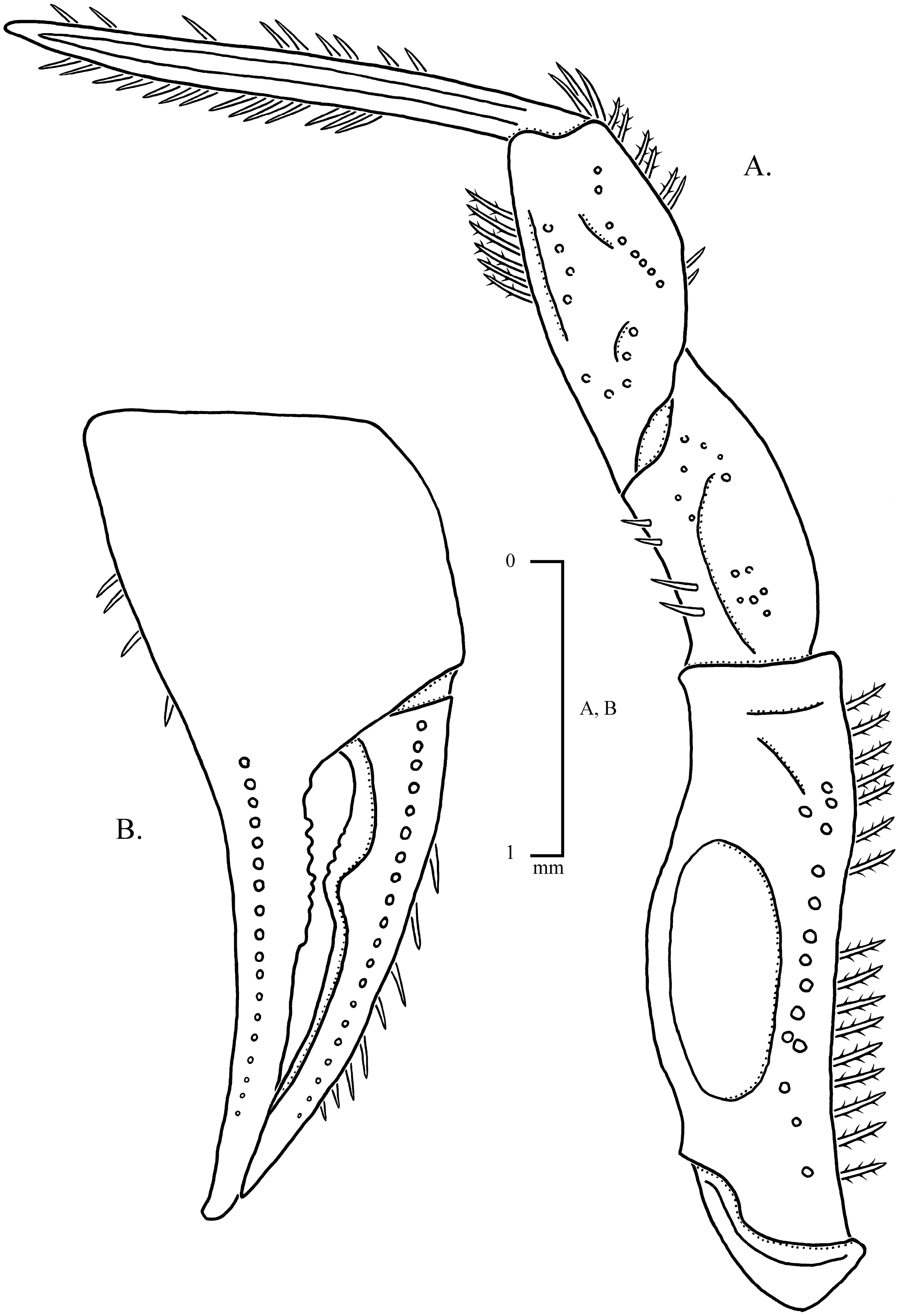


Fig. 2.4. *Dotilla intermedia* Laem Son National Park. A. Left fourth ambulatory leg, dorsal view; B. Right chela, anterior view

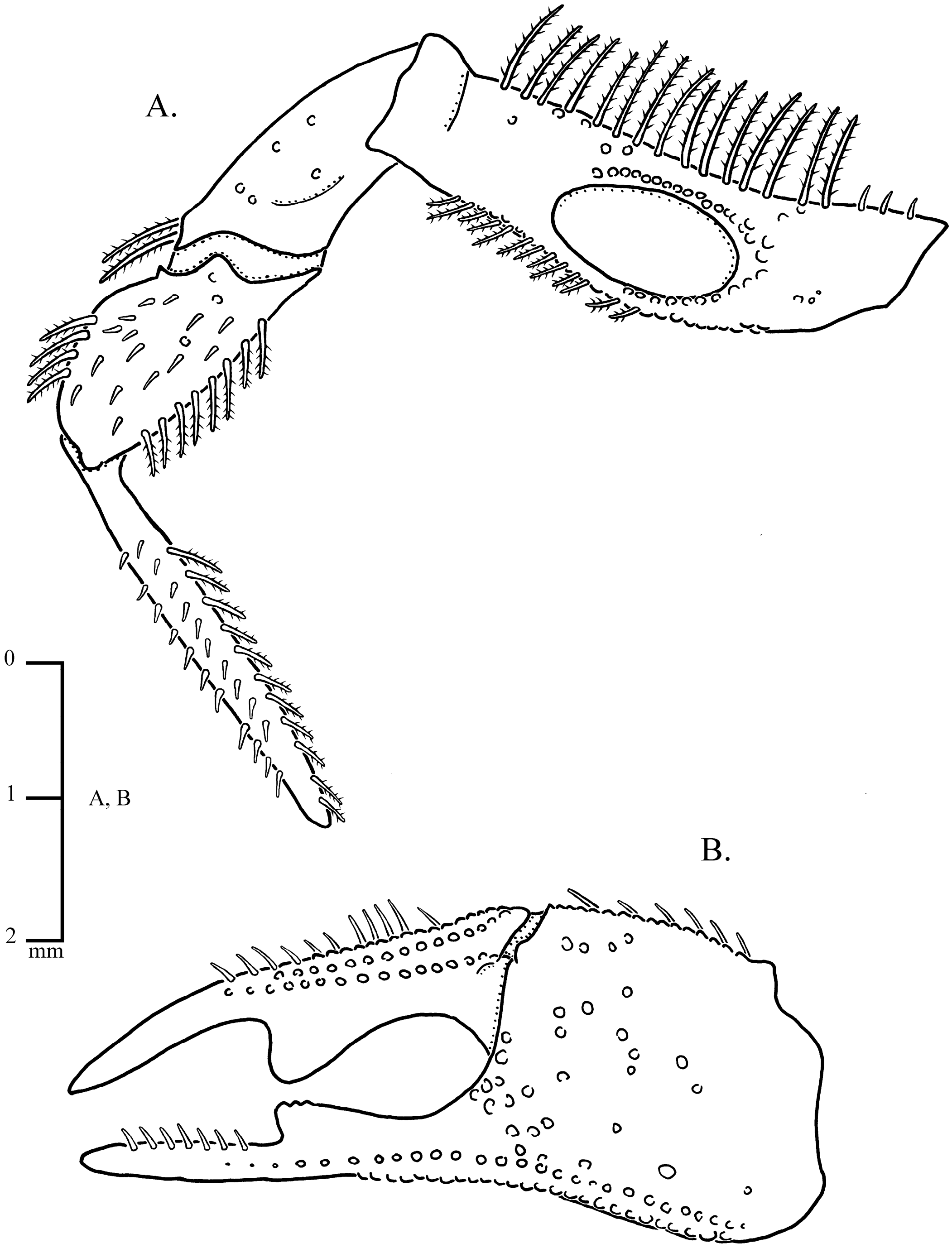


Fig. 2.5. *Dotilla intermedia* (from India, NHM 1919.11.1.95-100). A. Left fourth ambulatory leg, dorsal view; B. Left chela, anterior view

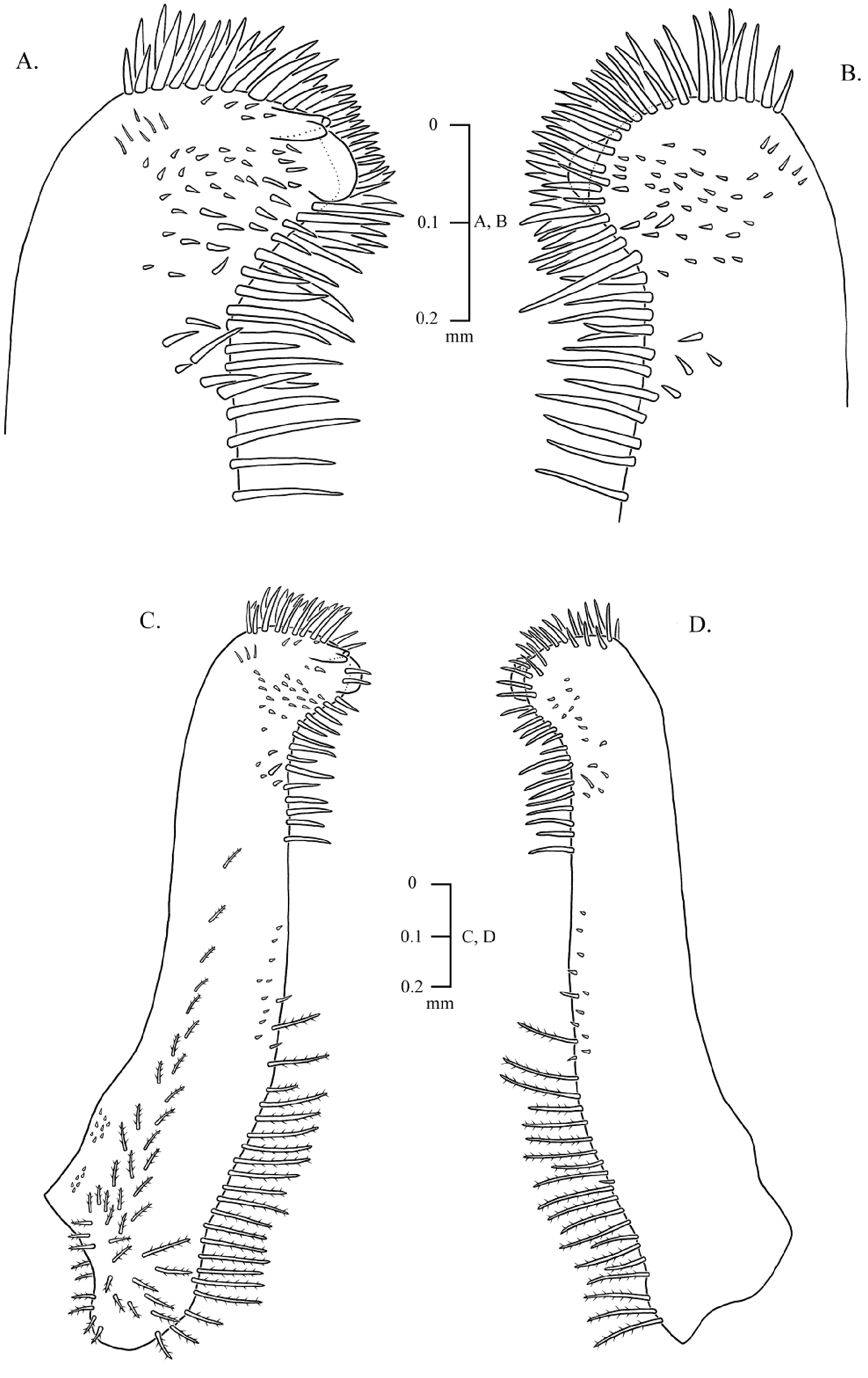


Fig. 2.6. *Dotilla intermedia* Laem Son National Park. A. C. Dorsal view, right first pleopod; B. D. Ventral view, right first pleopod

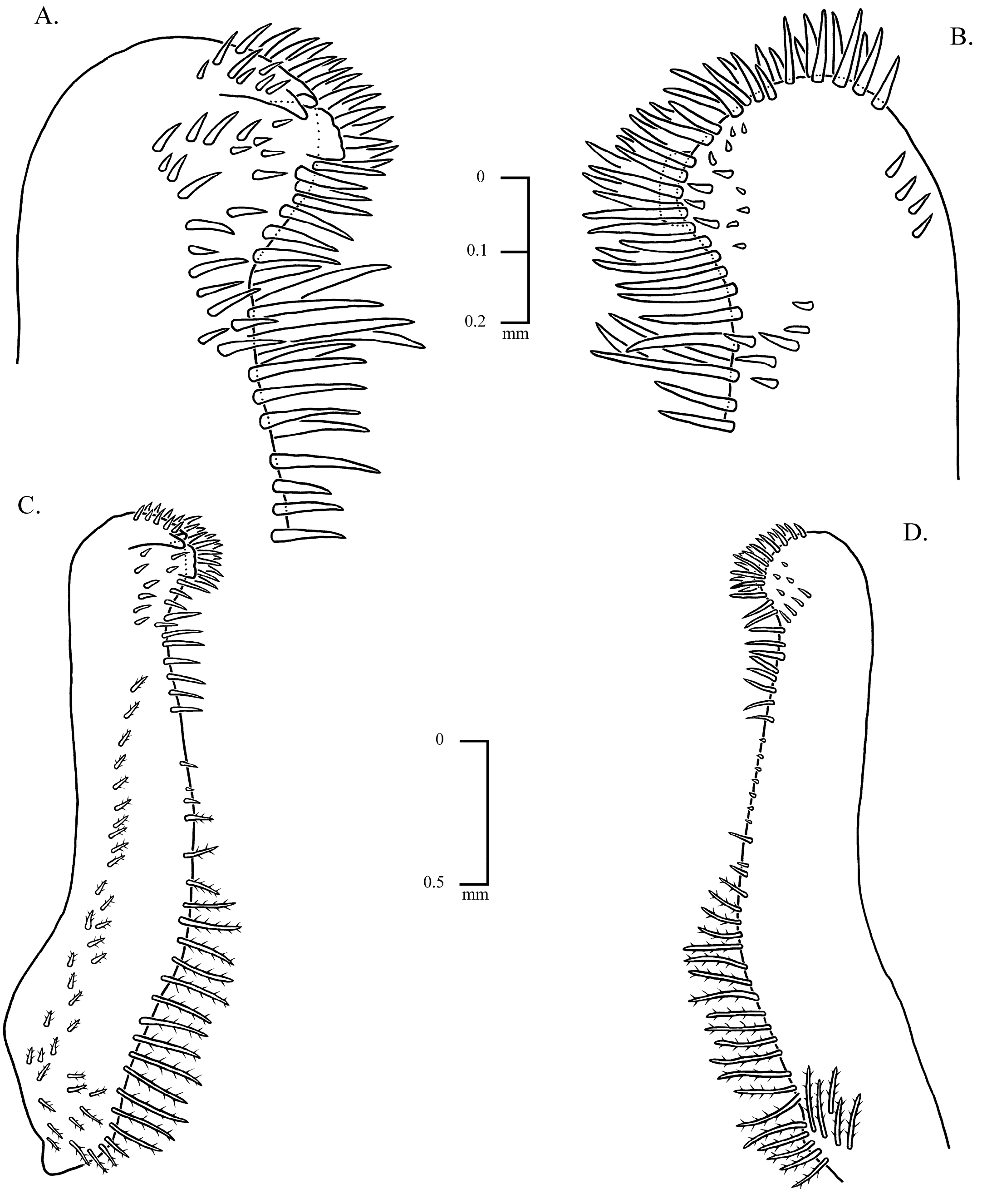


Fig. 2.7. *Dotilla intermedia* (from India, NHM 1919.11.1.95-100). A. C. Dorsal view, right first male pleopod; B. D. Ventral view, right first male pleopod

Dotilla blanfordi Alcock, 1900

Dotilla blanfordi Alcock 1900a: 366, pl. lxiii, Fig. 3, 3a; Kemp 1919: 330, Fig. 9e; Vogel 1984: 225, Fig. 2b, 4.

Material Examined

Bandra, Bombay, India, March 1980; 2 ♂ (CW 3.8 - 4.7 mm), 2 ♀ (CW 3.5 - 4.1 mm) (NHM 1983.456); Mangroves of Manora Island, Pakistan, April 1982; 3♂ (CW 6.0 - 7.0 mm), 2 ♀ (CW 4.8 - 5.0 mm) (NHM 1984.412).

Description

Carapace (Fig. 2.8): Broader than long, wide mid-dorsal groove running from front to posterior. Distinct unbranched groove parallel to lateral margin. Complete transverse groove parallel to posterior margin. Gastric region marked by five small tubercles, separated by grooves. Four of tubercles identical in size; fifth is slightly smaller, positioned between protogastric regions. Grooves run obliquely from corners of gastric area to antero-lateral angle and postero-lateral angle.

Second maxilliped: Spoon-tipped setae located on merus are four tined at distal end, not bowl-shaped. Peduncle of setae single stalked, with groove along axis (Vogel 1984).

Chela (Fig. 2.9b): Fingers slightly longer than length of propodus palm. Tooth absent from both fingers.

Fourth ambulatory leg (Fig. 2.9a): Dactylus ca. twice length of propodus. Tympana on both sides of merus. Tympanum on dorsal surface occupies ca. third to half of total area of merus.

Sternum: Somites devoid of features, sternal tympana absent.

Abdomen (Fig. 2.30c): Comprises seven separate moveable somites. Distal margin of fourth somite moderately emarginate and overlaps fifth somite. Fourth somite bears two tufts of setae on distal margin, separated by width of emarginated area. Male and female abdomens are similar, not v-shaped and u-shaped respectively.

First male pleopod (Fig. 2.10): Slightly sinuous, ca. uniform in width, slightly more slender towards tip. Tip dorso-ventrally flattened, covered with numerous unbranched setae. Proximal part fringed with plumose setae on outer edge. Plumose setae close to inner margin, running from proximal end halfway to tip.

Remarks

Stephensen (1945) tentatively reported *Dotilla wichmanni* from the Iranian Gulf. The determination was uncertain as the specimens were small and slightly damaged. Stephensen drew the first male pleopod and the abdomen, and compared these to Rathbun's (1910) drawings of the same characters for *D. wichmanni.* Although the form of the male abdomen appears to be similar, there is a distinct difference between the shapes of the pleopods, with Stephensen's specimens possessing a flattened tip. Stephensen commented that the carapace sculpture of his specimens was similar to *D. wichmanni*, but lacked a pair of lateral teeth on the side of the carapace. The sternal tympana that are present in *D. wichmanni* were also absent. This suggests that the specimens examined by Stephensen are more likely to be *D. blanfordi* than *D. wichmanni*.

Distribution

Dotilla blanfordi is found from Bombay in India, and occurs along the Sind coast into Pakistan, possibly reaching as far west as the Iranian Gulf.

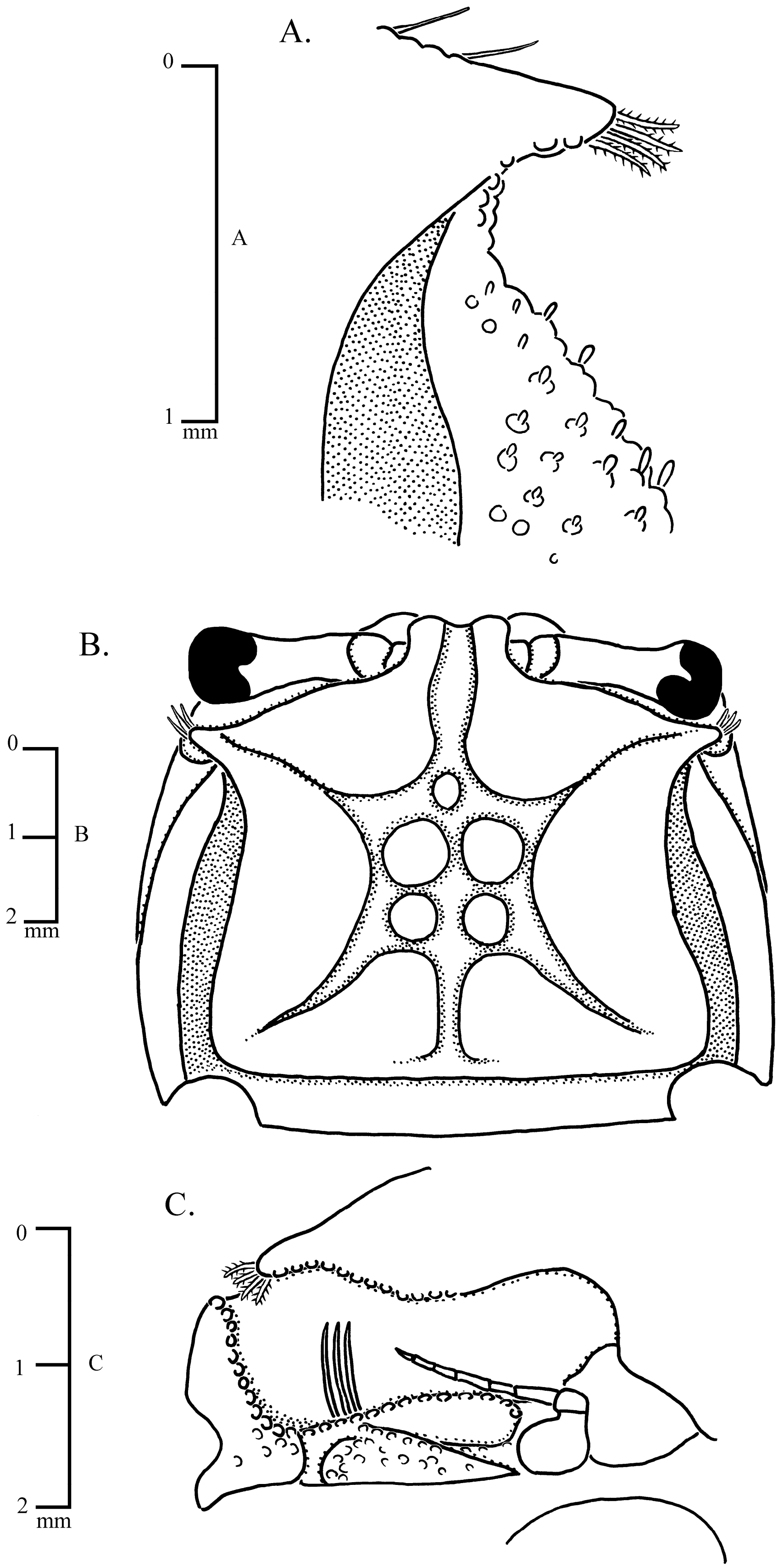


Fig. 2.8. *Dotilla blanfordi* (NHM 1984.412). A. Right antero-lateral margin, dorsal view; B. Carapace, dorsal view; C. Front, anterior view (eye stalk removed).

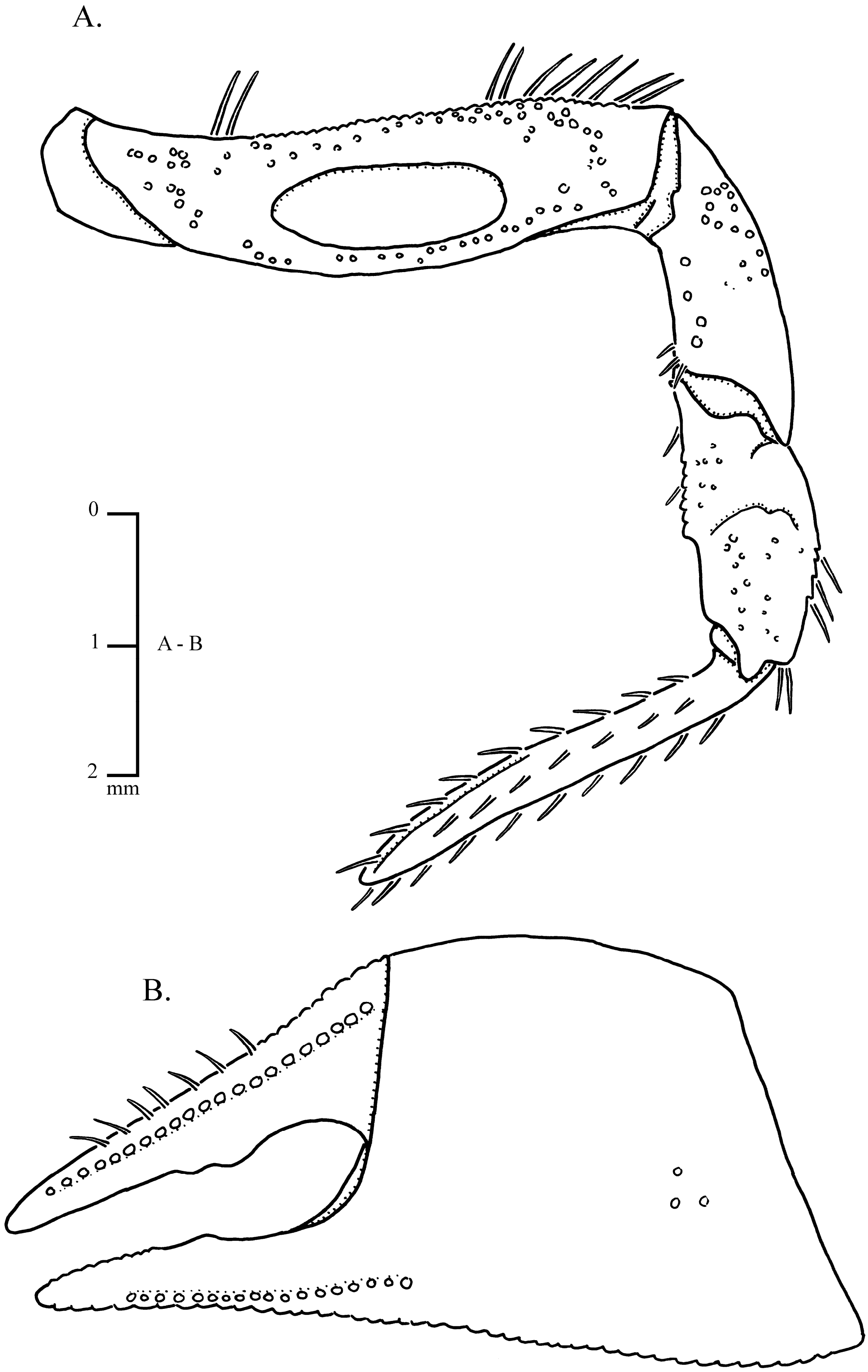


Fig. 2.9. *Dotilla blanfordi* (NHM 1984.412). A. Fourth right ambulatory leg, dorsal view; B. Left chela, anterior view.

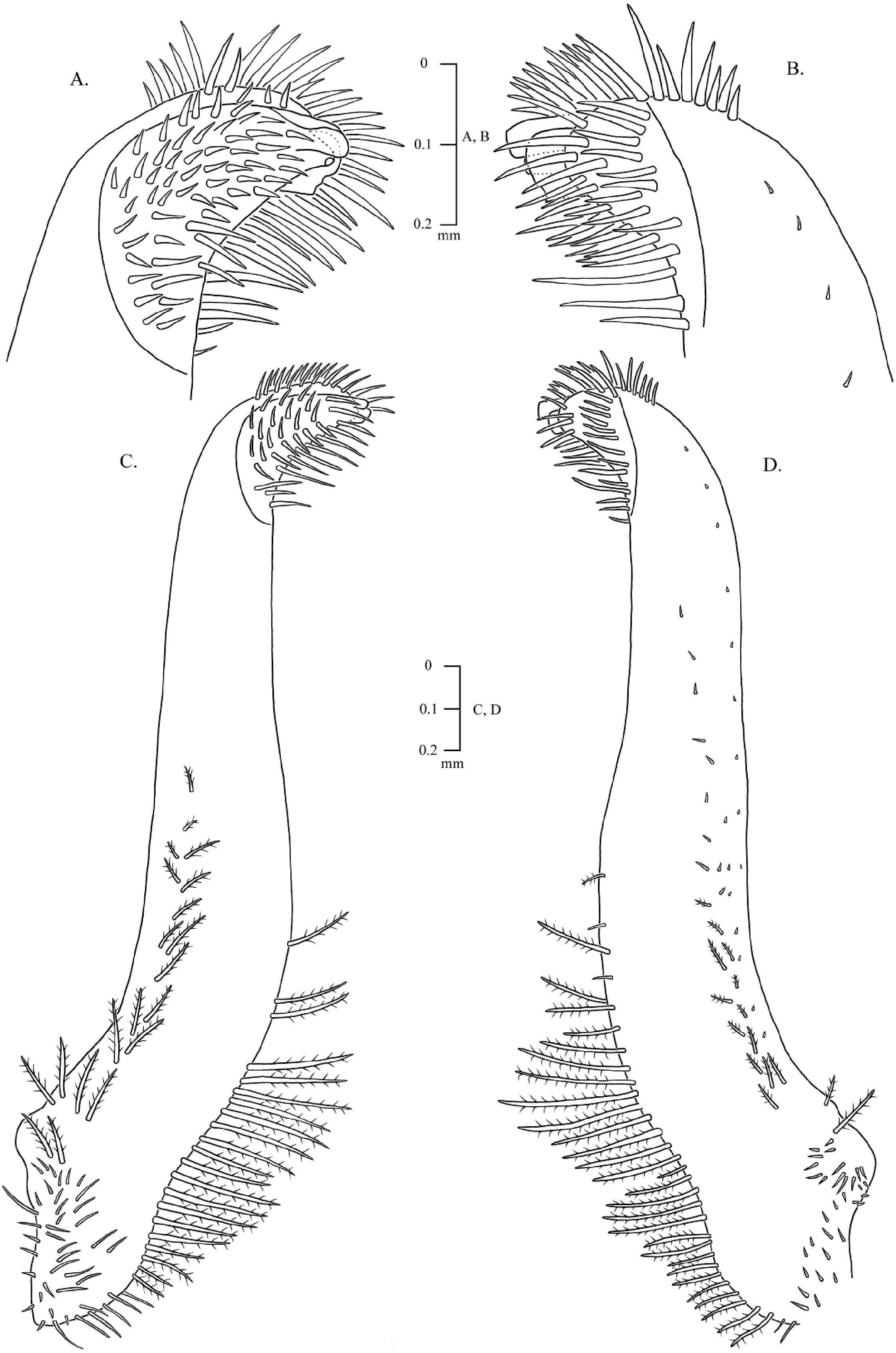


Fig. 2.10. *Dotilla blanfordi* (NHM 1984.412). A. C. Dorsal view, right first pleopod. B. D. Ventral view, right first pleopod.

Dotilla fenestrata Hilgendorf, 1869

Doto sulcatus Krauss 1843: 39.

Dotilla fenestrata Hilgendorf 1869: 85, pl. iii, Fig. 5; 1879: 806; Miers 1884: 543; Aurivillius 1893: 12, pl. i, Fig. 14, 15; Ortmann 1894: 748; Lenz 1905: 367; Kemp 1919: 327; Gordon 1941: 136, Fig. 11a; Vogel 1984: 225, Fig. 3a, 4.

Dotilla clepsydra Stebbing 1917: 18, pl. v.

Material Examined

Mombasa; 1 ♂ (CW 8.7 mm) (NHM 1910.12.16-24); Durban, Types; 3 ♂ (CW 8.6 - 10.4 mm) (NHM 1913.2.14.8-10); Presented by Natal Government Museum, ? location; 1 ♂ (CW 8.7 mm) (NHM 1917.6.19.53); South Africa, Durban Bay, Salisbury Island, Oct 1927; 31 ♂ (CW 5.8 - 10.6 mm), 3 ♀ (CW 4.4 - 7.1 mm) (ZMA 241629); South Africa, Durban Bay, Salisbury Island, Nov 1938; 15 ♂ (CW 5.9 - 9.9 mm), 3 ♀ ovig. (CW 6.4 - 7.1 mm) (ZMA 241635); South Africa, Durban Bay, Salisbury Island, 1938; 11 ♂ (CW 6.1 - 10.5 mm), 1 ♀ ovig. (CW 6.8 mm), 1 ♀ (CW 6.3 mm) (RMNH 3222); Morrumbene estuary, East Africa; 10 ♂ (CW 6.8 - 10.0 mm) (NHM 1955.3.5.82-89); Mida Creek, Kenya Coast; 4 ♂ (CW 6.8 - 8.7 mm) (NHM 1962.9.18); Mida Creek, Kenya Coast; 1 ♂ (CW 5.2 mm), 2 ♀ (CW 6.4 mm) (NHM 1962.9.18.18/21); Zanzibar Island; 8 ♂ (CW 4.8 - 6.6 mm), 3 ♀ (CW 4.2 - 5.2 mm) (NHM 1964.vii.i.32); Mozambique, Costa do Sol Lourenço Marques, Sep 1967; 6 ♂ (CW 5.7 - 8.0 mm), 1 ♀ (CW 5.7 mm) (RMNH 28244); Mozambique, Lourenço Marques, Sep 1967; 2 ♂ (CW 8.7 - 10.4 mm) (RMNH 28245); Mozambique, Costa do Sol Lourenço Marques, Sep 1967; 9 ♂ (CW 3.4 - 5.6 mm), 3 ♀ (CW 4.1 - 5.1 mm) (RMNH 28246); Dar es Salaam area; 15 ♂ (CW 5.6 - 8.7 mm), 2 ♀ ovig. (CW 5.2 - 5.3 mm), 2 ♀ (CW 7.4 - 7.5 mm), 1 juv. (CW 2.8 mm) (NHM 1973.66); Mozambique; 10 ♂ (CW 7.0 - 7.6 mm) (NHM 82.24); Mozambique, Maputa, Costa do Sol, July1983; 2 ♂ (CW 10.7 - 11.1 mm) (RMNH 40886); Durban, South Africa, Feb 1990 (NUS 1992.8579-8581); Mozambique, Lourenço Marques; 3 ♂ (CW 5.9 - 7.2 mm) (ZMA 241625); South Africa, Natal Bay; 1 ♂ (CW 11.1 mm) (ZMA 241630).

Description

Carapace (Fig 2.11): Broader than long, distinct anteriorly bifurcated groove parallel to lateral margin. Wide groove runs from front and branches before gastric region, continuing obliquely to postero-lateral angle, becoming narrower and fainter towards posterior. Groove runs from edge of mesogastric region to antero-lateral angle. Grooves run obliquely from postero-lateral angle and join before cardiac region, creating triangular plane with base formed by posterior margin. Mesogastric region marked by grooves forming a diamond shape, with anterior point elongated towards front.

Second maxilliped: Spoon-tipped setae located on merus are umbrella-shaped. Comprise three broad lobes ca. equal size, central lobe sometimes partially slit. Proximal region of setae serrated at margins of bowl formed by lobes (Vogel 1984).

Chelipeds: Merus bears pronounced spine at proximal end of lower surface. Fingers of chela (Fig. 2.12b) ca. twice length of propodus palm. Both fingers bear a tooth at mid-point of cutting edge in adult males.

Fourth ambulatory leg (Fig. 2.12a): Dactylus similar in size to propodus. Tympana on both sides of merus. Tympanum on dorsal surface of merus occupies third to half of total area of merus.

Sternum: Sternal tympana present on second and third somites.

Abdomen (Fig. 2.29d): Comprises seven separate moveable somites. Distal margin of fourth somite is moderately emarginate and overlaps fifth somite. Single tuft of setae extends over entire distal margin of fourth somite. Male and female abdomens are similar, not v-shaped and u-shaped respectively.

First male pleopod (Fig. 2.13): Slender and sinuous, distal end bent at an angle of ca. 90° to main shaft. Plumose setae run from proximal part along outer edge, ending at distal bend. On dorsal side setae run parallel to inner edge to distal bend. Tip has plumose setae on upper side, and unbranched setae on lower.

Remarks

A large amount of variation has been reported in the number and arrangement of the tympana on the somites of *Dotilla fenestrata*. As a general rule, *D. fenestrata* has tympana on the second and third somites. Gordon (1941) reported that some specimens from Durban had tympana only on the second somite, whilst individuals from Mozambique had tympana on somites one to four. Gordon (1941) also commented that specimens from Madagascar lacked the strong spine on the merus of the chelipeds.

Distribution

East coast of Africa, from South Africa northwards to Kenya. Also present on Madagascar and Comoro Islands.

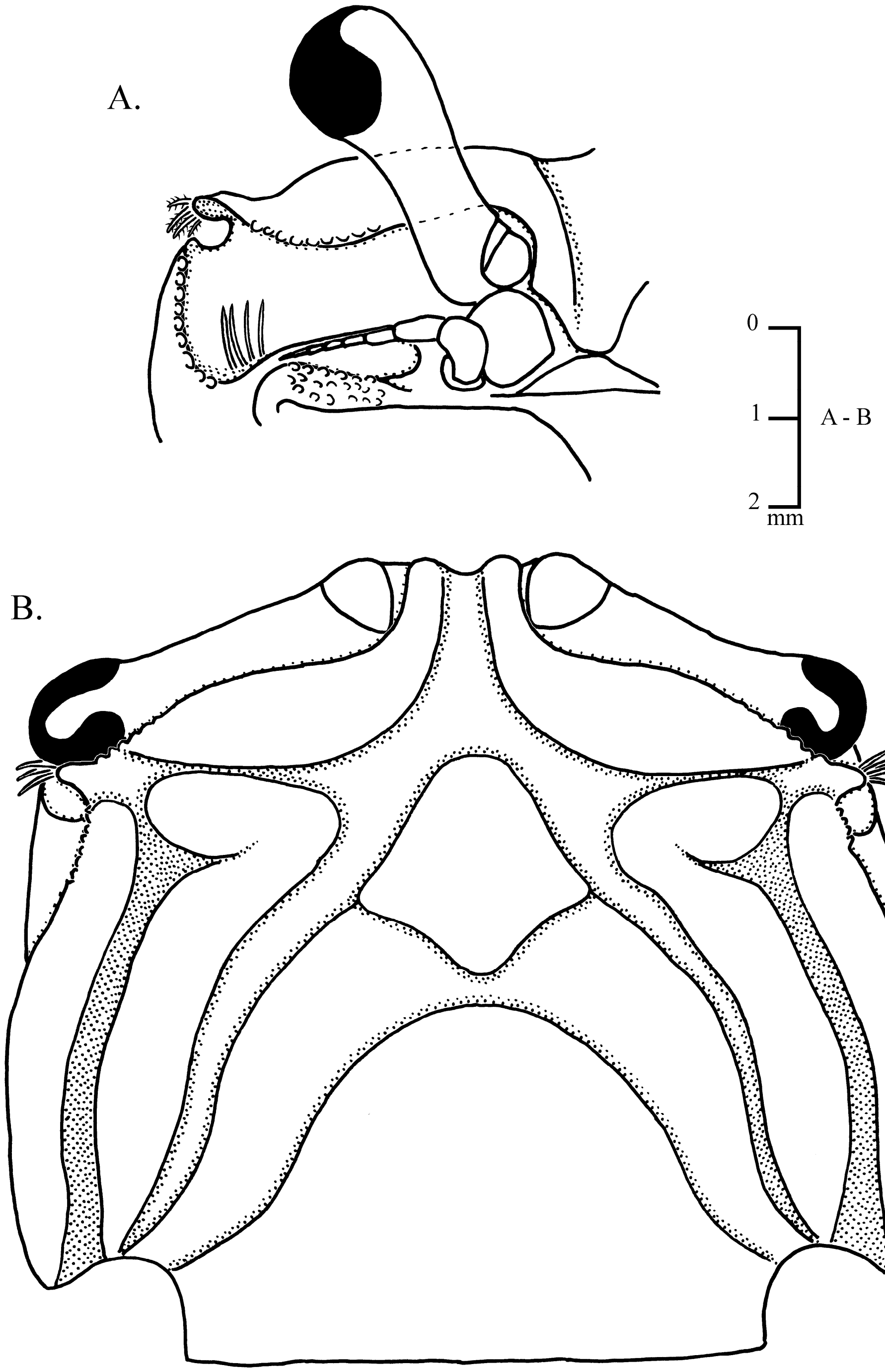


Fig. 2.11. *Dotilla fenestrata* (NHM 1913.2.14.8-10). A. Front, anterior view; B. Carapace, dorsal view.

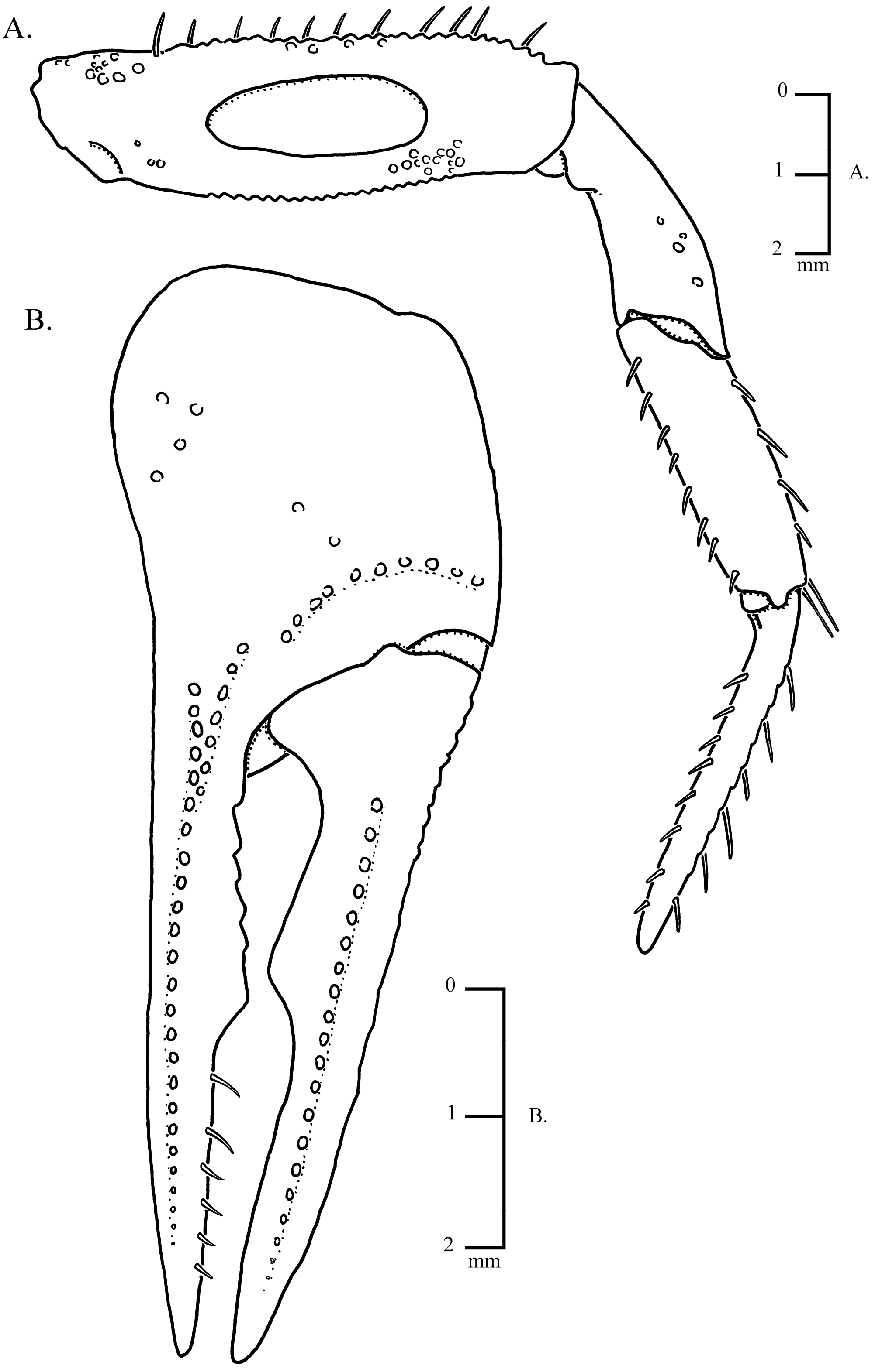


Fig. 2.12. *Dotilla fenestrata* (NHM 1913.2.14.8-10). A. Right fourth ambulatory leg, dorsal view; B. Right chela, anterior view.

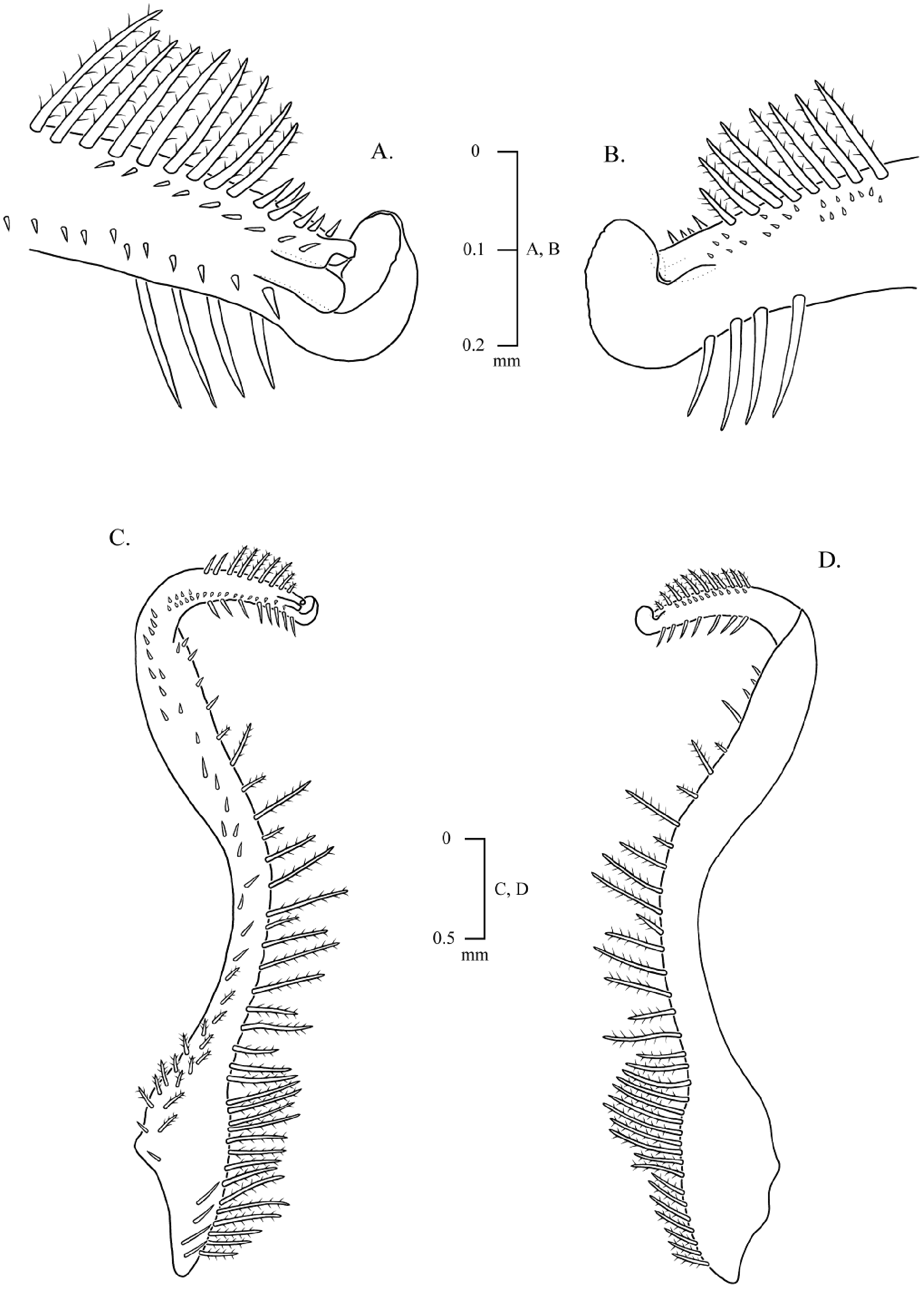


Fig. 2.13. *Dotilla fenestrata* (NHM 1913.2.14.8-10). A., C. Dorsal view, right first male pleopod; B., D. Ventral view, right first male pleopod.

Dotilla malabarica Nobili, 1903

Dotilla malabarica Nobili 1903: 20, Fig. 6; Gordon 1941: 137, Figs 12b, 13, 14a; Vogel 1983: 157, Figs 1-3, 4e; 1984: 225, Figs 2c, 4.

Material Examined

Chaliyam, Malabar Coast, India, April 1980; 3 ♂ (CW 5.0 - 5.9 mm), 1 ♀ (CW 5.2 mm) (NHM 1983.455).

Description

Carapace (Fig. 2.14): Broader than long, groove parallel to lateral margin bifurcated anteriorly. Wide groove runs from front, bifurcates in front of gastric region. Branches run obliquely to postero-lateral angles. Gastric region marked by five tubercles, forming a five-pointed star pattern. Groove runs obliquely from edge of mesogastric region to just below antero-lateral angle. Shallow groove delimits edge of cardio-intestinal region, and extends to posterior margin. No transverse groove parallel to posterior margin.

Second maxilliped: Spoon-tipped setae located on merus three-lobed, with central lobe broader than other two. ‘Bowl’ formed by lobes oblong in shape, and serrated on proximal edge (Vogel, 1984).

Chela (Fig. 2.15b): Dactylus bears a faint tooth, serrated on proximal half of cutting edge. Cutting edge of propodus also serrated on proximal half. Fingers 1.5 times length of propodus palm.

Fourth ambulatory leg (Fig. 2.15a): Dactylus twice length of propodus, bears conspicuous lateral groove. Tympanum absent from upper surface of merus but present on lower, unlike legs 1–3 that bear tympana on both sides of merus.

Sternum: Sternal tympana absent.

Abdomen (Fig. 2.30b): Comprises seven separate moveable somites. Distal margin of fourth somite moderately emarginate, overlaps fifth somite. Fourth somite bears two tufts of setae separated by width of emarginate area on distal margin. Male and female abdomens are similar, not v-shaped and u-shaped respectively.

First male pleopod (Fig. 2.16): Distal third more slender than main shaft, bent at ca. 40° angle. Plumose setae run on outer surface from the proximal end to distal bend. After this point setae become thicker and unbranched, extending to tip. Last few setae on upper edge of tip plumose.

Distribution

Dotilla malabarica has only been reported from three locations in India: Mahé on the Malabar Coast, Chaliyam, 65 km south of Mahé, and Travancore.

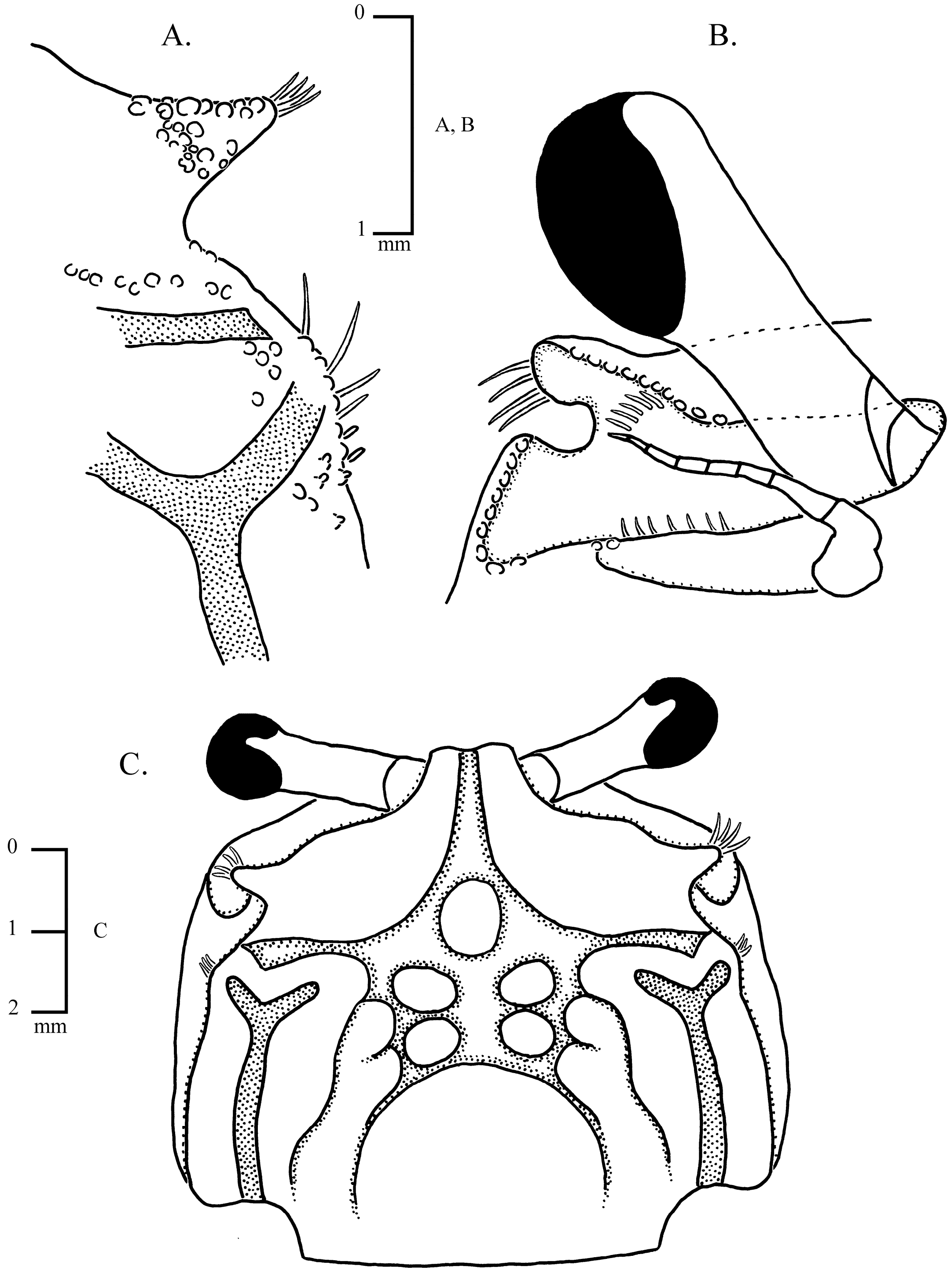


Fig. 2.14. *Dotilla malabarica* (NHM 1983.455). A. Right antero-lateral angle, dorsal view; B. Front, anterior view; C. Carapace, dorsal view.

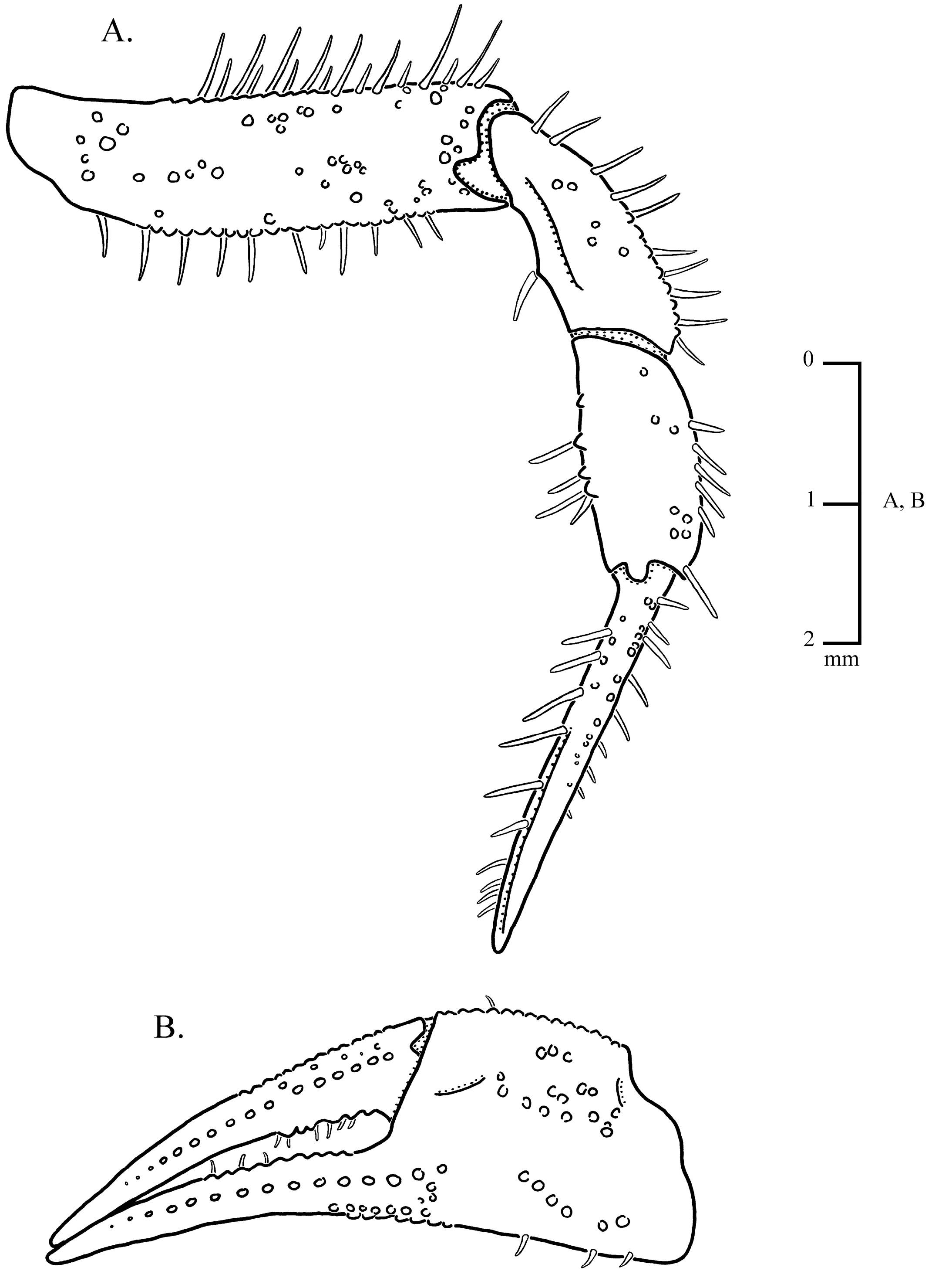


Fig. 2.15. *Dotilla malabarica* (NHM 1983.455). A. Right fourth ambulatory leg, dorsal view; B. Left chela, anterior view

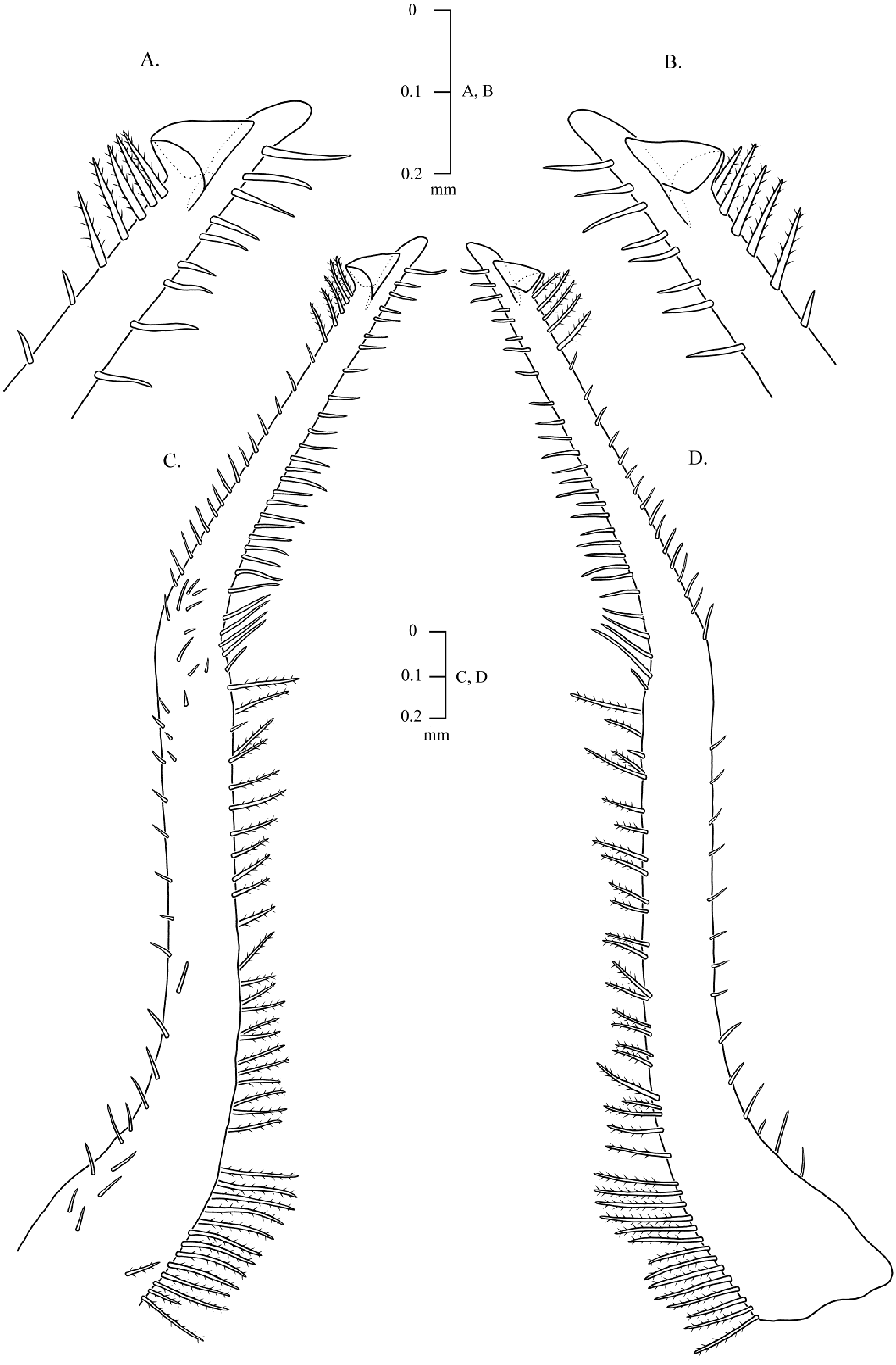


Fig. 2.16. *Dotilla malabarica* (NHM 1983.455). A. C. Dorsal view, right first male pleopod; B. D. Ventral view, right first male pleopod.

Dotilla myctiroides (H Milne Edwards, 1852)

Doto myctiroides H Milne Edwards 1852: pl. iv, fig. 24.

Dotilla myctiroides Henderson 1893: 390; Alcock 1900a: 368; Stimpson 1907: 101; Kemp 1915: 227, Fig. 8; 1919: 326, Fig. 9a; Gravely 1927: 149; Gordon 1941: 140, Fig. 15b; Chhapghar 1957: 511; Sankarankutty 1961: 114, Figs 3d-e, 4a; Vogel 1984: 225, Figs 2d, 4.

Scopimera myctiroides Lanchester 1900: 760, pl. xlvii, Fig. 14.

Material Examined

Billiton Island; 2 ♂ (NHM 1878.3); Billiton Island; 1 ♀ ovig. (CW 6.7 mm), 3 ♀ (CW 6.5 - 7.2 mm) (NHM 1900.10.22.295-98); Ceylon; 3 ♂ (CW 5.1 - 6.8 mm), 1 ♀ (CW 4.0 mm) (NHM 1934.1.16.160/61); Ceylon; 18 ♂ (CW 6.3 - 9.9 mm) (NHM 1907.5.22.384-388); Malaya, Port Dickson, 1946; 10 ♂ (CW 5.7 - 6.7 mm) (RMNH 5317); Changi Beach, Singapore, March 1953; 5 ♂ (NUS 1985.504-508); Katobai, Thailand, August 1970; 3 ♂ (CW 6.0 - 6.1 mm), 1 ♀ (CW 5.1 mm) (NHM 1985.412); Telok, Kemang Negri, Sembilam, Malaysia, August 1978; 5 ♂ (CW 5.4 - 6.9 mm), 1 ♀ (CW 6.2 mm) (NHM 2005.390-395); Panaji, Goa, India, March 1980; 4 ♂ (CW 3.6 - 7.6 mm) (NHM 1983.457); Thailand, Trang province, Ko Hard Toop near Ko Libong, Oct 1985; 4 ♂ (CW 4 - 7.1 mm) (RMNH 38218); Changi Beach, Singapore, Oct 1986; 11 ♂, 5♀ (NUS 1987.63-78); Morib Beach, Malaysia, June 1987; 17 ♂, 31♀ (NUS 1987.1228-1257); Phuket Marine Biology Centre beach, Phuket, Thailand, June 1987; 1 ♂, 3 ♀ (NUS 19871202-1205); Sungei Nibang, Penang Malaysia, June 1987; 1 ♂, 1 ♀ (NUS 1987.999-1000); Indonesia, Riau Archipelago, P. Binton, Tanjung Tondang, July 1995; 4 ♂, 2 ♀ (NUS 1999.0296); sandy rocky and mangrove shore, Cape Sei, Phuket, Thailand; 11 ♂ (CW 5.4 - 7.6 mm) (NHM 2000.1916-1928); Thailand, Phuket, Ao Tang Khen near Cape Panwa, Feb 2001; 3 ♂, 2 ♀ (NUS 2001.1073).

Description

Carapace (Fig. 2.17): As wide as long, if not slightly longer than wide. Distinct unbifurcated grooves parallel to lateral margin. Carapace almost devoid of sculpture. Groove runs from front and bifurcates before gastric region. Branches are faint, run obliquely to antero-lateral angle. Raised area covers cardio-intestinal and branchial regions on posterior half of carapace. Orbits shallow.

Second maxilliped: Spoon-tipped setae located on merus are bowl-shaped. Comprises three equal sized lobes, middle lobe possessing two slits (Vogel 1984).

Chelipeds: Long, between three and four times length of carapace. Chela (Fig. 2.18b) has serrated cutting edge to both fingers, neither bearing a conspicuous tooth. Fingers twice length of propodus palm.

Fourth ambulatory leg (Fig. 2.18a): Dactylus slightly longer than propodus. Merus bears large tympana on both sides. Tympanum on dorsal merus surface occupies almost all of merus surface area.

Sternum: Tympana present on all somites.

Abdomen (Fig. 2.29a): Comprises seven separate moveable somites. Distal margin of fourth somite shallowly emarginate, overlaps fifth somite. Fourth somite bears a single band of setae extending across entire distal margin. Male and female abdomens are similar, not v-shaped and u-shaped respectively.

First male pleopod (Fig. 2.19): Slightly sinuous, ca. uniform in diameter, more slender distally. Tip is blunt, furnished with numerous unbranched setae. Proximal end fringed with plumose setae on outer edge, running to mid point of appendage. Dorsal surface bears line of setae close to inner margin running from proximal end to tip.

Distribution

Dotilla myctiroides is widely distributed. The species can be found all along the Indian coast and around the Bay of Bengal to Thailand, Malaysia and Singapore. The species has also been recorded in Sri Lanka, the Andaman Islands and Java. *D. myctiroides* is frequently found in areas of brackish water and on muddy sand beaches.

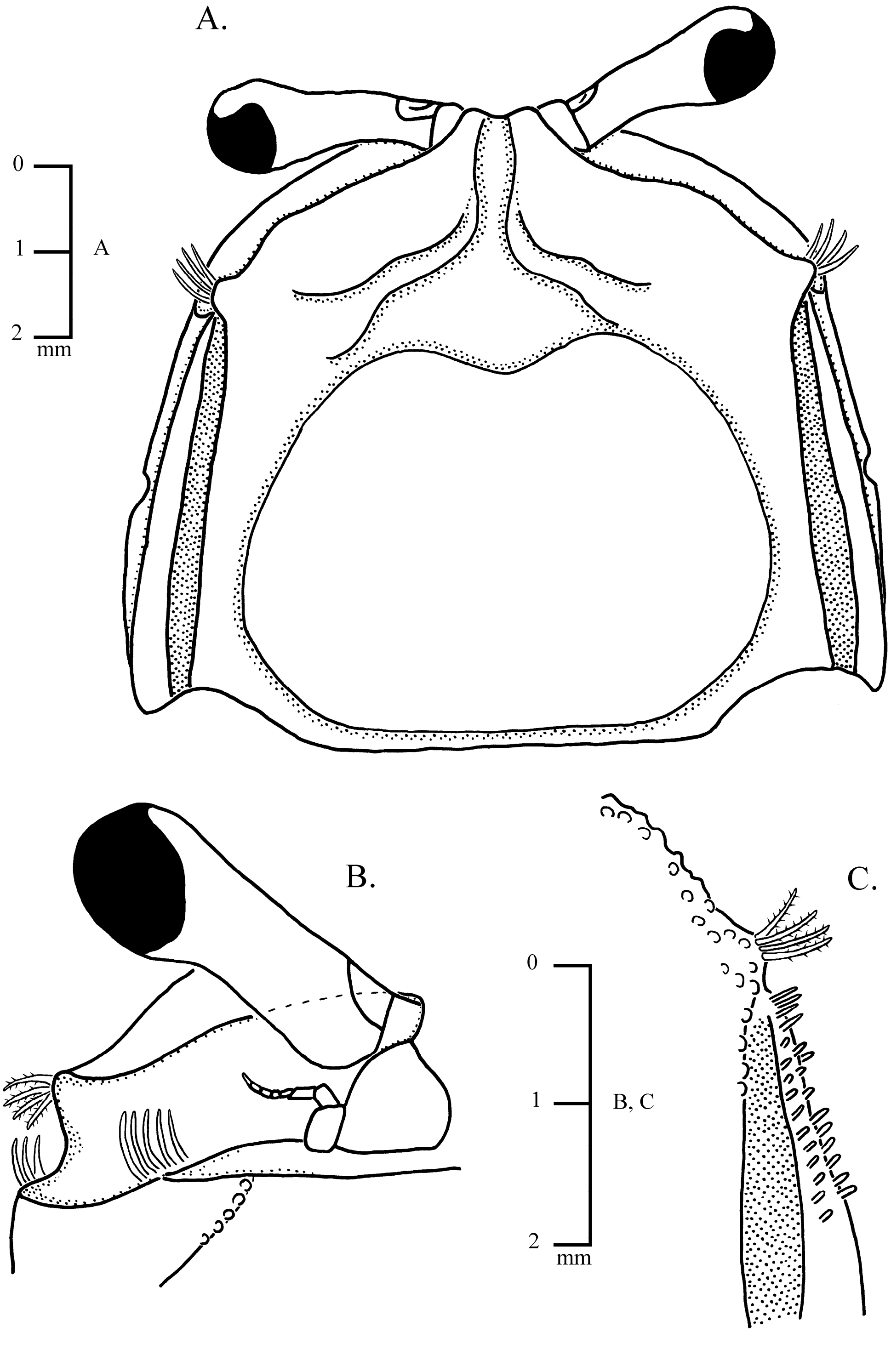


Fig. 2.17. *Dotilla myctiroides* (NHM 1907.5.22.384-388). A. Carapace, dorsal view; B. Front, anterior view; C. Right antero-lateral angle, dorsal view

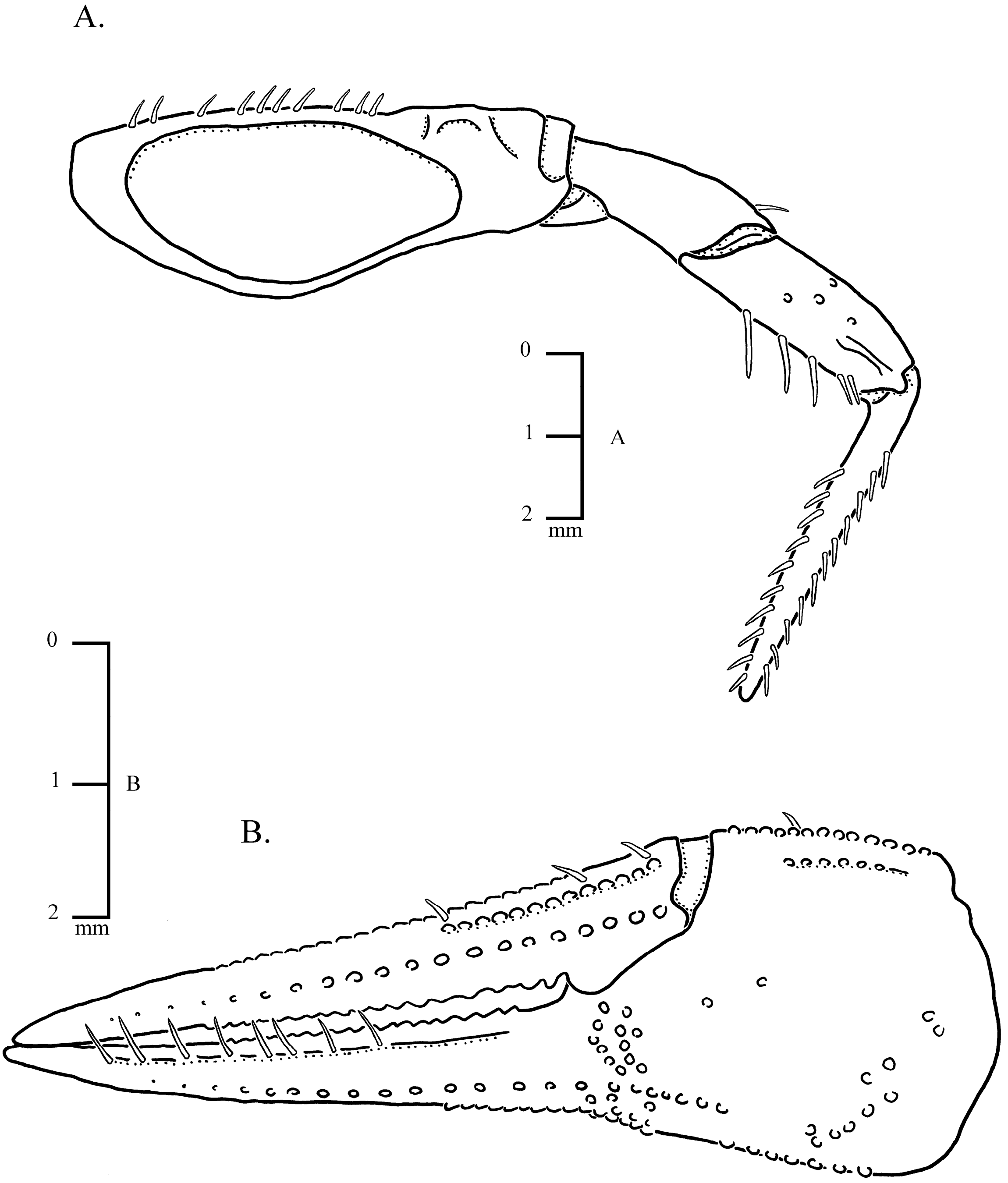


Fig. 2.18. *Dotilla myctiroides* (NHM 1907.5.22.384-388). A. Right fourth ambulatory leg, dorsal view; B. Left chela, anterior view

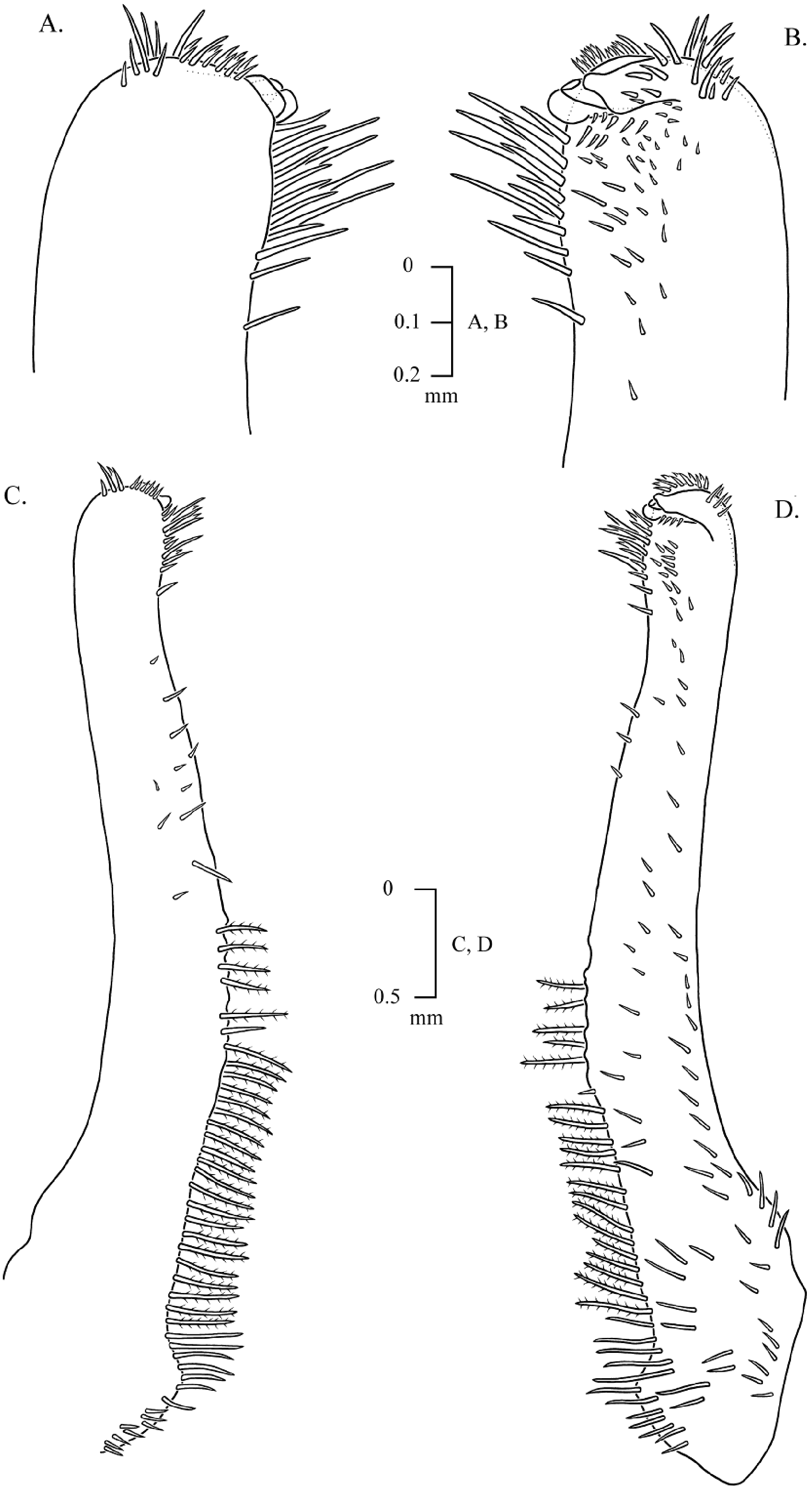


Fig. 2.19. *Dotilla myctiroides* (NHM 1907.5.22.384-388). A. C. Ventral view, left first male pleopod; B. D. Dorsal view, left first male pleopod.

Dotilla pertinax Kemp, 1915

Dotilla pertinax Kemp 1915: 222, pl. xii, Fig. 4, 7; 1919: 329, Fig. 9c; Gordon 1941: 139 Figs 12a, 14b-c; Vogel 1984: 225, Figs 3c, 4.

Material Examined

Puri, Orissa, Syntypes; 2 ♂ (CW 4.5 - 5.1 mm), 1 ♀ (CW 4.4 mm) (NHM 1919.11.1.101-103); Puri, Orissa; 1 ♀ (CW 5.7 mm) (NHM 1938.5.31.2); India, Orissa Coast, Puri; 2 ♂ (CW 4.5 - 5.5 mm), 2 ♀ (CW 4 - 5 mm) (ZMA 241628).

Description

Carapace (Fig. 2.20): Broader than long, distinct groove parallel to lateral margin, bifurcated anteriorly, ending short of antero-lateral angle. Deep groove runs from frontal margin and bifurcates immediately before gastric region. Branches run obliquely backwards to postero-lateral angles, becoming shallower distally. Groove runs transversely from edge of mesogastric region to antero-lateral angle, with a branch running forward towards middle of orbit. Triangular shape formed over mesogastric region in centre of carapace. Faint grooves delimit branchial and cardio-intestinal regions. Faint Y-shaped groove marks boundary of cardiac region. Faint complete transverse groove parallel to posterior margin.

Second maxilliped: Spoon-tipped setae located on merus formed of three lobes, with middle lobe larger than other two. Thumb-like structure present at upper end of setae peduncle (Vogel 1984).

Chela (Fig. 2.21b): Dactylus serrated at proximal end of cutting edge. Mid-point of cutting edge bears a tooth in adult males. Cutting edge of propodus serrated, no tooth present. Fingers twice length of propodus palm.

Fourth ambulatory leg (Fig. 2.21a): Propodus ca. two-thirds length of dactylus. Merus greatly extended, ca. twice length of propodus, and bears large tympana on both sides that occupy almost whole merus surface area.

Sternum: Sternal tympana absent.

Abdomen (Fig. 2.30a): Comprises seven separate moveable somites. Distal margin of fourth somite deeply emarginate, overlaps fifth somite. Fourth somite bears two tufts of setae on distal margin, separated by width of emarginate area. Male and female abdomens are similar, not v-shaped and u-shaped respectively.

First male pleopod (Fig. 2.22): Long and sinuous, distal third bent at ca. 40° angle to main shaft, more slender distally. Plumose setae fringe outer edge of proximal end. Small unbranched setae found on both edges in distal two-thirds of appendage. Tip simple, very pointed.

Distribution

Dotilla pertinax has only been reported from one small region in India, at Puri and on the outer channel of Chilka Lake, Orissa.

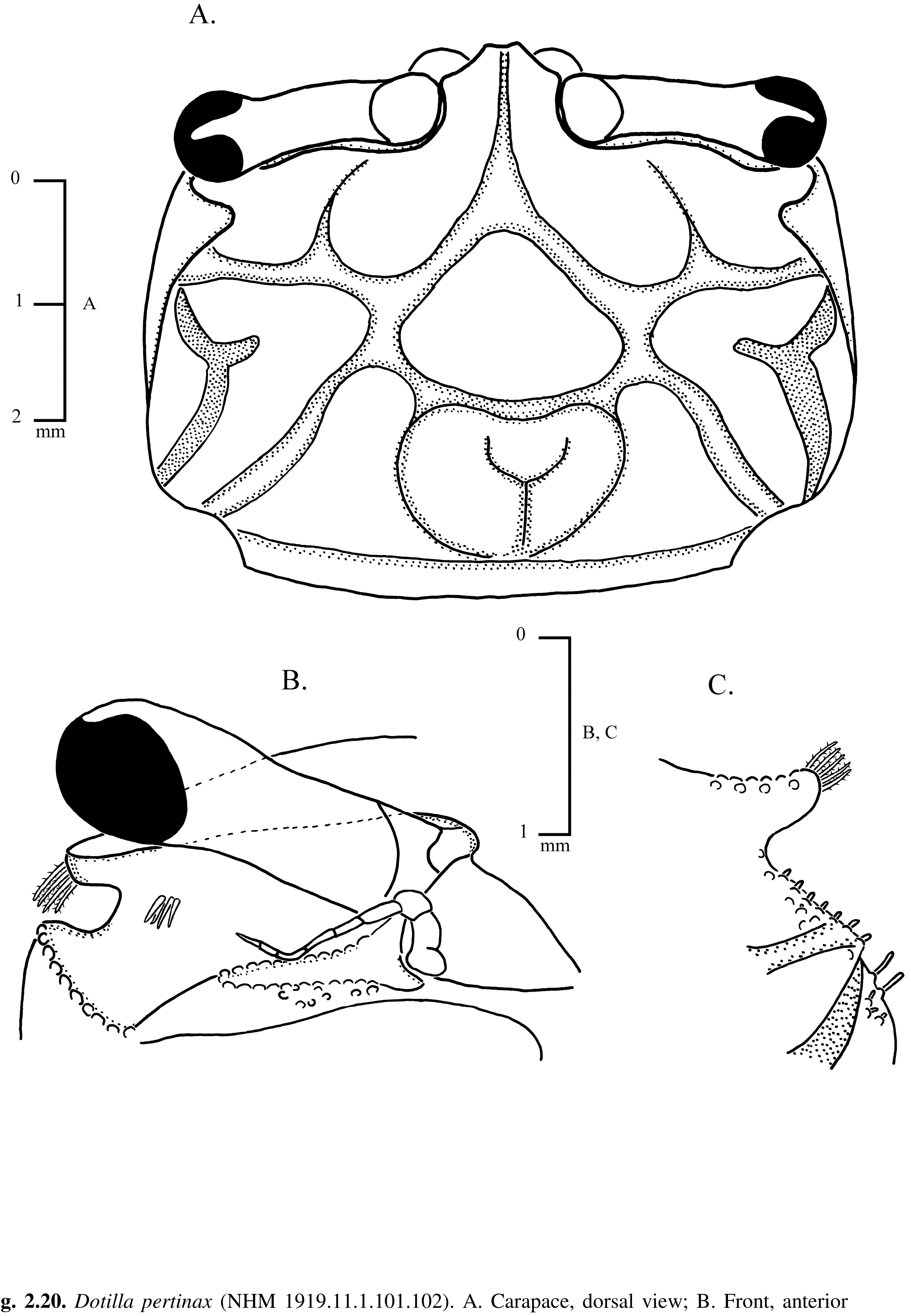


Fig. 2.20. *Dotilla pertinax* (NHM 1919.11.1.101.102). A. Carapace, dorsal view; B. Front, anterior view; C. Right antero-lateral angle, dorsal view

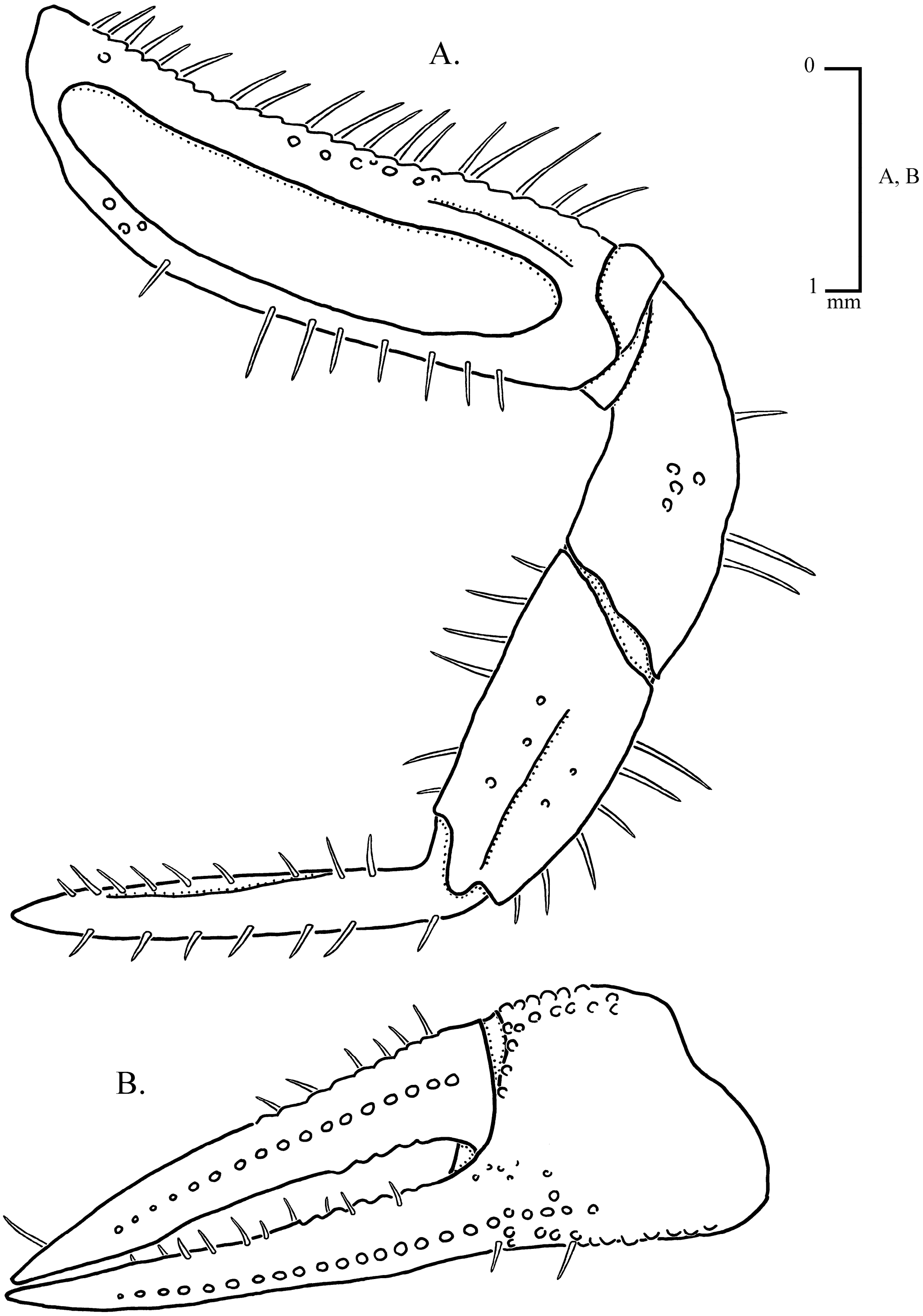


Fig. 2.21. *Dotilla pertinax* (NHM 1919.11.1.101.102). A. Right fourth ambulatory leg, dorsal view; B. Left chela, anterior view

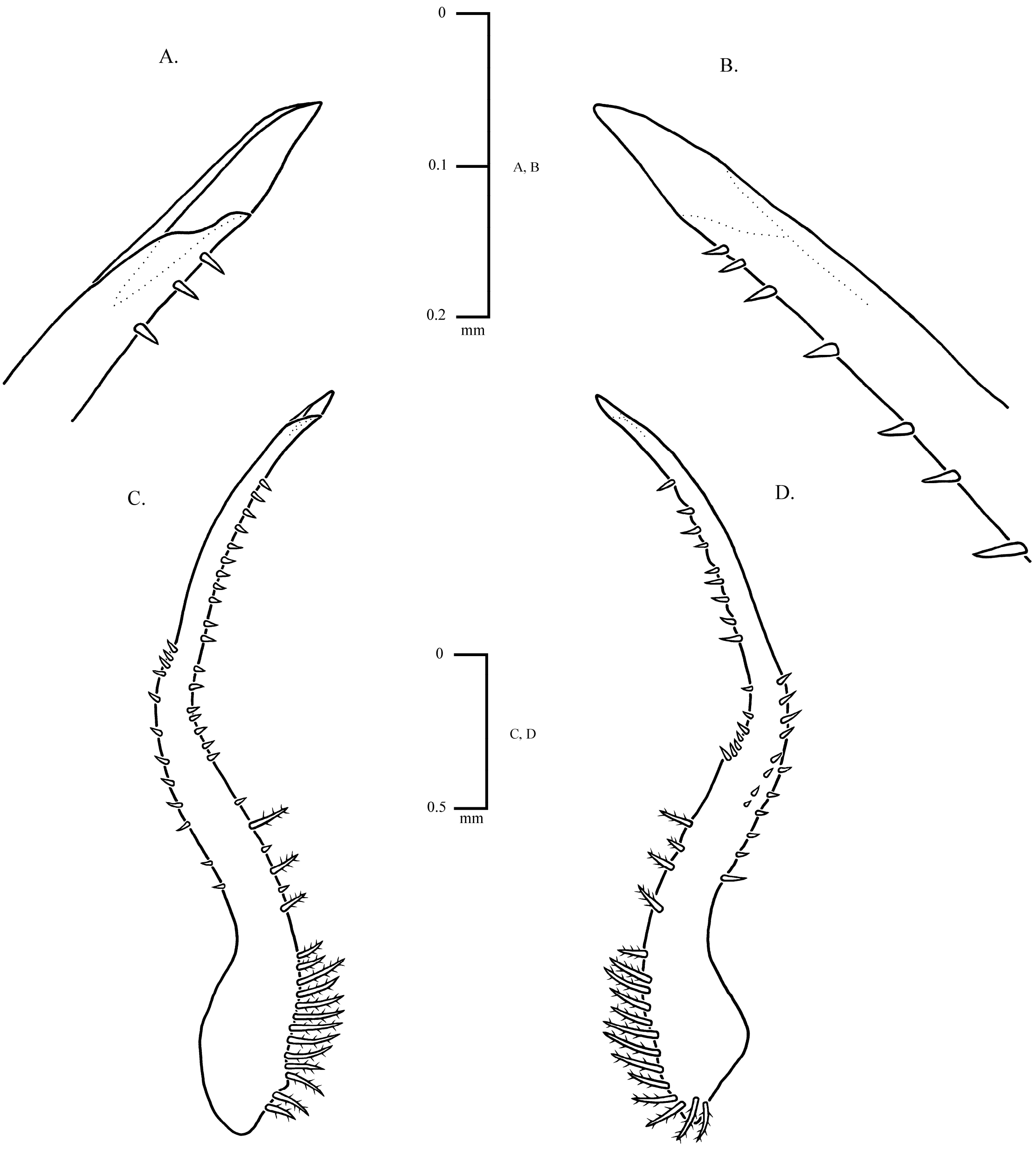


Fig. 2.22. *Dotilla pertinax* (NHM 1919.11.1.101.102). A. C. Dorsal view, right first male pleopod; B. D. Ventral view, right first male pleopod.

Dotilla sulcata (Forskål, 1775)

Cancer sulcatus Forskål 1775: 92.

Myctiris sulcatus Audouin 1809: 81.

Ocypode (Doto) sulcata de Haan 1833: 24.

Doto sulcatus H. Milne-Edwards 1837: 92; Lucas 1850: 61, pl. ii, Fig. 1; Heller 1861: 361; Cano 1889: 249.

Dotilla sulcata De Man 1888: 130; 1892: 309; Nobili 1906: 315; Laurie 1915: 467; Kemp 1919: 328, Fig. 9b; Gordon 1941: 137, Fig. 11b; Vogel 1984: 225, Figs 3d, 4.

Dotilla affinis Alcock 1900a: 365, pl. lxiii, Fig. 1, 1a-b.

Material Examined

Djeddah, 1880; 3 ♂ (CW 5.6 - 6.4 mm), 5 ♀ (CW 5.3 - 5.8 mm) (RMNH 279); Saudi Arabia, Djeddah, 1881; 2 ♂ (CW 7.5 - 7.7 mm), 1 ♀ (CW 5.6 mm) (ZMA 202765); Suez; 3 ♂ (CW 8.1 - 8.5 mm), 3 ♀ (CW 7.0 - 7.6 mm) (NHM 1913.10.30.1-5); Suez Canal; 9 ♂ (3.7 - 10.8 mm), 5 ♀ (CW 3.1 - 5.9 mm), 1 juv. (CW 2.5 mm) (NHM 1926.1.26.118-127); Sudanese Red Sea; 8 ♂ (CW 6.2 - 7.9 mm), 4 ♀ (CW 6.2 - 6.6 mm) (NHM 1934.1.19.119-130); Red Sea; 6 ♂ (CW 4.1 - 6.8 mm), 2 ♀ (CW 5.9 - 6.1 mm) (NHM 1938.1.28.11-16); Israel, Red Sea, Sinai Peninsula, Abu Zabad, 1957; 2 ♂ (CW 7.7 - 8.5 mm), 1 ♀ (CW 7.1 mm) (RMNH 14183); Ethiopia, Camping Bay, Dahlak Archipelago, Museri Island, Oct 1965; 1 ♂ (CW 3.8 mm), 5 ♀ (CW 2.8 - 4.5 mm) (RMNH 26879); Ethiopia, Camping Bay, Dahlak Archipelago, Museri Island, Oct 1965; 9 ♂ (CW 3.1 - 3.8 mm), 2 ♀ (CW 3.3 - 3.4 mm) (RMNH 26880); Ethiopia, Camping Bay, Dahlak Archipelago, Museri Island, Oct 1965; 2 ♂ (CW 5.8 - 6.7 mm), 1 ♀ (CW 4.2 mm) (RMNH 26881); Egypt, Gulf of Aqaba, Marsa Murach, Aug 1967; 4 ♂ (CW 8.3 - 11.0 mm), 2 ♀ (CW 3.8 - 8.7 mm) (RMNH 24820); Egypt, Gulf Of Aqaba, Shurat al Manqata, Sep 1967; 10 ♂ (CW 3.2 - 6.5 mm), 7 ♀ (CW 3.2 - 5.3 mm) (RMNH 28421); Egypt, Gulf of Aqaba, Marsa Murach, Aug 1967; 7 ♂ (CW 4.6 - 10.6 mm), 1 ♀ (CW 4.8 mm) (RMNH 28214); Egypt, Gulf of Aqaba, Wasi Muran, Apr 1968; 1 ♂ (CW 6.2 mm) (RMNH 28215); Egypt, Al-Ghardaqa, July 1968; 8 ♂ (CW 7.0 - 9.9 mm), 6 ♀ (CW 6.0 - 7.2 mm) (RMNH D 25511); Egypt, Gulf of Suez, El-Bilaiyim, June 1968; 18 ♂ (CW 5.1 - 9.8 mm) (RMNH 27445); Egypt, Gulf of Aqaba, Nabq, June 1969; 2 ♂ (CW 8.0 - 8.6 mm) (RMNH 28216); Sudan, Muhammed Qol 160 km N of Port Sudan, Aug 1969; 3 ♂ (CW 4.1 - 5.0 mm), 8 ♀ (CW 2.0 - 5.1 mm) (RMNH 26878); Egypt, Gulf of Suez, El-Bilaiyim, Jan 1970; 1 ♂ (CW 7.8 mm) (RMNH 30831); Egypt, North part Gulf of Suez, Ras el Misalla, Aug 1970; 2 ♂ (CW 3.0 - 3.1 mm), 3 ♀ (CW 2.1 - 3.8 mm) (RMNH 30831); Egypt, Gulf of Suez, Ras Matarma, Aug 1970; 1 ♀ ovig. (CW 6.4 mm), 2 ♀ (CW 4.3 - 4.5 mm) (RMNH 28218); Egypt, Gulf of Suez, Ras Matarma, Sep 1970; 22 ♂ (CW 3.5 - 5.8 mm), 19 ♀ (CW 3.0 - 7.1 mm) (RMNH 28217); Egypt, Gulf of Aqaba, Dahab, Apr 1973; 2 ♂ (CW 7.0 - 7.8 mm), 1 ♀ ovig. (CW 5.3 mm), 1 ♀ (CW 7.0 mm) (RMNH 29246); Dungunab, Sudan, May 1973; 1 ♂ (CW 5.5 mm) (NHM 1974.14); near Ras Al Khalman, Tricial Coast, Persian Gulf, intertidal mud of lagoon; 4 ♂ (CW 6.8 - 8.3 mm), 1 ♀ ovig. (CW 6.1 mm) (NHM 1973.463); Israel, Gulf of Aqaba, Eilat, Oct-Nov 1975; 1 ♂ (CW 9.8 mm) (RMNH 30980); Egypt, Shurat al Manqata, Apr 1976; 2 ♂ (CW 5.0 - 6.1 mm), 2 ♀ (CW 4.2 - 6.0 mm) (RMNH 31078); East Sinai, Neviot; 2 ♂ (CW 4.5 - 5.1 mm), 1 ♀ (CW 4.4 mm) (NHM 19.ii-5-iii 1981); Red Sea, Egypt, 10 km South of Safaga, Aug 1988; 5 ♂ (CW 6.3 - 7.8 mm) (RMNH 38540); Red Sea; 1 ♂ (NHM 1998.2761.49.66).

Description

Carapace (Fig. 2.23): Broader than long, distinct grooves parallel to lateral margin, bifurcated anteriorly. Groove runs from front and bifurcates before gastric region, with branches running obliquely to postero-lateral angles. Groove runs transversely from edge of mesogastric region to antero-lateral angle. Short groove runs backwards from middle of orbit towards gastric region, ending before transverse groove is reached. Faint groove longitudinally bisects gastric region and bifurcates at edge of cardiac region, with branches running obliquely to postero-lateral angles. Branches form triangular shape, with base of the triangle formed by posterior margin. No transverse posterior groove.

Second maxilliped: Spoon-tipped setae located on merus are bowl-shaped and three-lobed, central lobe slightly broader and possessing two slits. Thumb-like protrusion present on distal end of setal peduncle (Vogel 1984).

Cheliped: Merus bears small spine at proximal end of lower surface. Fingers of chela (Fig. 2.24b) slender and slightly longer than propodus palm. No teeth present on either finger.

Fourth ambulatory leg (Fig. 2.24a): Dactylus slightly longer than propodus. Merus bears tympana on both sides. Tympanum on dorsal merus surface not more than half total area of merus.

Sternum: Sternal tympana absent.

Abdomen (Fig. 2.29c): Comprises seven separate moveable somites. Distal margin of fourth somite deeply emarginate, overlaps fifth somite. Fourth somite bears single tuft of setae extending across entire distal margin. Fifth somite marked by transverse ridge close to distal margin. Small tooth extends distally, situated between overlapping sides of fourth somite. Male and female abdomens are similar, not v-shaped and u-shaped respectively.

First male pleopod (Fig. 2.25): Slender and sinuous, distal fourth bent at 90° angle to main shaft. Long plumose setae fringe outer margin on proximal half. Small unbranched setae run close inner margin from proximal end to tip. Plumose setae present on upper surface of bent distal end, and unbranched setae on lower.

Distribution

Throughout the Red Sea, also reported at Ras Al Khalman in the Persian Gulf.

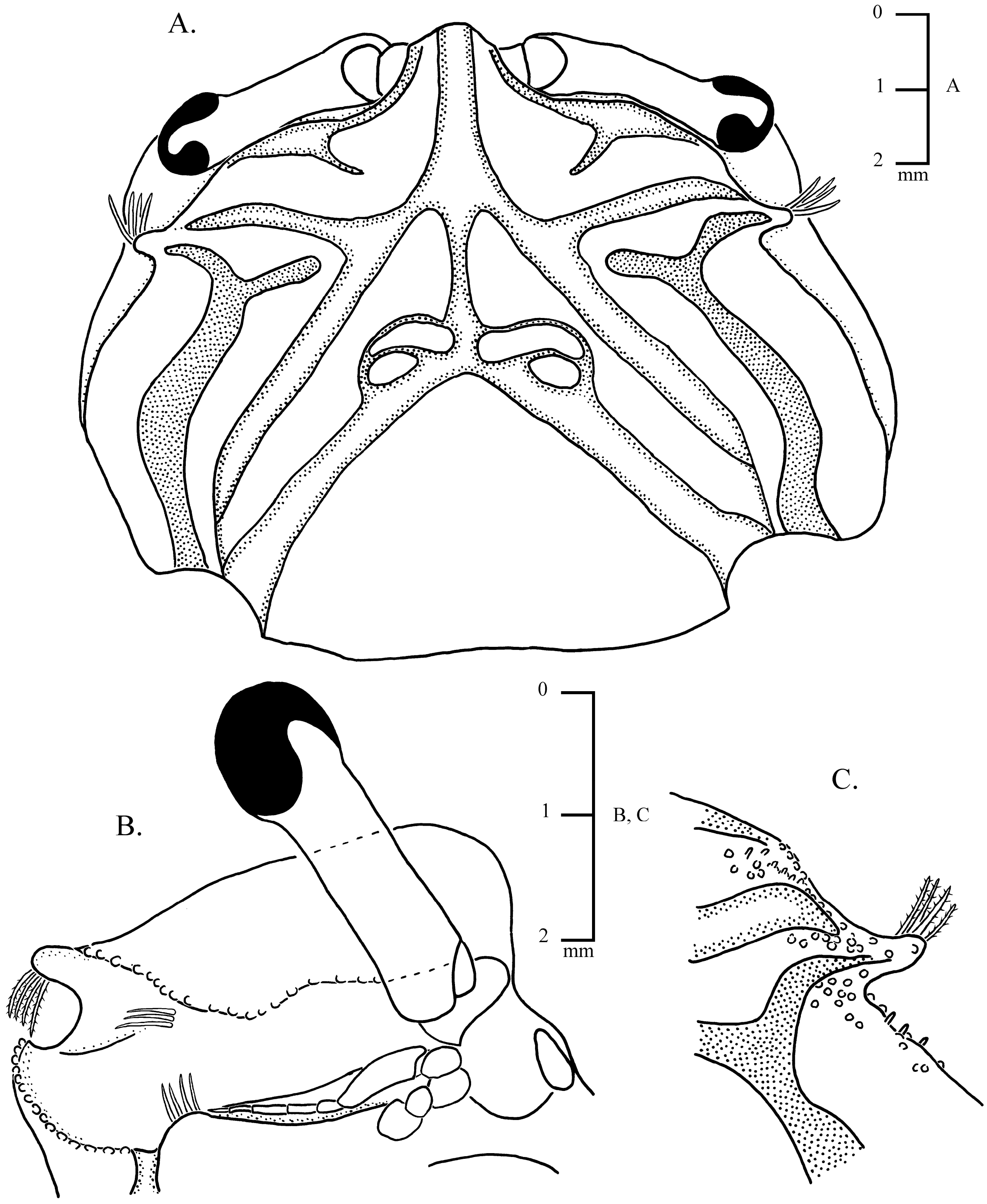


Fig. 2.23. *Dotilla sulcata* (1936.1.28.11-16). A. Carapace, dorsal view; B. Front, anterior view; C. Right antero-lateral angle, dorsal view

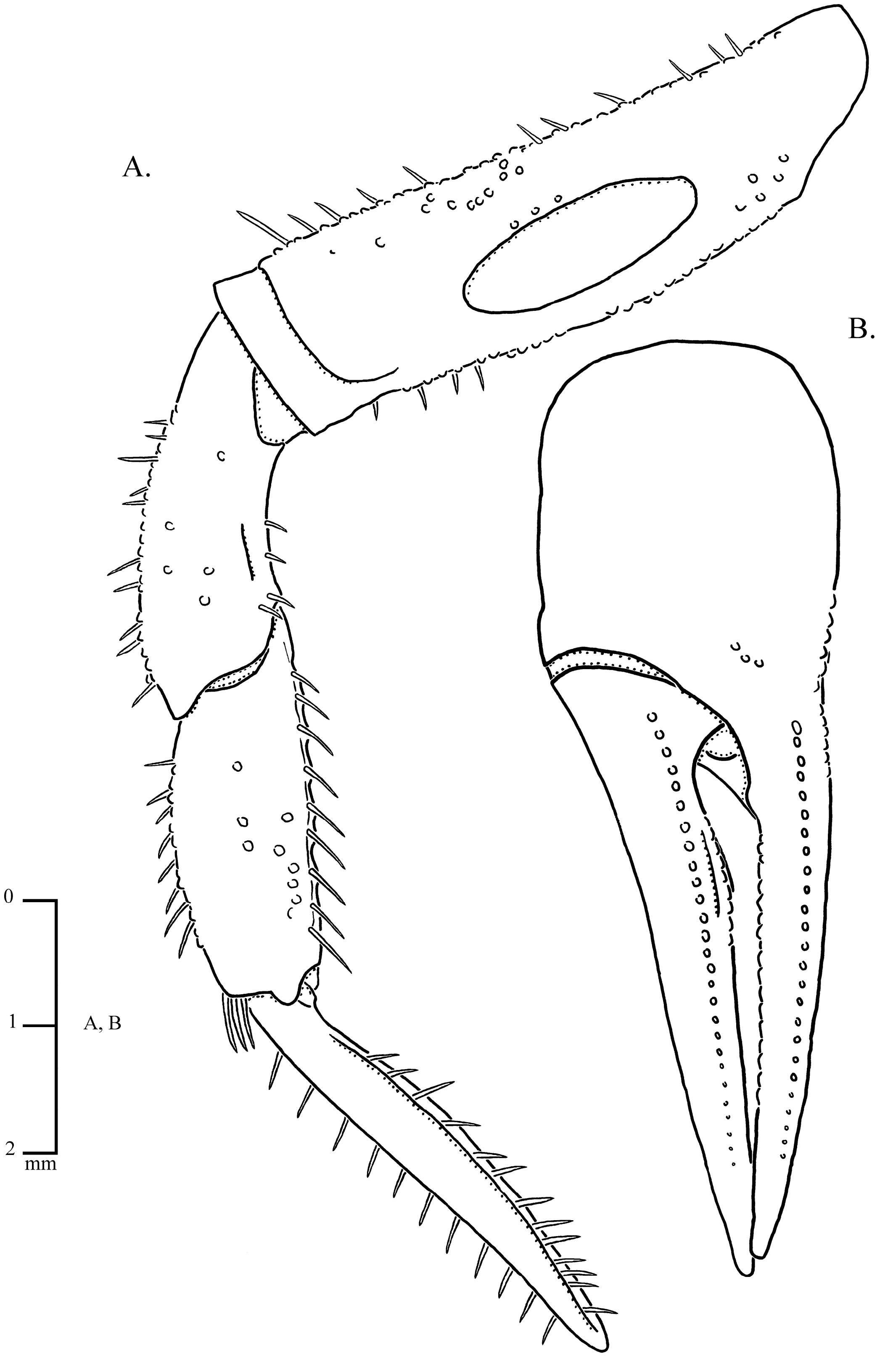


Fig. 2.24. *Dotilla sulcata* (NHM 1936.1.28.11-16). A. Left fourth ambulatory leg, dorsal view; B. Left chela, anterior view

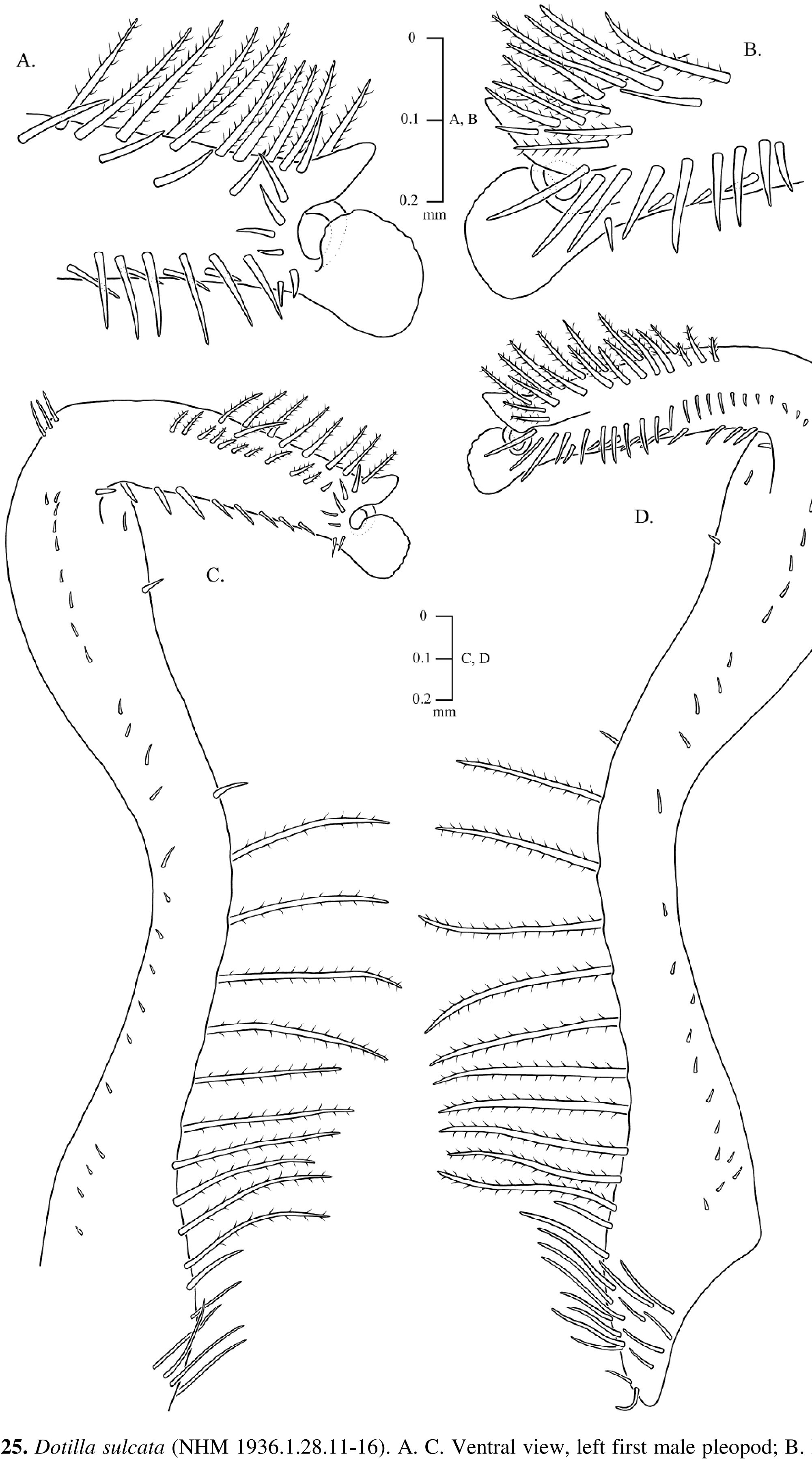


Fig. 2.25. *Dotilla sulcata* (NHM 1936.1.28.11-16). A. C. Ventral view, left first male pleopod; B. D. Dorsal view, left first male pleopod.

Dotilla wichmanni De Man, 1892

Dotilla wichmanni De Man 1892: 308, pl. xviii, Fig. 8; 1895: p. 577, Rathbun 1910: 324; Tesch 1918: 45; Kemp 1915: 227, Fig. 1; 1919: 330, Fig. 9d; Gordon 1941: 140, Fig. 15; Sankarankutty 1961: 114, Fig. 1d, 3a, 4b; Aiyun & Siliang 1991: 499, pl. lxiv, Fig. 255; Vogel 1984: 225, Figs 3b, 4.

Material Examined

Indonesia Karakelang Islands; 1 ♂ type (CW 7.4 mm) (ZMA 241623); Indonesia, Sulawesi, Ujung Pandang; 2 ♂ syntypes (CW 3.5 - 3.7 mm), 3 ♀ syntypes (CW 2.7 - 3.6 mm) (ZMA 102582); Indonesia, Sulawesi, Ujung Pandang, river near Tello; 2 ♂ syntypes (CW 3.5 - 3.7 mm) (RMNH 1279); Amoy, China; 2 ♂ (CW 5.3 - 5.9 mm) (RMNH 2078); Indonesia, Sulawesi, Ujung Pandang, river near Tello; 2 ♂ syntypes (CW 3.4 - 3.5 mm) (RMNH 2623); Indonesia, Java, near Jakarta, Dec 1927; 2 ♀ (CW 3.7 - 4.9 mm) (ZMA 241632); Indonesia, Bay of Jakarta, Muara Kalong, Aug 1929; 2 juv. (CW 1.8 - 1.9 mm), 7 ♂ (CW 2.4 - 3.5 mm), 8 ♀ (CW 2.7 - 4.0 mm) (ZMA 241631); Indonesia, Sulawesi, Ujung Pandang, Dec 1934; 1 juv. (CW 1.7 mm), 4 ♂ (CW 3.3 - 3.9 mm), 1 ♀ (CW 2.8 mm) (ZMA 241641); Indonesia, Sumatra, Atjeh; 2 ♂ (CW 3.7 - 3.9 mm), 1 ♀ (CW 5.2 mm) (ZMA 241627); Singapore; 3 ♂ (CW 5.8 - 8.0 mm), 1 ♀ ovig. (CW 6.4 mm) (NHM 1973.11.15.24-27); Kuala Ibai, Trengganu, Malaysia, Aug 1950 (NHM 1965.7.15.94-103); Bedok Beach, Singapore March 1953; 2 ♀ (NUS 1985.530-531); Mersing Karam, March 1963; 2 ♂ (NUS 1985.537-538); Thailand, Koh Samet, Sriracha Beach, Mar 1970; 23 ♂ (CW 2.8 - 4.9 mm), 13 ♀ (CW 3.5 - 4.7 mm) (RMNH 28207); Indonesia, Java, Palabuhanratu, Oct 1976; 14 ♂ (CW 5.7 - 7.7 mm), 1 ♀ ovig. (CW 7.0 mm), 8 ♀ (CW 5.8 - 7.7 mm) (RMNH 31592); Lim Chu Kang, Singapore, March 1986; 3 juv. (NUS 1985.536); Changi Becah, Singapore, July 1986; 2 ♂, 3 ♀ (NUS 1987.154-158); Changi Beach, Singapore, Aug 1986; 4 ♀ (NUS 1987.150-153); Sembawang, Singapore, Oct 1986; 4 ♀ (NUS 1987.161-164); Indonesia, Bali, Feb 1988; 5 ♂ (CW 6.5 - 7.6 mm), 5 ♀ (CW 4.0 - 6.5 mm) (RMNH 37312); Indonesia, Bali, Feb 1988; 12 ♂ (CW 6.4 - 8.1 mm) (RMNH 37313); Indonesia, Bali, Feb 1988; 3 ♂ (CW 6.9 - 7.9 mm) (RMNH 37314).

Description

Carapace (Fig. 2.26): Broader than long, distinct grooves parallel to lateral margin, slightly bifurcated anteriorly. Large males have three angular projections on either side of carapace. Two projections are situated on outer side of lateral groove, behind each other. Third projection is situated on side wall immediately beneath small tooth that defines upper and outer limit of orbit. Tubercles absent from gastric region. Boundary of mesogastric region is marked by grooves. Groove runs sinuously from edge of mesogastric area to antero-lateral angle. Hexagonal shape on posterior half of carapace, with upper edge formed from transverse groove marking posterior boundary of mesogastric region. Grooves run sinuously from end of this transverse groove to postero-lateral angle. As groove curves, it branches, and branch runs obliquely towards posterior margin. Lower edge of hexagonal shape formed by posterior margin. Transverse groove parallel to posterior margin incomplete in middle.

Second maxilliped: Spoon-tipped setae located on merus are tri-lobed, two small slits on middle lobe. Peduncle of setae terminates without any special differentiations (Vogel 1984).

Chela (Fig. 2.27b): Dactylus bears small tooth in middle of cutting edge. Both fingers serrated at proximal end of cutting edge. Fingers slightly longer than propodus palm.

Fourth ambulatory leg (Fig. 2.27a): Dactylus ca. 1.5 times length of propodus. Tympana large, present on both sides of merus. Tympanum on dorsal side of merus occupies at least two-thirds of area of merus.

Sternum: Sternal tympana present on all somites.

Abdomen (Fig. 2.29b): Comprises seven separate moveable somites. Distal margin of fourth somite shallowly emarginate, overlaps fifth somite. Fourth somite bears single tuft of setae extending across entire distal margin. Male and female abdomens are similar, not v-shaped and u-shaped respectively.

First male pleopod (Fig. 2.28): Short, stout, slightly curved and more slender distally. Tip has a covering of unbranched setae. Outer margin bears fringe of plumose setae on proximal third. Line of unbranched setae run close to inner margin on dorsal side, extending from proximal area towards tip.

Remarks

Tentatively identified by Stephensen (1945) in the Iranian Gulf, although more likely to be *Dotilla blanfordi* (see remarks under *D. blanfordi*).

Distribution

Hainan Island, the coast of the Gulf of Thailand, Malaysia, Singapore, throughout Indonesia.

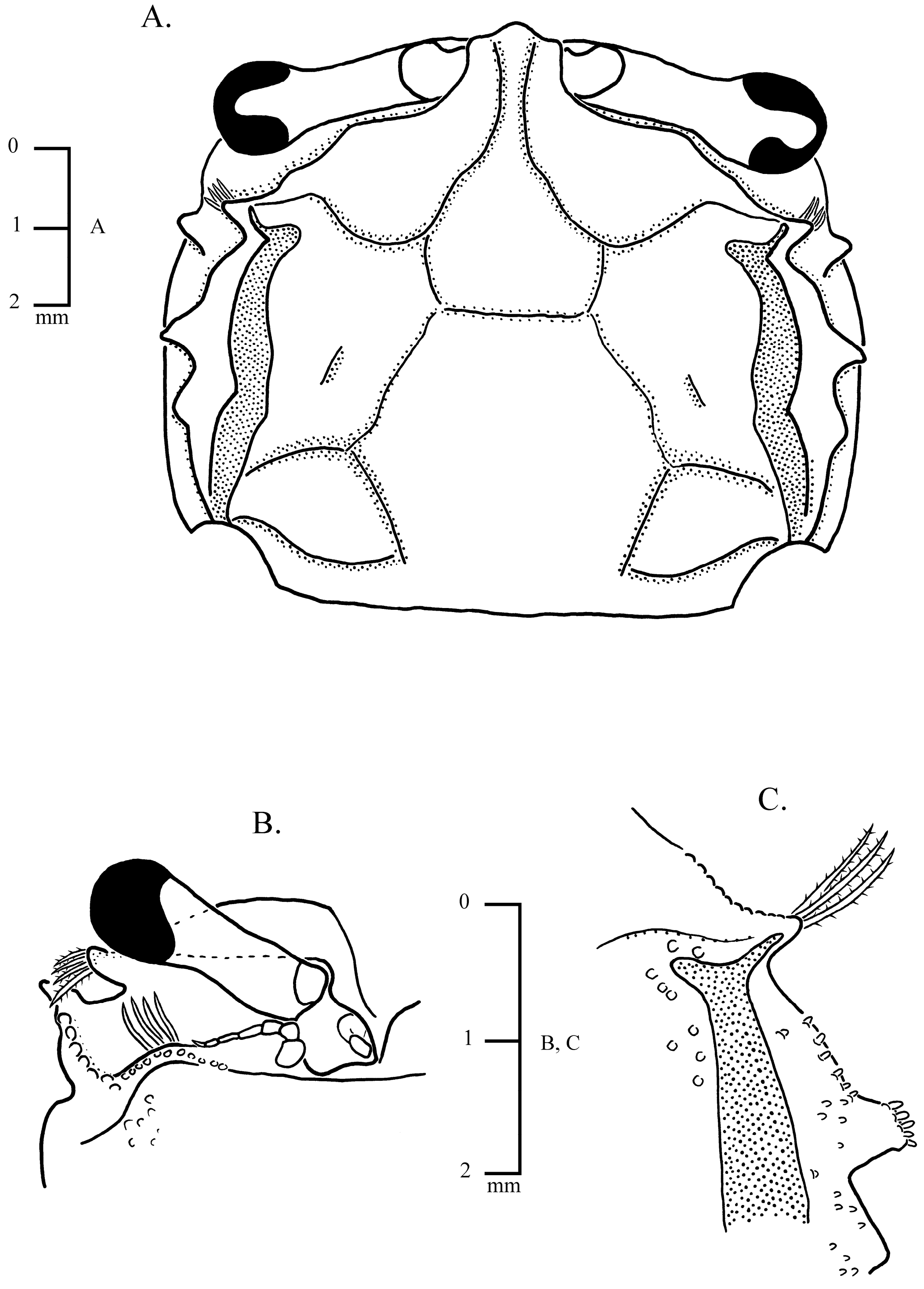


Fig. 2.26. *Dotilla wichmanni* (NHM 1937.11.15.24-27). A. Carapace, dorsal view; B. Front, anterior view; C. Right antero-lateral angle, dorsal view

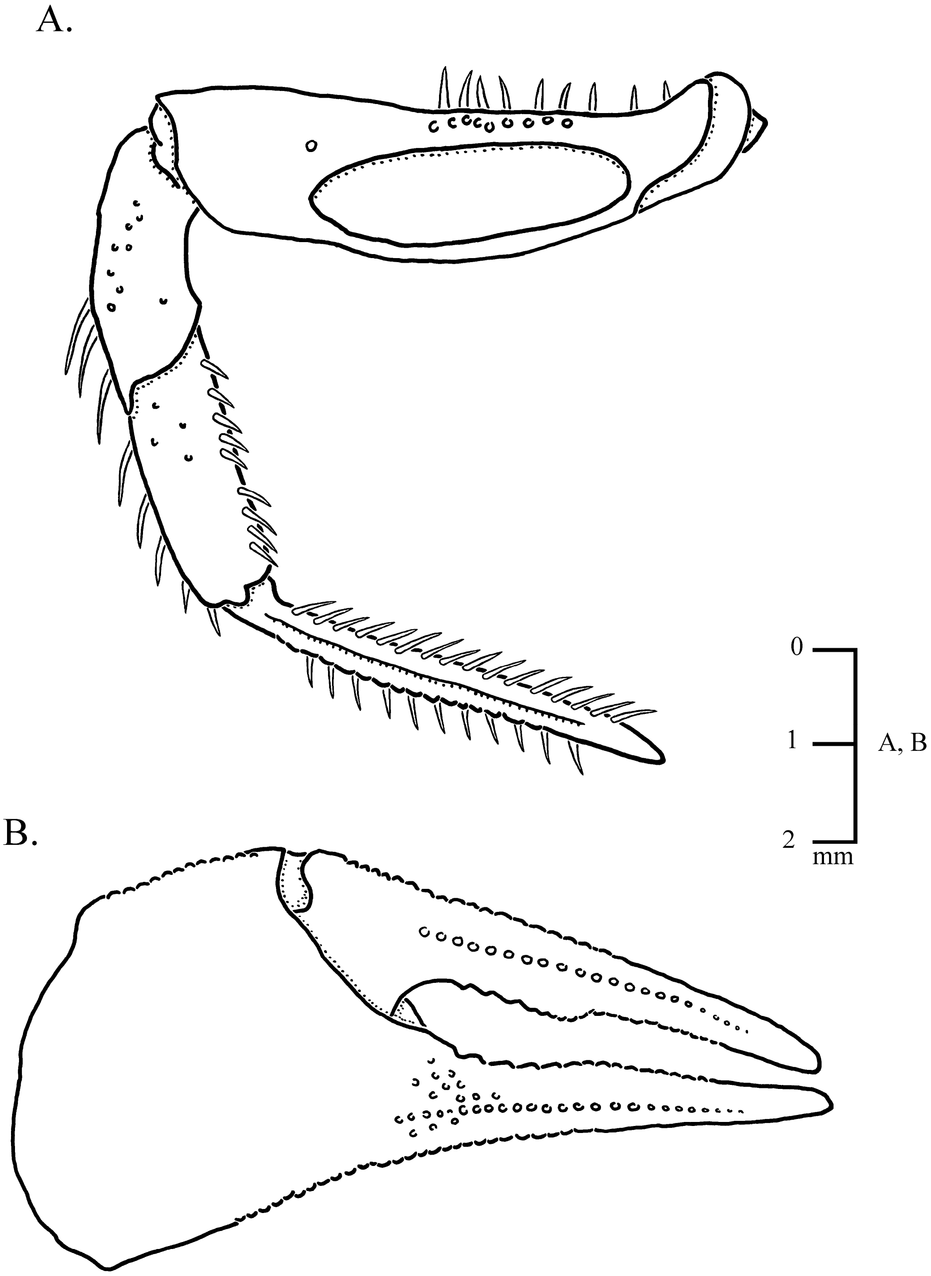


Fig. 2.27. *Dotilla wichmanni* (NHM 1937.11.15.24-27). A. Left fourth ambulatory leg, dorsal view; B. Right chela, anterior view

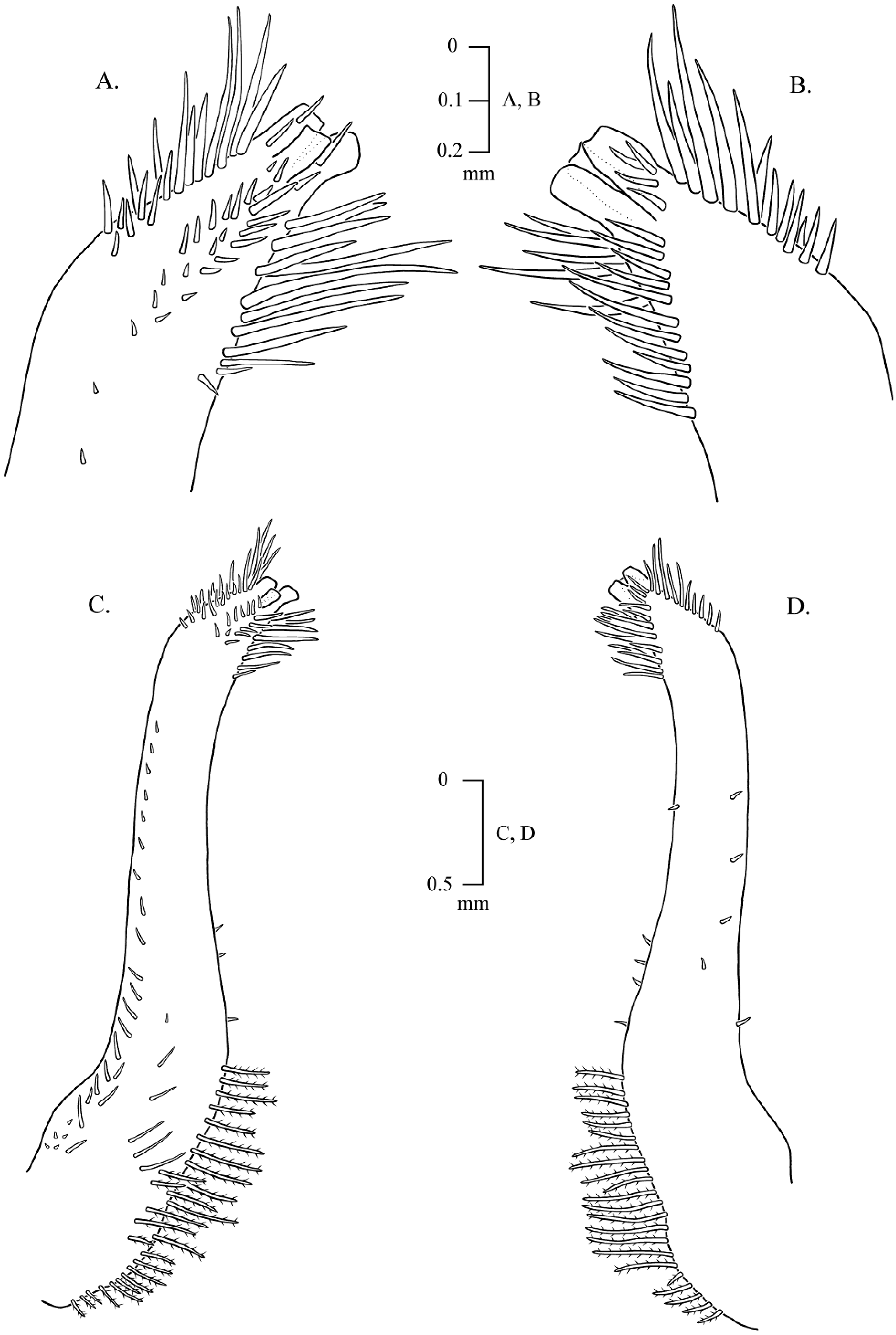


Fig. 2.28. *Dotilla wichmanni* (NHM 1937.11.15.24-27). A. C. Dorsal view, right first male pleopod. B. D. Ventral view, right first male pleopod.

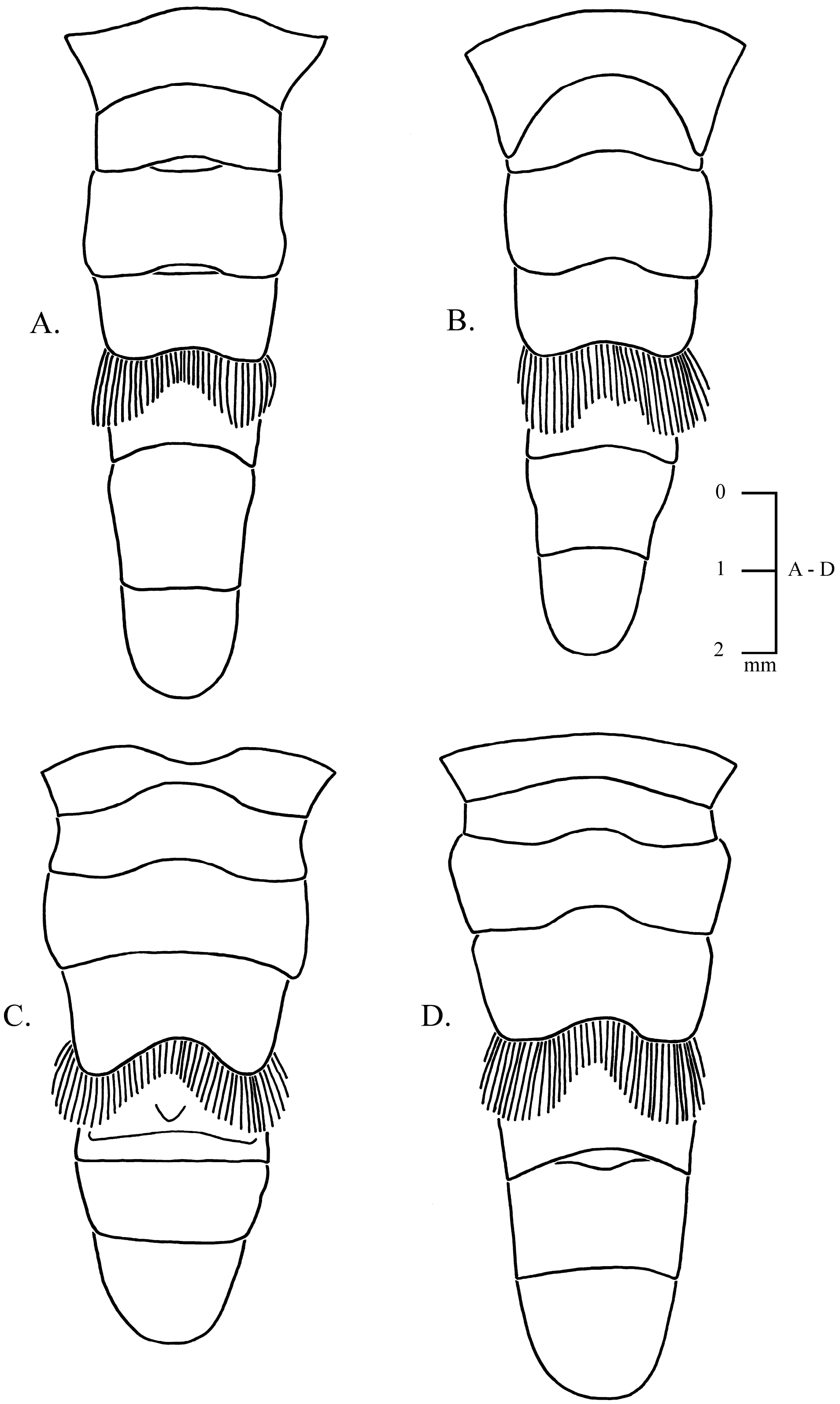


Fig. 2.29. Male abdomens of *Dotilla*. A. *D. myctiroides*; B. *D. wichmanni*; C. *D. sulcata*; D. *D. fenestrata.*

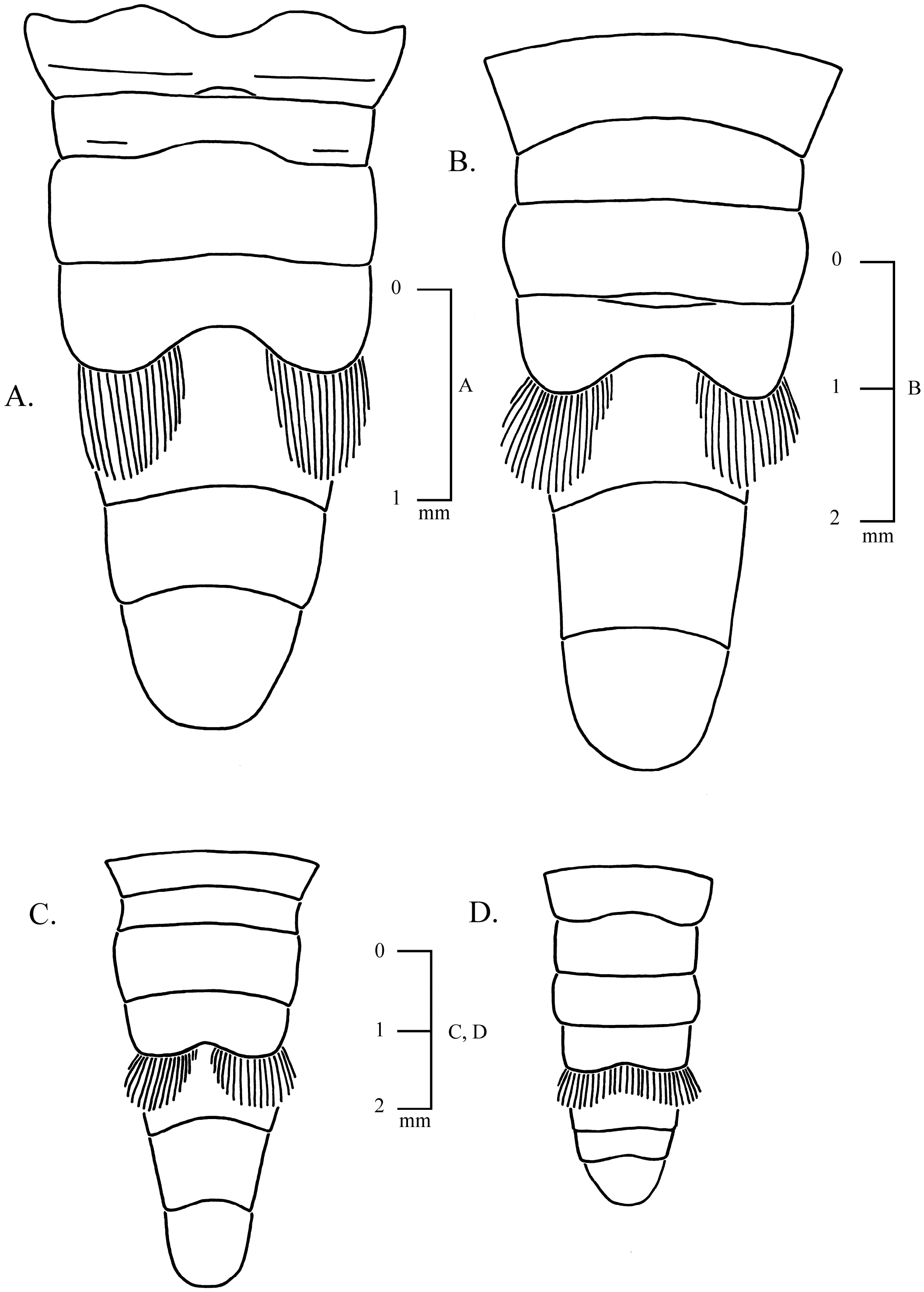


Fig. 2.30. Male abdomens of *Dotilla*. A. *D. pertinax*; B. *D. malabarica*; C. *D. blanfordi*; D. *D. intermedia*.

2.3.2. PHYLOGENETIC ANALYSIS

The molecular phylogenies for the 16S region (See Appendix *III*) showed that the raw sequence data for *Dotilla intermedia* collected from Prapas beach was identical to those collected from Bang Ben. The sequences for *D. intermedia* from the exposed sandy beaches in the Laem Son did not match any of the other *Dotilla* species in the phylogeny, and were quite distinct from the *D. wichmanni* and *D. myctiroides* sequences. This offers support for the morphological diagnosis of the crabs from the Laem Son being *D. intermedia*, rather than either of the other species of *Dotilla* previously recorded from Thailand.

2.3.3. MORPHOLOGICAL PHYLOGENY

The consensus tree estimated for *Dotilla* based on 22 morphological characters can be seen in Fig. 2.32. There is strong support for the grouping of *D. fenestrata* with *D. sulcata* (74.3) and *D. intermedia* with *D. blanfordi* (87.1). However, most of the bootstrapping values in the tree are not strong, and a much larger number of characters should be added to increase resolution and create a much more robust tree. In addition, further outgroups representing other genera of the Ocypodidae (i.e. *Uca*, *Ocypode* or *Ilyoplax*) should be included in the phylogeny.

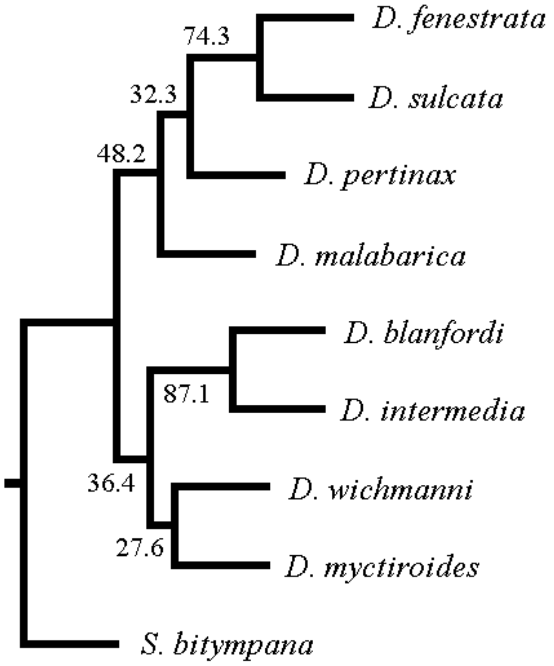


Fig. 2.31. Consensus maximum parsimony tree for the genus *Dotilla* constructed using PHYLIP v. 3.69. *Scopimera bitympana* was set as an outgroup. Numbers on branches indicate bootstrapping values

2.3.4. BIOGEOGRAPHICAL DISTRIBUTION

A biogeographical map for *Dotilla* has been constructed using literature records of species distributions and collection information from museum material (Fig. 2.33). Some species have only been recorded from a very limited number of locations, increasing the difficulty of defining their ranges and establishing boundaries between the distributions of species. The majority of species of *Dotilla* occur in India, suggesting that the genus may have originated there, before radiating out to the east and west.

Dotilla fenestrata is the western most representative of the genus, running from the South Africa along the east coast, and on Madagascar and the Comoro Islands, until it is replaced by *D. sulcata* in the Red Sea. *Dotilla sulcata* probably runs from the Red Sea around the coast of Yemen and Oman to the Gulf of Oman. At some point along the coastline of the Persian Gulf *D. sulcata* is replaced by *D. blanfordi*, which ranges east to Mumbai in India. The boundary between *D. blanfordi* and *D. malabarica* is also uncertain, with *D. malabarica* found from Mahé to the tip of India. *Dotilla intermedia* can be found from Porto Novo and eastwards around the Bay of Bengal to the west coast of Thailand. This study represents the first time *D. intermedia* has been recorded from Thailand. The populations of *D. intermedia* from the Laem Son appear to represent the southern most limit of the distribution of this species on the eastern side of the Bay of Bengal.

Dotilla pertinax has only been reported from one location, Puri in Orissa, which is within the distribution range of *D. intermedia*. The distribution of *D. myctiroides* overlaps several of the other *Dotilla* species; it can be found eastwards from Goa all around the coast of India, around the Bay of Bengal, and into Malaysia. *Dotilla wichmanni* can be found from the east coast of Malaysia northwards through Thailand to China, and throughout Indonesia. *Dotilla myctiroides* has often been recorded from the same regions as other *Dotilla* species, most notably with *D. intermedia* as seen at Porto Novo in India (McIntyre 1968) and in Thailand (this study). The two different species were found on beaches characterised by differing sediment grain sizes, with *D. myctiroides* favouring finer muddier sediments, suggesting that adaptations to sediment of a particular particle size range allows the overlap in the distributions of these species. The division of habitats according to differential preferences for sediment of certain particle sizes has been reported for species *Uca* (Robertson & Newell 1982; Lim 2005).

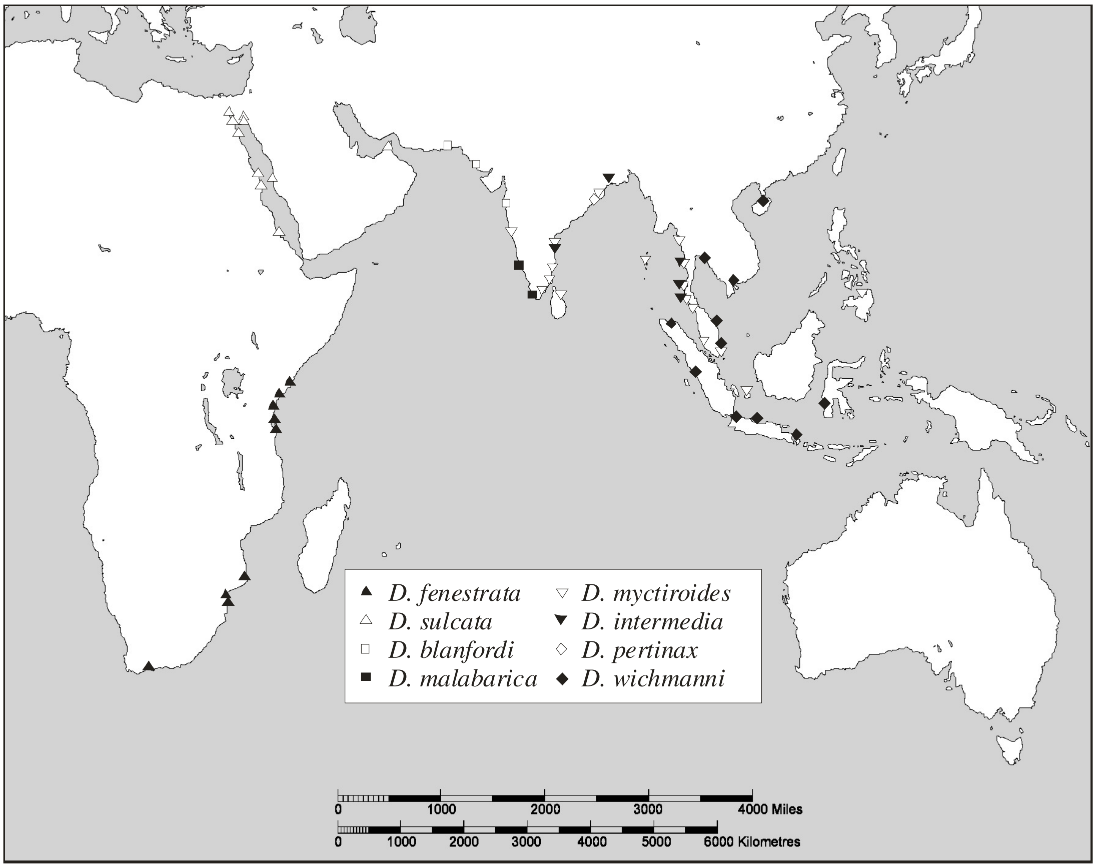


Fig. 2.32. Biogeographical distribution of the genus *Dotilla* based on museum collection material and records from the literature

2.4. DISCUSSION

2.4.1. IDENTIFICATION OF THE GENUS *DOTILLA*

The only key to the *Dotilla* (Kemp 1919) relies on the use of the carapace sculpture to distinguish between the different species. Each species does have very distinct carapace sculpture, and the key is robust for species identification providing these features can be easily observed. However, in juvenile and small specimens, or those that have been damaged, identification by examination of the carapace can prove to be problematic (e.g. Stephensen 1945). In these situations, other morphological characters must be used. Certain characters do vary between the species, such as the presence of sternal tympana, whether the setal band on the margin of the fourth abdominal somite is complete or forms two separate tufts, or if the merus of the chelipeds bears a spine at the proximal end of the lower surface. The shape of the first male pleopod does vary distinctly between all the species. This character is strong enough to be used in isolation to distinguish between *Dotilla* species, or used in conjunction with other features to confirm the identity of a species.

There are affinities between shapes of the first male pleopod in some of the species of the *Dotilla*. *Dotilla fenestrata, D. sulcata, D. pertinax* and *D. malabarica* all possess long, slender and sinuous pleopods. The distal fourth of the pleopod is bent at a 90° angle to the main shaft in *D. fenestrata* and *D. sulcata*, whilst in *D. pertinax* and *D. malabarica* the distal third is bent at an angle of around 40°. The pleopod of *D. fenestrata* is slightly more slender than that of *D. sulcata*, and the tip ends in a cup shape, as opposed to a bifid tip in *D. sulcata.* The tip of the pleopod in *D. malabarica* is characterised by a triangular projection. Unfortunately in all the specimens of *D. pertinax* available for examination the tip of the pleopod was damaged. If alternative material for *D. pertinax* can be obtained further examination should be made to determine the exact form of the first male pleopod.

Dotilla blanfordi, D. myctiroides and *D. wichmanni* all have short and robust pleopods, where the tip is heavily furnished with unbranched setae. The tip of the pleopod in *D. blanfordi* is dorso-ventrally flattened, whilst *D. wichmanni* has a bifid tip. *D. myctiroides* has a blunt tip to the pleopod.

The main complication with examining the male pleopods lies with the dimorphic forms of *Dotilla intermedia*. The ‘high’ form shows affinities with *D. blanfordi, D. myctiroides* and *D. wichmanni*. The ‘high’ pleopod is short and stout, with a blunt tip covered in unbranched setae, and is shorter and thicker than that seen in *D. myctiroides.* The ‘low’ form of *D. intermedia* has a shape closer to that shown by *D. fenestrata* and *D. sulcata*. It is long, slender and sinuous, and the distal fourth of the pleopod is bent at 90° angle to the main shaft.

The spoon-tipped setae found on the second maxilliped also have potential as an important diagnostic character. Vogel (1984) reported that the spoon-tipped setae are species-specific *Dotilla*. The *D. intermedia* specimens from the Laem Son certainly showed strong agreement with Vogel’s figures. However, no examination of the spoon-tipped setae in the other *Dotilla* has been undertaken in this study, so it is not possible to confirm how accurate these figures are for the entire genus. The spoon-tipped setae are generally all bowl-shaped, except in *D. blanfordi* and *D. intermedia*, where the tip of the setae resembles a bent fork shape. These bent-fork setae appear to be simpler in form than the bowl-shaped setae, and have been shown to be less efficient in retaining organic and silt particles than the bowl-shaped setae

(Altevogt 1957), suggesting that this may be a primitive form for these setae. Morphological examination of other Ocypodidae genera like *Scopimera* may help identify what constitutes a more primitive state for this particular character

The shape and setation of the abdomen also appears to be potentially species-specific. The composite figures illustrating the male abdomens (Figs 2.29 & 2.30) show that the relative sizes, shapes and sculpture of the abdominal somites vary between the species. *Dotilla blanfordi*, *D. malabarica* and *D. pertinax* all have two separate tufts of setae on the distal margin of their fourth somite, as opposed to the complete band found in the other species of *Dotilla.* The female abdomen has been seen to be slightly wider than the male in *D. myctiroides* (Kemp 1915) and *D. malabarica* (Vogel 1983), although only to a very slight extent. In general, the female abdomen appears to be indistinguishable in shape from the male abdomen for all *Dotilla*. The abdomen has rarely been illustrated for *Dotilla*, and this study represents the first time the abdomens of all eight species have been figured together. Due to the similarity between the male and female abdomens, reliable sex determination is almost impossible without examination of the pleopods (e.g. Kemp 1915, 1919; Vogel 1984).

The taxonomic descriptions of *Dotilla* were used to identify some mutual affinities between the different species, which have been listed in Table 2.4. Mutual affinities can also be seen between the carapace sculptures of the different *Dotilla* species, but as carapace sculpture is a complex character, these affinities are best illustrated figuratively (Fig. 2.34). *Dotilla fenestrata* and *D. sulcata* have very similar carapace sculpture, as do *D. intermedia* and *D. blanfordi*. Three species, *D. pertinax*, *D. malabarica* and *D wichmanni*, have carapaces that are distinct from each other, but yet share features with both *D. fenestrata*/ *D. sulcata* and *D. intermedia*/ *D. blanfordi*. The five tubercles on the anterior part of the carapace on *D. malabarica* resemble *D. intermedia*/ *D. blanfordi*, whilst the sculpture of the carapace posterior is more akin to *D. fenestrata*/ *D. sulcata*. The posterior sculpture of *D. pertinax* and *D. wichmanni* shows an affinity to *D. intermedia*/ *D. blanfordi*, whilst the anterior sculpture resembles that of *D. fenestrata*/ *D. sulcata*. The remaining species, *D. myctiroides*, has little to no carapace sculpture, and thus does not show any particular affinity to any other *Dotilla* species with regards to carapace sculpture.

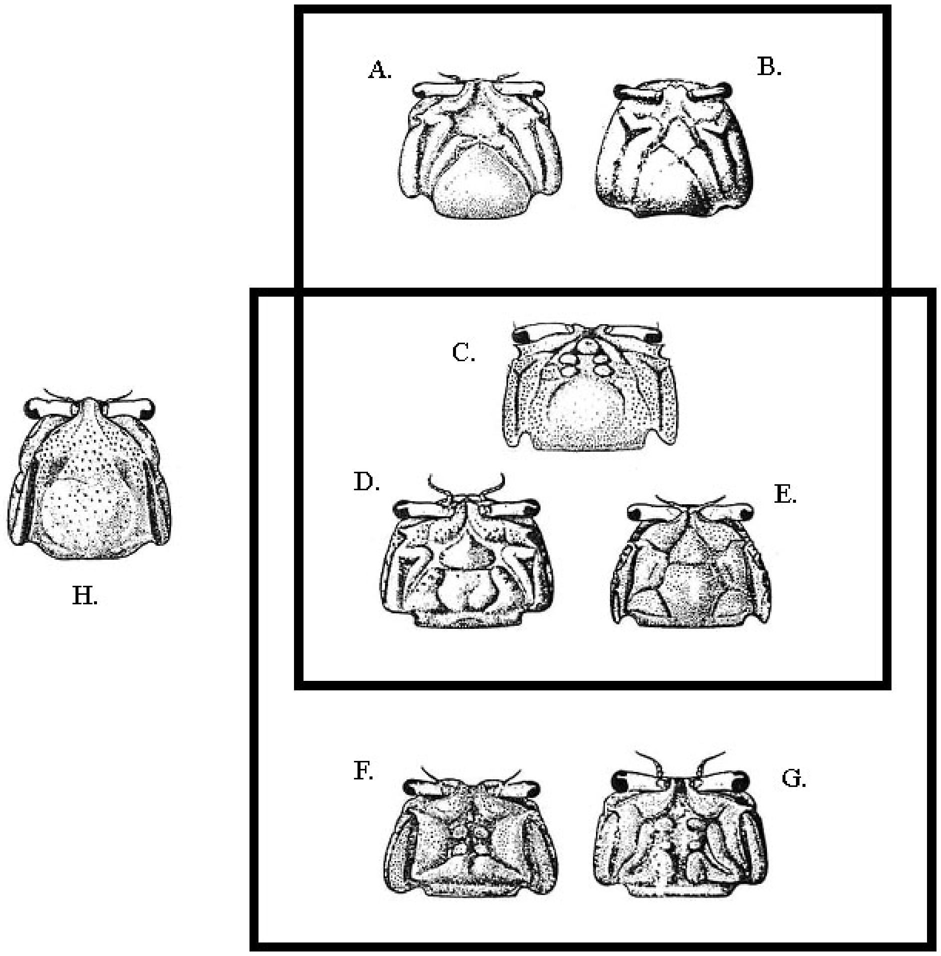


Fig. 2.33. Mutual affinities between carapace sculpture for *Dotilla* species, showing similarities for *D. blanfordi* & *D. intermedia*, and for *D. fenestrata* & *D. sulcata*. A. *D. sulcata*; B. *D. fenestrata*; C. *D. malabarica*; D. *D. pertinax*; E. *D. wichmanni*; F. *D. blanfordi*; G. *D. intermedia*; H. *D. myctiroides*. Carapace images from Kemp (1919) and Vogel (1983)

Table 2.4. Mutual affinities between *Dotilla* species for several morphological characters

Morphological Character	*Dotilla blanfordi*	*Dotilla fenestrata*	*Dotilla intermedia*	*Dotilla malabarica*	*Dotilla myctiroides*	*Dotilla pertinax*	*Dotilla sulcata*	*Dotilla wichmanni*
First male pleopod								
Short, stout, tip covered in setae	×		× (high form)		×			×
Long, slender, distal third bent at 40° to main shaft				×		×		
Long, slender, distal quarter bent at 90° to main shaft		×	× (low form)				×	
Sternal tympana present		×			×			×
Spine present on merus of cheliped		×					×	
Setal band on fourth somite								
Complete		×	×		×		×	×
Two separate tufts	×			×		×		
Spoon-tipped setae								
Bowl-shaped		×		×	×	×	×	×
Not bowl-shaped	×		×					

2.4.2. BIOGEOGRAPHICAL DISTRIBUTION AND THE PHYLOGENY OF *DOTILLA*

The affinities shown between *Dotilla* species are reflected in the morphological phylogeny, and are matched to a certain extent by their biogeographical distributions. The two western species (*D. fenestrata* and *D. sulcata*) are similar in the majority of characters, and group closely together in the phylogeny. The two eastern most species (*D. myctiroides* and *D. wichmanni*) also demonstrate affinities to one another for several characters, and although they group together in the morphological phylogeny, the bootstrapping support is weak. These two grouping represent species from the western- and eastern-most areas in the range of *Dotilla*, and occur on opposite sides of the phylogenic tree. However, the mixed distributions of the species from India do not coincide with some of the morphological affinities, and do not coincide with the patterns seen in the phylogenic tree. For example *D. blanfordi* and *D. intermedia* show many morphological affinities to each other, and group very closely together in the phylogenetic tree, but their distributions are separated by *D. malabarica*.

The molecular phylogeny was rather limited in that only four species of *Dotilla* were represented, and thus did not provide much information beyond confirming that *Dotilla* sp. from the exposed sandy beaches in the Laem Son was definitely not either *D. myctiroides* or *D. wichmanni*, the only other *Dotilla* species previously recorded from Thailand. Thus, the molecular phylogeny offers further support to the diagnosis that the species of *Dotilla* from the Laem Son National Park was *D. intermedia*. The molecular phylogeny supports some of the patterns seen in the morphological phylogeny, such as *D. sulcata* being quite distant from *D. intermedia*, *D. wichmanni* and *D. myctiroides*. The molecular phylogeny does show *D. myctiroides* being closer to *D. intermedia* rather than *D. wichmanni* as shown by the morphological phylogeny, although the bootstrap values supporting these branches were quite low. The molecular phylogeny also segregates along biogeographical lines, with *D. sulcata* from the Red Sea at the base of the tree. *Dotilla wichmanni* from the Gulf of Thailand occurs next on the tree, then *D. myctiroides* from the Indian Ocean followed by *D. intermedia* also from the Indian Ocean.

Currently, there is no published phylogeny for the species of *Dotilla*. If genetic material could be obtained for all the *Dotilla* species, a molecular phylogeny could be a good approach for constructing a phylogeny of the genus to help confirm the preliminary morphological phylogeny attempted in this study. Previous molecular studies on Ocypodidae have favoured the use of 16S as a marker for constructing phylogenies (e.g. Kitaura *et al.* 1998, 2002; Kitaura & Wada 2006), and the small molecular phylogeny constructed in this thesis supports using 16S for the *Dotilla.* Further outgroups should also be included in the phylogeny to add more resolution, using other genera from the Ocypodidae (e.g. *Uca*, *Ilyoplax*, *Dotillopsis*) in addition to *Scopimera*. Another potential marker to consider using for constructing a phylogeny would be COI. A good approach would be to create phylogenies using both markers and compare the topologies to generate a consensus phylogeny. Phylogenies should be built using several tree-building methods in order to create a consensus topology for each marker, with Bayesian inference and maximum parsimony suggested to be used as a minimum. Once a molecular phylogeny for the whole genus has been constructed, comparisons could be made with geographical distributions and morphological phylogeny of the species to help elucidate the evolutionary history and speciation within the *Dotilla*.

2.5. SUMMARY

The identity of the *Dotilla* species found on the exposed oceanic sandy beaches in the Laem Son National Park has been diagnosed as *Dotilla intermedia*; this represents the first time that this species has been recorded from Thailand. The molecular phylogeny confirmed that the crab was not either of the two previously recorded *Dotilla* species from Thailand. The carapace sculpture was the most obvious morphological feature that can be used to distinguish between the species of *Dotilla*, and as such the key to the genus in Kemp (1919) remains valid and robust. In specimens where the carapace sculpture is unclear, it is necessary to use alternative characters. The first male pleopod was easily distinguishable between all eight species of the *Dotilla*, and should be a first point of consideration after examination of the carapace. The shape of the abdomen and the spoon-tipped setae on the second maxillipeds are also good characters to identify between the species within the genus. A biogeographical map for the genus has been constructed from literature records and museum collection material. Some mutual affinities between the morphology of different *Dotilla* species have been identified, and a morphological phylogeny was attempted for the genus. The morphological phylogeny showed some relation to the biogeographical ranges of the species, and was partially supported by the molecular phylogeny. The construction of a molecular phylogeny for the whole genus would help to give support to the attempted morphological phylogeny of the genus *Dotilla*.

3. THE ECOLOGY OF *DOTILLA INTERMEDIA* FROM THE LAEM SON

3.1. INTRODUCTION

Exposed sandy beaches have been described as being amongst the harshest aquatic ecosystems on Earth (McLachlan *et al.* 1993). In these physically controlled environments biological interactions are minimal and communities are structured by the independent responses of individual species to the physical environment, as suggested by the Autoecological Hypothesis set out by Noy-Meir (1979) and McLachlan (1990). The review by McLachlan and Dorvlo (2005) indicates that the most recent studies of sandy beach ecology have correlated the physical shore attributes with biotic community-level indices, such as species diversity, total abundance and biomass. However, these biotic measures reflect the summation of the responses of individual populations. The investigation of the relationship between the distribution of key species and their physical environment can provide an insight into the processes that structure community-level patterns. The present study departs from the usual community level approach to beach ecology by investigating the autoecology (i.e. the ecology of the population of a single species) of a key macrofaunal species of a tropical sandy shore.

Crabs of the genus *Dotilla* are ecologically important members of tropical sandy-shore intertidal communities. *Dotilla* are found on tropical shores and mudflats from East Africa and the Red Sea eastwards towards Japan (Alcock, 1900b). Several *Dotilla* species have been extensively examined, such as *D. myctiroides* (e.g. Tweedie 1950; Halis & Yaziz 1982; Takeda *et al.* 1996; Bradshaw & Scoffin 1999) and *D. fenestrata* (e.g. MacNae & Kalk 1962; Hartnoll 1973; Gherardi *et al.* 1999). However, there have been no detailed ecological studies on *D. intermedia*.

The aims of this study were twofold: firstly, to gain an understanding of the autoecological zonation of *Dotilla intermedia* De Man, 1888 on two oceanic exposed beaches from the Laem Son National Park in Ranong Province, Thailand; secondly, to use the data gathered from this investigation as a case study of a littoral species to examine the current hypotheses concerning community structure on sandy beaches.

The relationship between the beach gradient and the extent and position of the *Dotilla* zone was examined, along with physical factors associated with the beach gradient that may be responsible for driving the observed patterns. The distribution of crabs within the *Dotilla* zone was also examined to see if there was any evidence of size segregation and whether this was, in turn, related to available foraging time (governed by tidal immersion/exposure), population density or sediment particle size and organic content. The sex ratio and any evidence for sexual asymmetry were also established for *D. intermedia*.

3.2. METHODS

3.2.1. BEACH GRADIENT

Both study beaches were very long, so the sampling strategy adopted was to take transects that were representative of the beach as a whole, but sufficiently separated to ensure that some degree of heterogeneity and changes in beach topology were captured.

Transects were established perpendicular to the shoreline from the extreme high water spring (EHWS) mark to the low water mark. Starting at EHWS mark, the vertical drop in height (cm) was measured every metre to construct beach profiles. The heights at which the boundaries of the *Scopimera* sp. zone and the *Dotilla* zone occurred were recorded for each transect. The transects were spaced 200 m apart. All transects on each beach were measured at low water during a spring tide on a single day. Nine transects were established on Prapas beach in November 2007, and five were repeated again in April 2008. Three transects were established on Bang Ben in April 2008 (the extra width of Bang Ben compared to Prapas meant that fewer transects could be completed over a single low tide). Transects were measured only over a single day to avoid any temporal changes. The sampling periods were timed to avoid the monsoon season in Thailand, as *Dotilla* crabs retreat and plug their burrows during rainfall. Rain also acts to destroy any feeding pellets, effectively removing any trace of where the crabs were located on the beach. For each transect, the gradient was compared to the height of the upper and lower boundaries of the *Dotilla* zone.

3.2.2. QUADRAT ANALYSIS

Quadrats (0.25 m^2) were placed at approximately 2 m intervals across the *Dotilla* zone on each transects. After being placed, quadrats were photographed from above using a Canon EOS 400D digital SLR camera, ensuring the quadrats were square in the photograph. The photographs were then analysed using 'MapInfo Professional ver. 9'. The corners of the quadrat were registered as co-ordinates in a 50x50 cm square, allowing measurements to be made within the quadrat using the programme relative to this 2-D scale. The numbers of burrows present in the quadrat were counted, and the density of crabs (ρ) per m^2 was calculated:

$$\rho = \frac{n}{0.25}$$

where n = number of individuals in the quadrat

The burrow diameter (*BD)* of every burrow was measured, and then converted into crab carapace width (*CW*) using a regression equation (see *3.2.3*). The area each crab fed in around its burrow was measured, with the feeding area identified by examining the arrangement of feeding pellets and the feeding trench leading out from the burrow. In addition, the distance to the next nearest burrow was measured from the centre of each burrow.

The nearest neighbour distances were used to analyse the spatial patterns within each quadrat using an established method (Clark & Evans 1954), generating an *R*-value for each quadrat. The index of aggregation (*R*) is defined as a ratio between the observed mean nearest neighbour distance (r_a) and the expected value if the distribution were random (r_e):

$$R = \frac{r_a}{r_e}$$

$$r_a = \frac{\sum_{i=1}^{n} r_i}{n}$$

where r_i = measured nearest-neighbour distance for each individual burrow

$$r_e = \frac{1}{2\sqrt{\rho}}$$

The *R*-value takes a range between 0 and 2.1, and describes the spatial pattern within an area. An *R*-value of 0 indicates a completely aggregated distribution, whilst an *R*-value of 1 shows a random distribution, and 2.1 demonstrates a completely regular distribution.

In order to eliminate the potential that an observed spatial pattern may have been achieved by random chance, the z statistic was calculated:

$$z = \frac{r_a - r_e}{s_r}$$

where s_r = standard error of the expected nearest neighbour distance

$$s_r = \frac{0.26136}{\sqrt{n\rho}}$$

If the magnitude of z was greater than 1.96, then the observed spatial distribution pattern could be accepted as significant at the 95% confidence level; z values below 1.96 indicate that the spatial pattern indicated by the *R*-value were not significantly different from a random distribution.

Mean crab *CW* and the mean feeding area size within each quadrat were log transformed and correlated using Pearson's Correlation Coefficient. The mean feeding area size and the nearest neighbour *R*-value was compared to the density of crabs within each quadrat.

3.2.3. BURROW DIAMETER AND CRAB SIZE

Ocypodidae crab carapace width (*CW*) is closely related to burrow diameter (*BD*) (e.g. Zwarts 1985; Dray & Paula 1998; Lee & Lim 2004), with the exact relationship varying amongst species. Creating a regression equation between burrow diameter and crab *CW* allows for quick estimation of crab size without having to excavate every individual crab. A preliminary survey was conducted to establish the relationship between *CW* and *BD* for *Dotilla intermedia.* The diameters of 30 burrows, covering a range of sizes, were measured to the nearest 0.01 mm with a pair of digital vernier callipers, along with the *CW* of the resident crab. *BD* was regressed against *CW*. There was a highly significant linear relationship between crab *CW* and *BD* ($R^2 = 0.934$; $P < 0.0001$); therefore *BD* was used as a proxy for *CW* in analysing body size (Fig. 3.1).

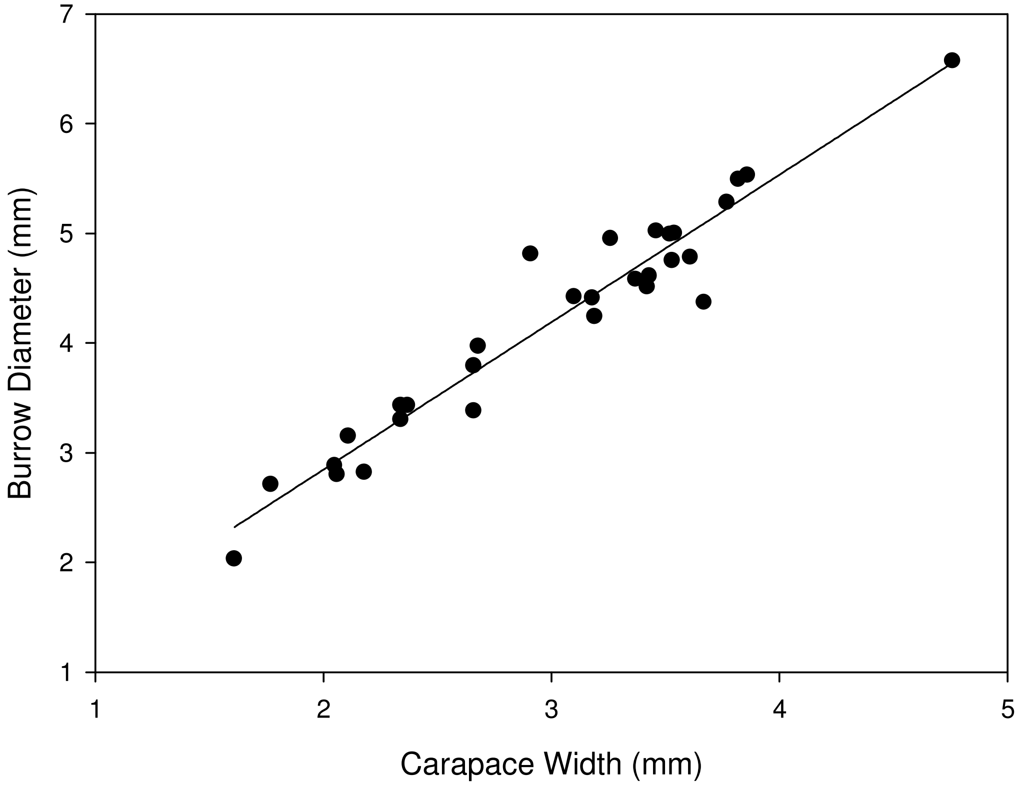


Fig. 3.1. Linear regression of burrow diameter (*BD*; mm) against resident crab carapace width (*CW*; mm) for *Dotilla intermedia*. $n = 30$
$CW = 0.695 \times BD + 0.089 \quad R^2 = 0.934$

3.2.4. SEDIMENT ANALYSIS

Sediment samples were collected on Prapas beach and Bang Ben in 2008. At five points along each transect single 5cm diameter sediment cores were taken to a depth of 10 cm, along with a single surface sediment sample:

- One metre above the upper edge of the *Dotilla* zone (Above)
- One metre inside the high shore edge of the *Dotilla* zone (High)
- In the middle of the *Dotilla* zone (Mid)
- One metre inside the low shore edge of the *Dotilla* zone (Low)
- One metre outside the low shore edge of the *Dotilla* zone (Below)

Sediment samples were weighed and oven dried at 60ºC to constant weight. The total water content of the sediment was measured as the percentage weight loss of the sample. The dried samples were then sieved through a sieve stack (2 mm - 0.064 mm mesh sizes) and each fraction weighed separately. The Gradistat program (Blott & Pye 2001) was used to calculate the average sediment grain size (MDΦ) and the sediment sorting (QDΦ) from each sample.

The surface sediment samples were taken to analyse for organic content, and were comprised of surface scrapes to a 3 mm depth. This depth was chosen to represent the depth of sediment that *Dotilla* spp. scrape into their mouths when feeding (Luschi *et al.* 1997). Each sample was oven dried at 60ºC to a constant weight, before being placed into a muffle furnace at 450ºC for 6 hours. Samples were then reweighed, with organic content of the sediment measured as the percentage weight loss of the sample.

To test whether there were any significant differences in mean sediment total water content and mean sediment surface organic content between the different areas of the *Dotilla* zone, and between the two different beaches, the data were arcsine transformed, and nested ANOVA tests were performed. The Scheirer-Ray-Hare extension of the Kruskal-Wallis Test (Scheirer *et al.* 1976) was used to test if the median particle size varied significantly between beaches and between each area within the *Dotilla* zone. This test was repeated for sediment sorting.

3.2.5. PRINICPAL COMPONENT ANALYSIS

Principal Component Analysis (PCA) (Pearson 1901) was used to examine which of the four sediment variables measured were most important in controlling the distribution of *Dotilla intermedia* on the beaches, with sediment data from Prapas beach and Bang Ben collected in 2008 combined together. The four variables examined were: water content of the sediment, surface sediment organic content, average sediment grain size (MDΦ), and sediment sorting (QDΦ), with each variable taken from the above, high, middle, low and below areas of the *Dotilla* zone on each transect.

3.2.6. POPULATION SIZE-FREQUENCY AND SEX RATIO

Size-frequency distributions were constructed using all crab *CW* estimates from every quadrat for Prapas 2007, Prapas 2008 and Bang Ben 2008, with *CW* measurements divided up into 0.3 mm size classes. All crabs with a *CW* less than 2 mm were grouped together. Kolmogorov-Smirnov tests (Sokal & Rohlf 1995) were used to test for any significant difference between the population size structure from Prapas 2007, Prapas 2008 and Bang Ben 2008.

The sex ratio of *Dotilla intermedia* was assessed by excavating 317 burrows from the high to low shore and capturing the resident crabs. The *CW* of the crabs was measured using vernier callipers, and the crabs were then sexed by examination of the pleopods. Crabs with a *CW* less than 2 mm were too small to reliably sex, and thus were classified as juveniles. The observed numbers of male and female crabs were tested to see if they deviated significantly from an expected 1:1 ratio using a chi-squared test with Yates' Correction for Continuity (Yates 1934). As the data were not normally distributed, a Mann Whitney *U*-test was used to test whether the median *CW* differed significantly between male and female crabs.

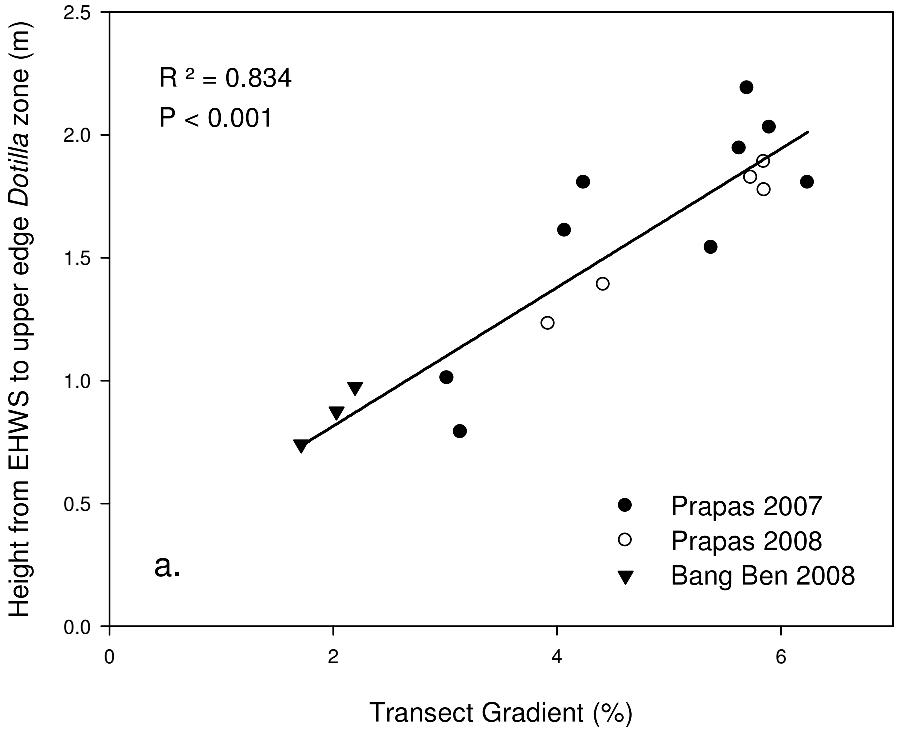
3.2.7. SIZE SEGREGATION

To assess whether there was any evidence of population size segregation across the beaches, the *Dotilla* zone on each beach (Prapas 2007, Prapas 2008 and Bang Ben 2008) was split into three equal areas (upper, middle and low) and crab size-frequency distributions were constructed using the quadrat data from each area. Kolmogorov-Smirnov tests (Sokal & Rohlf 1995) were used to compare the size-frequency distribution between each of the areas on each beach, and to compare distributions between each area with its corresponding area on the other beaches.

3.3. RESULTS

3.3.1 BOUNDARIES OF THE *DOTILLA* ZONE

The height of the upper edge of the *Dotilla* zone had a significant relationship to the gradient of the beach calculated from each transect ($P < 0.001$) from Prapas beach in November 2007 and April 2008, and in Bang Ben April 2008. The lower boundary of the *Dotilla* zone was also significantly related to the beach gradient ($P < 0.005$). The R^2 values show that the beach gradient explained more of the variance in the high shore boundary of the *Dotilla* zone than the low shore boundary (Fig. 3.2).



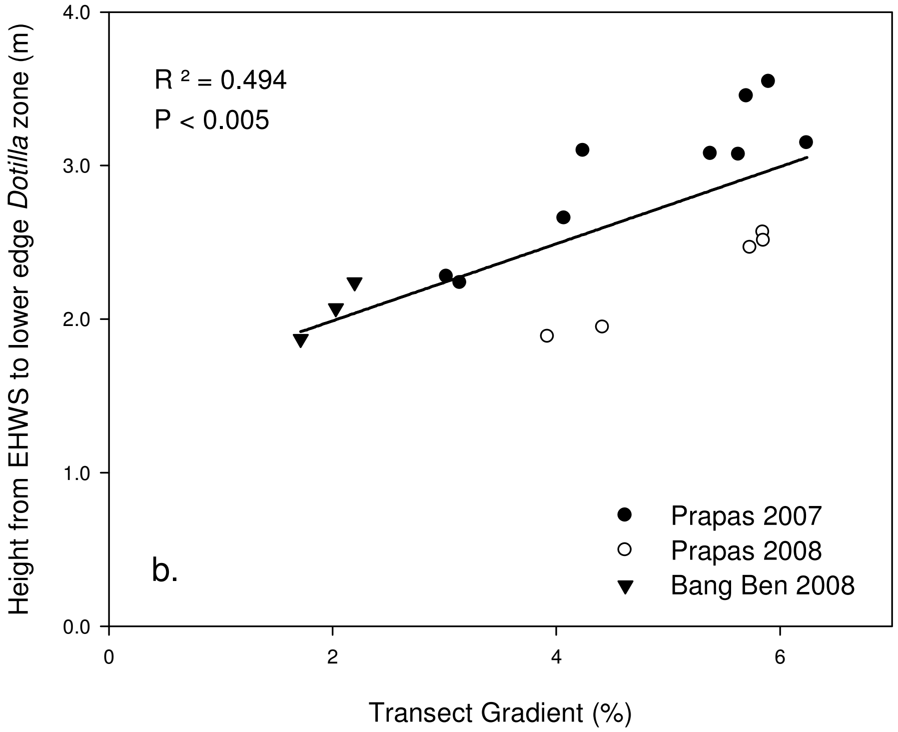


Fig. 3.2. Transect gradient against the height from extreme high water spring (EHWS) of the boundaries of the *Dotilla* zone. a.) Upper *Dotilla* boundary b.) Lower *Dotilla* boundary

Height upper edge *Dotilla* zone = 0.2822 x transect gradient + 0.2501

Height lower edge *Dotilla* zone = 0.2508 x transect gradient + 1.4867

3.3.2. SEDIMENT WATER AND ORGANIC CONTENT

The mean total water content of the sediment increased from above the high water boundary of the *Dotilla* zone to the low water boundary (Fig. 3.3). Nested ANOVA showed that there was a significant difference between the water content of the five areas of the *Dotilla* zone ($F_{4, 5} = 42.079$; $P < 0.001$), and that there was no significant difference between the Prapas beach and Bang Ben within each beach area ($F_{5, 30} = 0.820$; ns). Holm-Sidak pairwise comparisons (Sidak 1967) showed that there was no significant difference between the mean water content from the low and below areas of the dotillid zone (~22.5 %). *Dotilla intermedia* was only present in areas where total water content of the sediment was in excess of 15 %.

Mean total surface organic content of the sediment was also shown be to significantly different between the five areas of the *Dotilla* zone ($F_{4, 5} = 37.578$; $P < 0.001$), with no significant difference found between Prapas beach and Bang Ben within each area of the *Dotilla* zone ($F_{5, 30} = 1.545$; ns). However when looking at the pairwise comparison, the only significant difference occurred between the above zone and below zone samples. The mean surface organic content increased from the high shore to the low shore, although the surface organic content was highly variable across the *Dotilla* zone (Fig. 3.4).

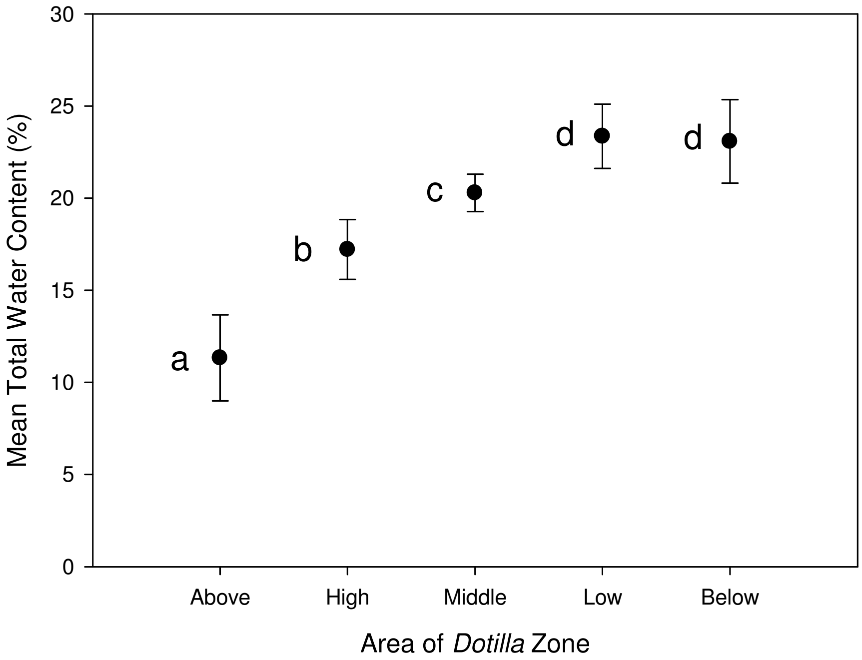


Fig. 3.3. Sediment mean total water content from different areas of the *Dotilla* zone from Prapas and Bang Ben 2008. As nested ANOVA showed no significant difference between beaches, data from Prapas and Bang Ben have been pooled together. Error bars ± 1 SD. *n* = 8. Lower case letters denote statistically significant groups

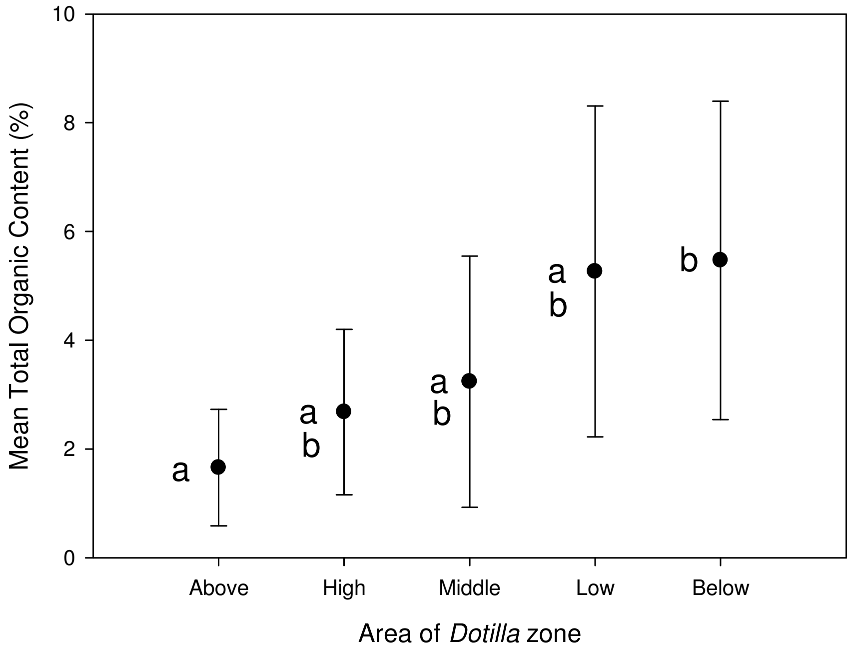


Fig. 3.4. Sediment mean total surface organic content from different areas of the *Dotilla* zone from Prapas and Bang Ben 2008. As nested ANOVA showed no significant difference between beaches, data from Prapas and Bang Ben have been pooled together. Error bars ± 1 SD. *n* = 8. Lower case letters denote statistically significant groups

3.3.3. SEDIMENT PARTICLE SIZE AND SORTING

Median sediment particle size had a large range in each area of the *Dotilla* zone, from around 175 to 350 μm. Samples from Bang Ben generally had finer sediment than on Prapas beach, ranging from 175 to 220 μm (Fig. 3.5). The Scheirer-Ray-Hare extension of the Kruskal-Wallis Test showed that although there was a significant difference between median grain size from Prapas and Bang Ben (H = 17.71, P < 0.001), there was no significant difference in median grain size at each *Dotilla* zone area on the two beaches (H = 0.67, ns) or between different areas in the *Dotilla* zone across both beaches (H = 0.28, ns).

Sediment sorting was very varied overall in each *Dotilla* zone area. Bang Ben had much more well-sorted sediment, hence the lower range of median particle sizes encountered on the beach. Sediment was more moderately sorted on Prapas beach (Fig. 3.6). There was a significant difference between sediment sorting on the two beaches (H = 26.62, P < 0.001), but there was no significant difference in sediment sorting at each *Dotilla* zone area on the two beaches (H = 0.15, ns) or between different areas in the *Dotilla* zone across both beaches (H = 0.88, ns).

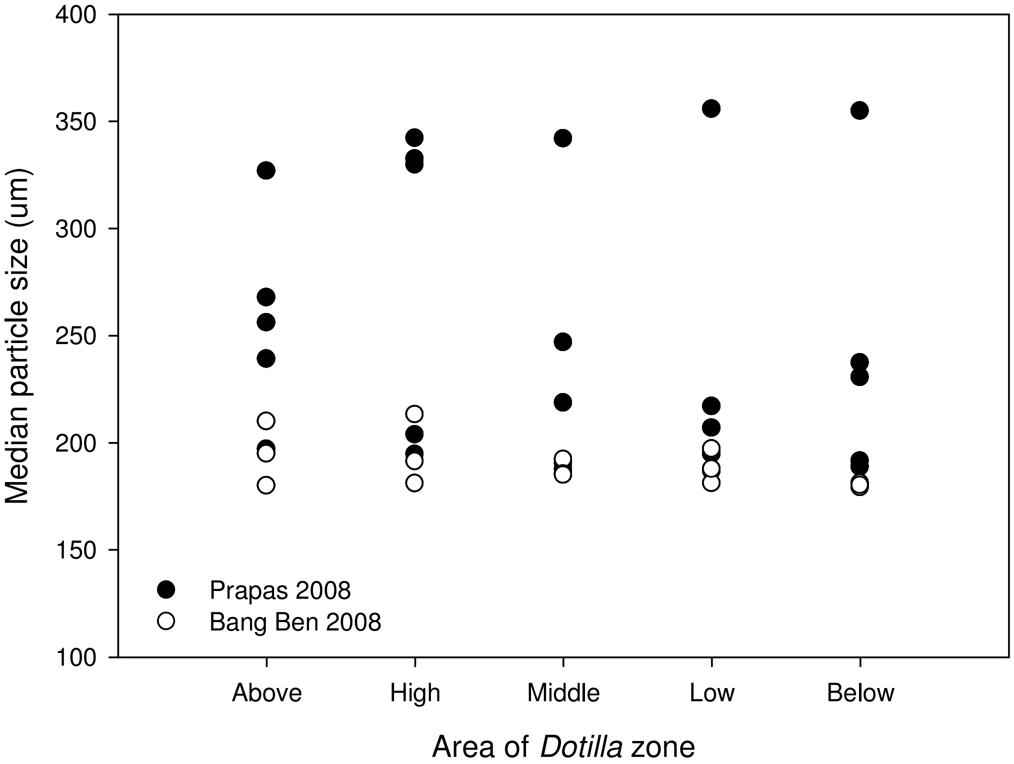


Fig. 3.5. Median sediment particle size (µm) from different areas of the *Dotilla* zone. *n*=8

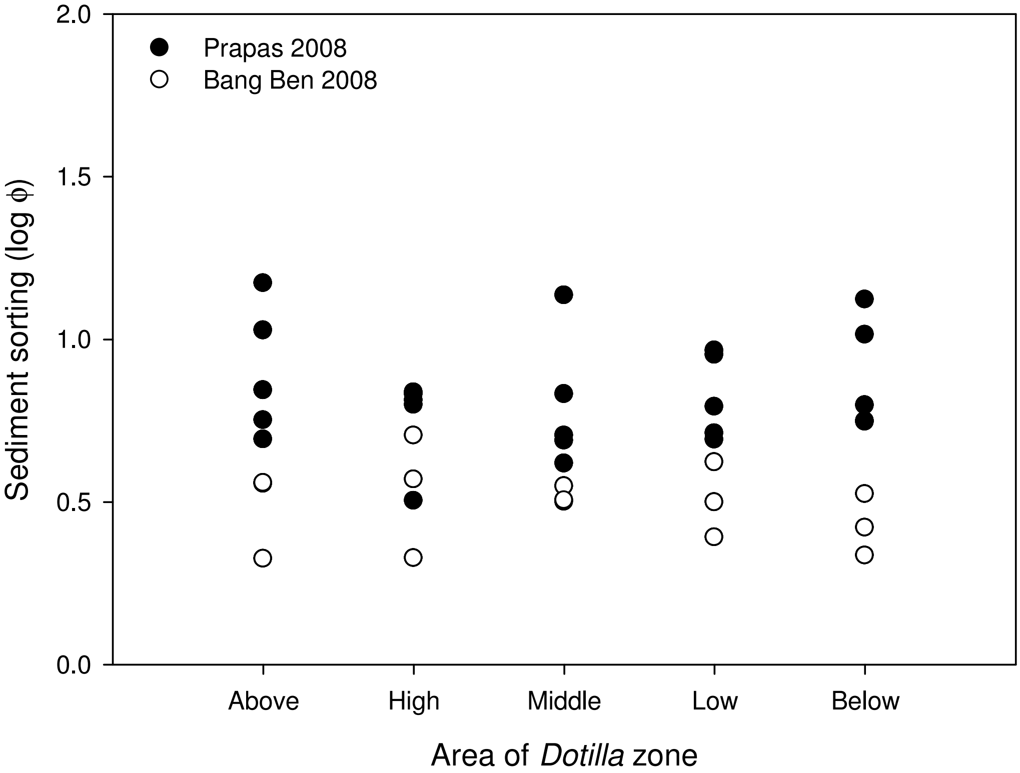


Fig. 3.6. Sediment sorting (log Φ) from different areas of the *Dotilla* zone. *n* = 8

3.3.4. PRINCIPAL COMPONENT ANALYSIS (PCA)

Tables 4.1 and 4.2 summarise the eigenvalues and eigenvectors from the PCA. The eigenvalues show how much of the observed variation between the samples can be explained by each principal component (PC). The eigenvectors show which variables contributed the most in each PC, with larger numbers (irrespective of sign) indicating a higher contribution. The first two PCs were responsible for the majority of the variation between the samples, with PC1 and PC2 representing 82.7 % and 16.9 % of the variation respectively. PC1 was dominated by the influence of the total water content of the sediment, whilst the total organic content of the surface sediment was the most important vector in PC2. On a plot of PC 1 against PC 2 (Fig. 3.7) the samples from above the *Dotilla* zone clustered together, and there was some grouping with the high- and middle-zone samples, although they were not as clearly defined as the above-zone samples. The low- and below-zone samples appeared to be very poorly separated from each other, suggesting a variable not examined in the PCA was responsible for defining the lower limit of the *Dotilla* zone.

Table 3.1. Eigenvalues from Principal Component Analysis

PC	Eigenvalues	% Variation	% Cumulative Variation
1	26	82.7	82.7
2	5.32	16.9	99.6
3	0.103	0.3	99.9
4	0.0376	0.1	100

Table 3.2. Eigenvectors from Principal Component Analysis

Variable	PC1	PC2	PC3	PC4
MDΦ	0.018	-0.028	0.925	0.380
QDΦ	-0.004	0.019	-0.379	0.925
Water content	0.940	-0.340	-0.029	-0.001
Organic content	0.341	0.940	0.025	-0.008

The total water content of the sediment dominated the separation of the samples by their relative position within the *Dotilla* zone (i.e. above, high, middle, low and below). There was a large amount of variation in the total surface organic content of the sediment, which differentiated between samples from within the same section of the *Dotilla* zone, rather than between the different areas of the *Dotilla* zone. Sediment grain size and sorting represented very little of the observed variation in the zonation of *Dotilla intermedia*.

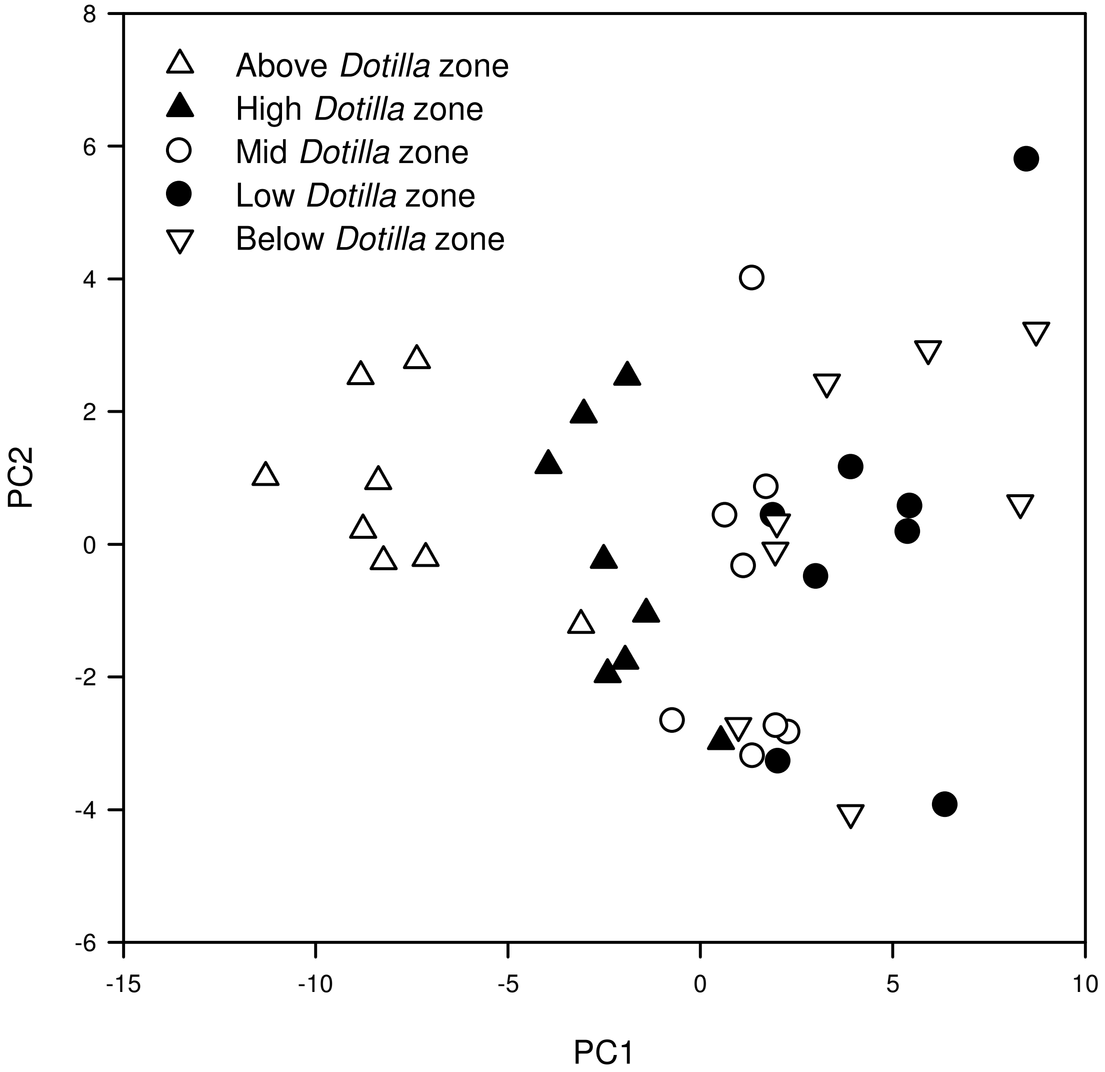


Fig. 3.7. Principal component (PC) 1 vs. PC 2 from Prapas beach and Bang Ben, April 2008

3.3.5. POPULATION DENSITY, CRAB SIZE AND FEEDING

The density of *Dotilla intermedia* within each 0.25 m^2 quadrat had a close relationship with the mean size of the feeding area of the crabs. As density increased, the size of the mean feeding area decreased (Fig. 3.8). The upper limit of the mean feeding area followed an inverse power curve which designated a maximum size to the feeding area at a given density of *D. intermedia* (maximum mean feeding area (cm^2) = 425 x density crabs $^{-0.695}$). Thus at a density of 100 crabs 0.25 m^{-2} each crab would have a mean maximum sized feeding area of 17.3 cm^2, whilst at 50 crabs 0.25 m^{-2} the maximum mean feeding area would be 28 cm^2. The values falling under this curve may have resulted from sampling proceeding before the crabs had had enough time after tidal emersion to feed sufficiently to reach the maximum extent of their feeding area. There was a significant relationship between the log mean *CW* and the log mean feeding area within a quadrat (Pearson Correlation Coefficient = 0.713; $P < 0.0001$). The size of the mean feeding area increased as the mean *CW* width in a quadrat increased (Fig. 3.9).

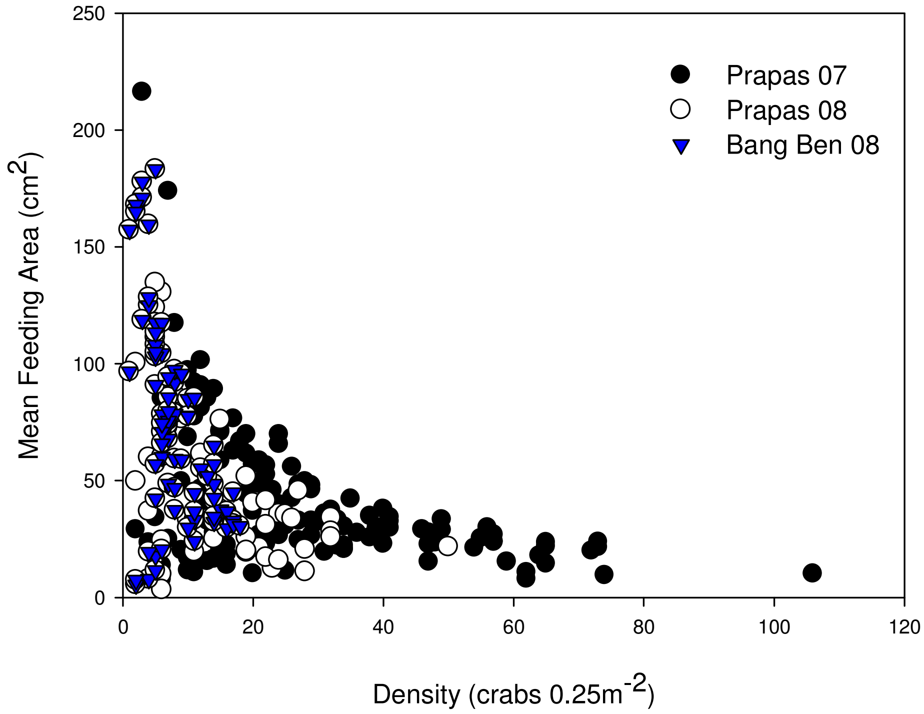


Fig. 3.8. Density of *Dotilla intermedia* against mean feeding area within a quadrat (0.25m ^{2})

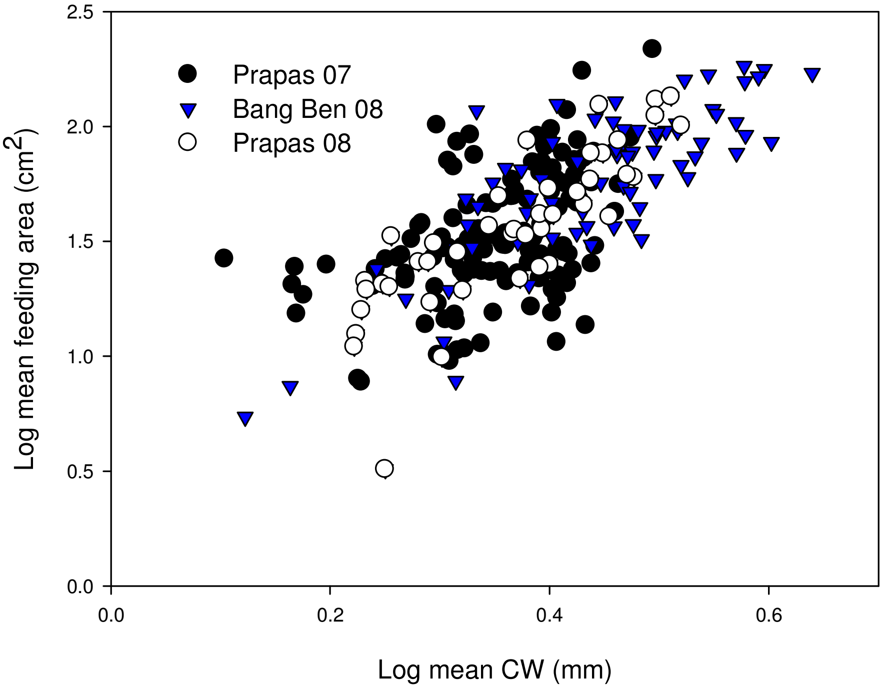


Fig. 3.9. Log mean *Dotilla intermedia* crab carapace width (*CW*) against log mean feeding area within a quadrat (0.25m ^{2})

3.3.6. NEAREST NEIGHBOUR ANALYSIS

At low densities, there was a range of spatial patterns shown, from random to regular distributions. As density increased, the range in the *R*-value decreased, with distribution patterns becoming less regular and more random. By an abundance of around 10 individuals 0.25 m^{-2} the *R*-values had stabilised at around 1.3 - 1.5, representing a distribution that showed a trend that tends towards regular spacing within each quadrat, as opposed to aggregation. These trends were common from both beaches in 2008, and at Prapas beach in both 2007 and 2008 (Fig. 3.10).

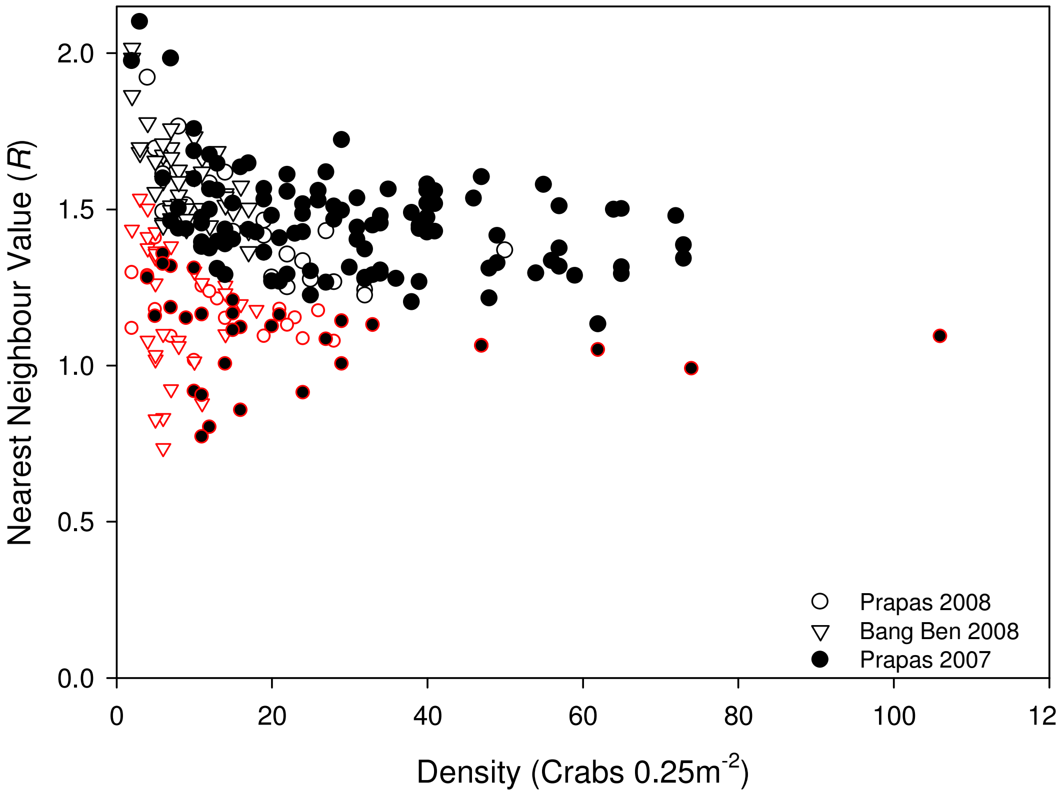


Fig. 3.10. Density of *Dotilla intermedia* per quadrat (0.25m ^{2}) against nearest neighbour *R*-value. Symbols with a red outline failed the *z*-statistic test; the spatial distributions in these quadrats were not significantly different from a random distribution

3.3.7. POPULATION SIZE DISTRIBUTION

The population size distribution for *Dotilla intermedia* can be seen for Prapas beach in 2007 and 2008, and for Bang Ben in 2008 in Figure 3.11. Kolmogorov-Smirnov tests showed that there were significant differences between the population size structure of Prapas beach in 2007 and 2008 ($P < 0.001$), and between Bang Ben 2008 and Prapas beach in both 2007 and 2008 ($P < 0.001$). Crabs with *CW* less than 2 mm numerically dominated the population on Prapas beach in both 2007 and 2008. The numbers of crabs in each *CW* size class declined as *CW* increased on Prapas in 2007. Although there appears to be peak in the number of crabs with a *CW* between 2.6 and 2.9 mm in 2008 on Prapas, the percentage values (~ 17%) for this size class are very similar to Prapas 2007. Lower numbers of crabs were found with *CW* ranging from 2.0 - 2.6 mm on Prapas 2008, and there were higher percentages of very small crabs (less than 2 mm *CW*) and slight increases in the percentage of crabs with *CW* greater than 2.9mm compared to 2007. Bang Ben showed a similar pattern to Prapas 2008, but without the very high percentages of crabs with *CW* 2 mm or smaller. There were higher percentages of crabs present in all other *CW* size classes on Bang Ben, and the largest crabs measured were found here.

3.3.8. SEX RATIO AND SIZE

Table 3.3 shows the number of male and female crabs collected from Prapas beach, and their median *CW*. There was no significant deviation from a 1:1 sex ratio (χ^2 = 1.21; ns). There was a significant difference in the median size of the *CW* male and female crabs (Mann Whitney $U = 11122.50$, $P > 0.0001$).

Table 3.3. Numbers of male and female *Dotilla intermedia* from Prapas beach and their respective median *CW*

	Male	**Female**
n	143	125
Median *CW* (mm)	3.4	3.1

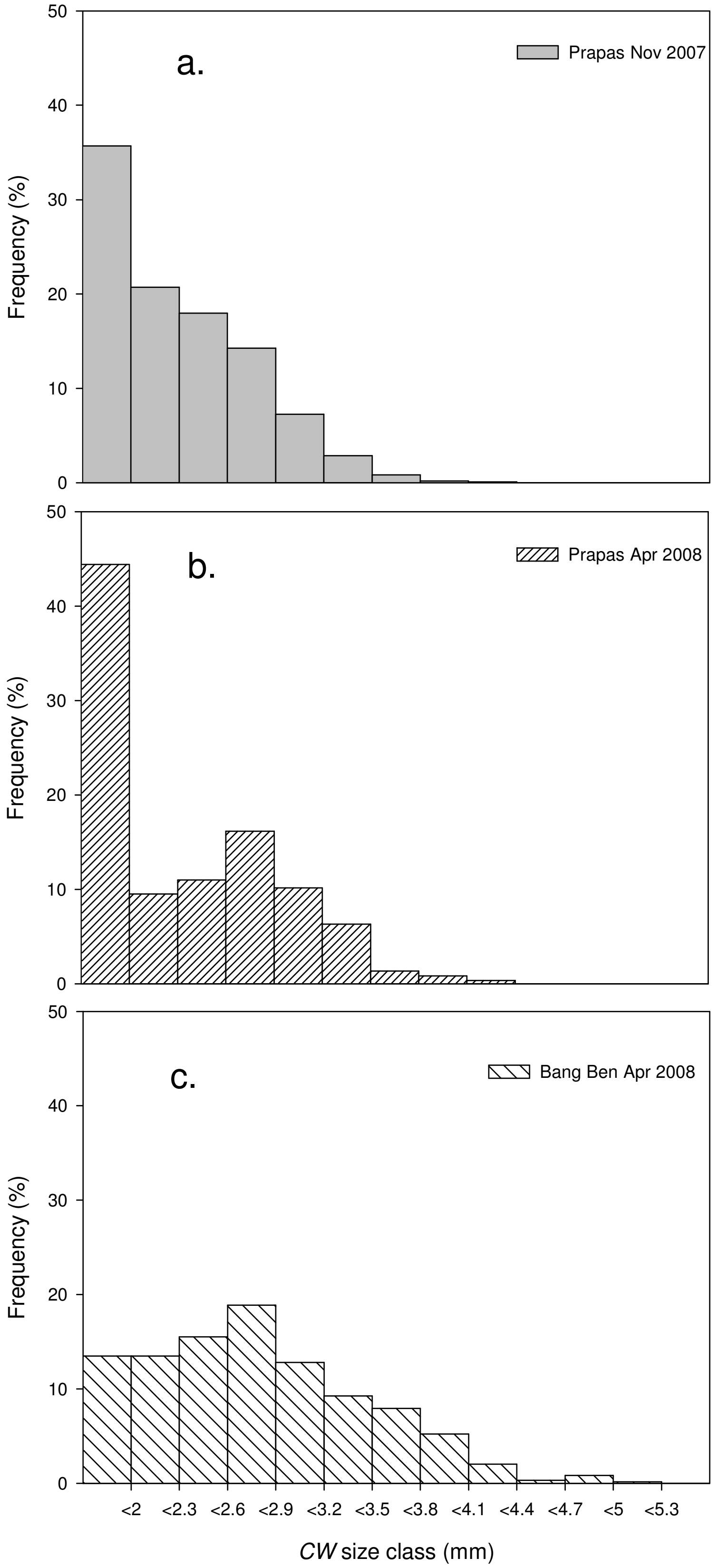


Fig. 3.11. Population size structure for *Dotilla intermedia.* (a) Prapas beach, November 2007 (*n* = 3863); (b) Prapas beach, April 2008 (*n* = 601); (c) and Bang Ben, April 2008 (*n* = 593)

3.3.9. SIZE RANGE IN *DOTILLA*

Dotilla carapace width (*CW*) was measured for all the museum collection material examined (see Chapter 2), and combined with data gathered for *D. intermedia* in this study (Fig. 3.12). The *CW* size ranges for the *Dotilla* species from museum collections were limited by the specimens available and do not accurately represent the range of *CW* that would be seen in natural populations. The collection material is probably skewed towards larger individuals, so the mean, median and minimum *CW* values for each of these species may not be accurate. However, the maximum *CW* should be a good estimate for the largest size reached by individuals of each species. There is a large range in *CW* within certain species of *Dotilla*, especially *D. sulcata*, *D. fenestrata* and *D. myctiroides*. Compared to the other *Dotilla* species, *D. intermedia* is one of the smaller species within the genus. The maximum *CW* measured for the museum collection material of *D. intermedia* is up to 2 mm greater than the largest *D. intermedia* individuals from the Laem Son.

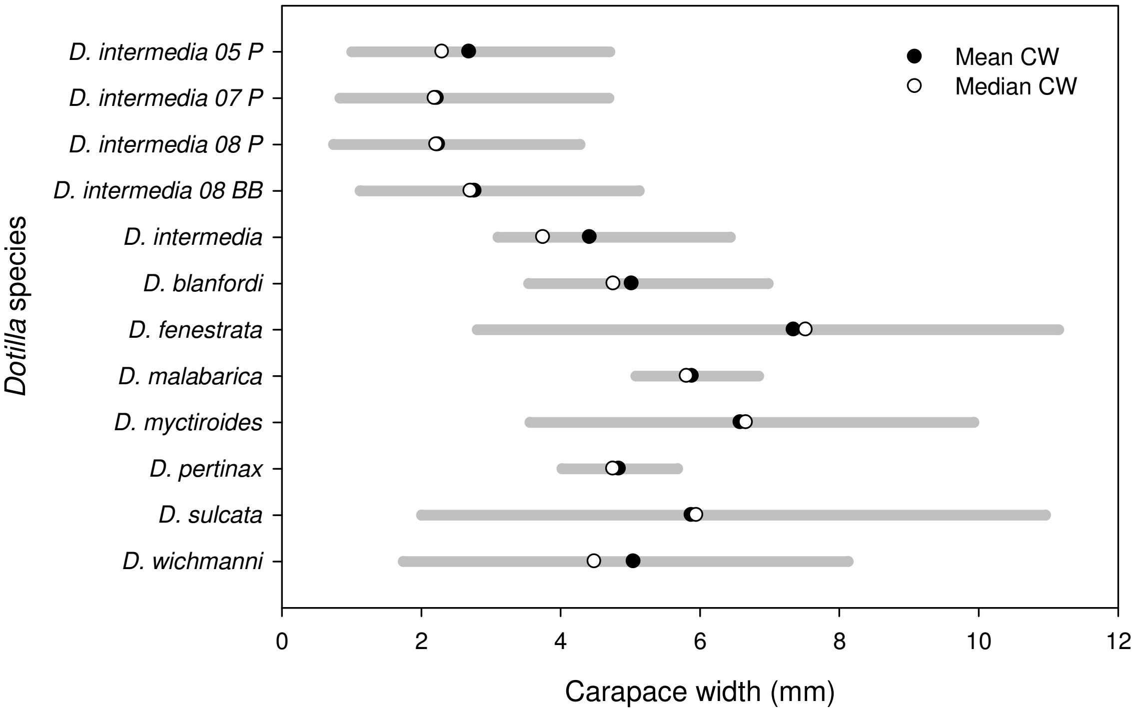


Fig. 3.12. Carapace width (*CW*) for species of *Dotilla*, showing mean (closed circles), median (open circles), and minimum to maximum size range (grey bar). Data includes measurements made on *D. intermedia* on Prapas beach (P) from three years (2005, 2007 & 2008), and from Bang Ben (BB) in 2008. All other species measurements were made from museum collection material

3.3.10. SIZE SEGREGATION

Figure 3.13 shows the beach profiles for Prapas 2007, Prapas 2008 and Bang Ben 2008, along with crab *CW* size-frequency distributions for each area of the *Dotilla* zone (upper, middle and lower). Each beach profile represents the mean of all transects from that beach. Kolmogorov-Smirnov tests showed significant differences ($P < 0.001$) between the size frequency distributions of *Dotilla intermedia* from the upper, middle and low shore areas on each beach. The size distribution in the upper area was skewed towards crabs with a large *CW*, whilst the low shore area was characterised by a distribution skewed towards smaller *CW*. The middle area demonstrated a normal distribution in crab *CW* size classes. Table 3.4 shows the mean and median crab *CW* at each area within the *Dotilla* zone for the three beaches.

Table 3.4. Mean and median crab carapace width (mm) for different areas within the *Dotilla* zone on Prapas beach 2007, Prapas beach 2008 and Bang Ben 2008

	Prapas 2007		**Prapas 2008**		**Bang Ben 2008**	
Area of *Dotilla* zone	**Mean**	**Median**	**Mean**	**Median**	**Mean**	**Median**
Upper	2.43	2.46	2.83	2.85	3.25	3.23
Middle	2.23	2.19	2.21	2.17	2.89	2.84
Lower	1.98	1.90	2.06	1.85	2.34	2.31

There were also significant differences ($P < 0.001$) between the size-frequency distributions of the same shore areas from the different beaches. There was a higher percentage of larger size classes at all shore heights in Prapas 2007 compared to Prapas 2008, whilst Bang Ben was occupied by larger crabs still.

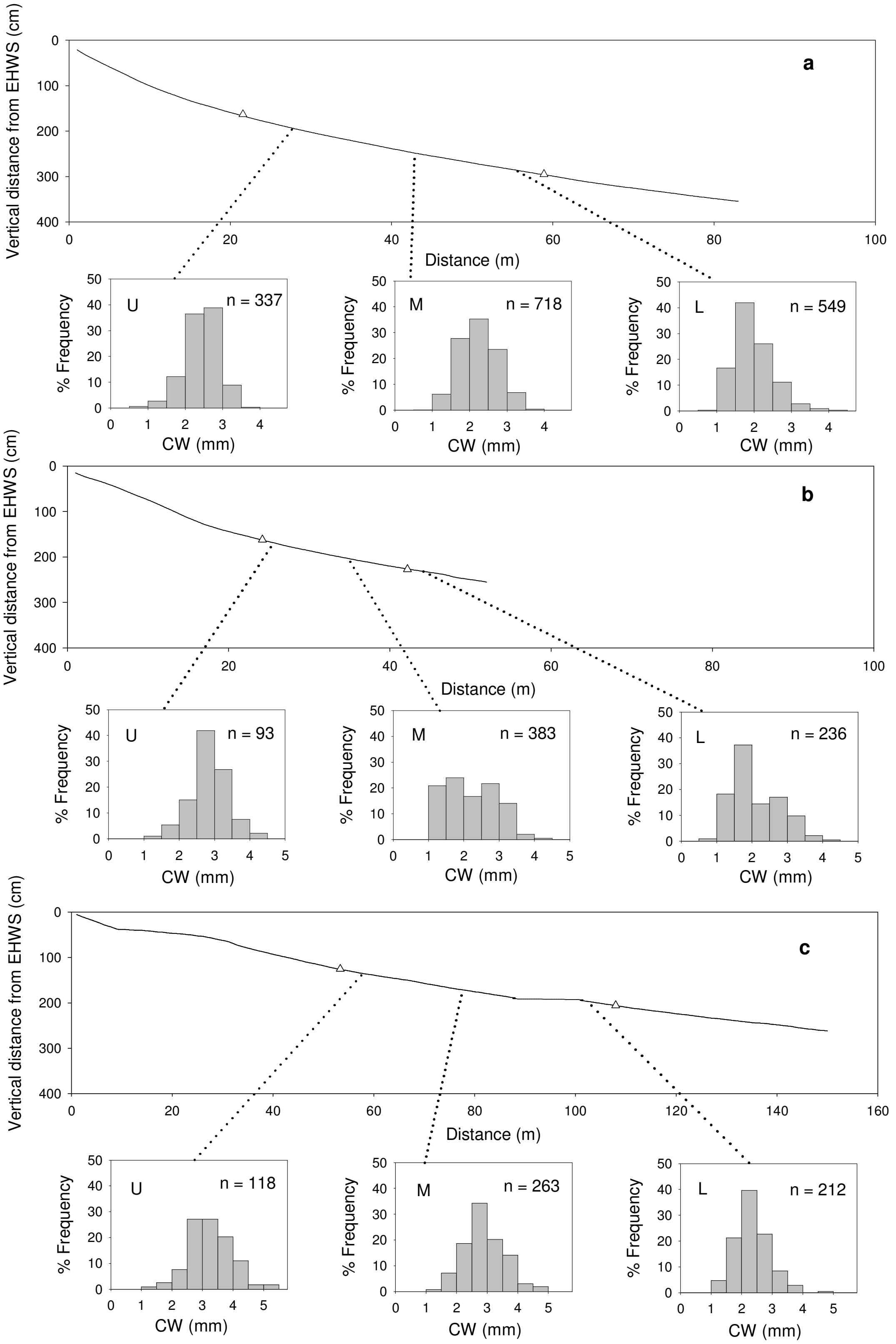


Fig. 3.13. *Dotilla intermedia* carapace width (*CW*) size-class frequency at different beach zones. a Prapas 2007; b Prapas 2008; c Bang Ben 2008. Triangles denote boundaries of *Dotilla* zone on each beach. Histograms show *CW* distribution in three areas of the *Dotilla* zone on each beach: upper (U), middle (M) and lower (L). Beach profiles are means of all transects for that beach

3.4. DISCUSSION

3.4.1. ZONATION IN *DOTILLA INTERMEDIA*

The heights of the upper and lower boundaries of the *Dotilla* zone were closely related to the gradient of the beach. However, beach gradient does not directly govern crab zonation itself, instead acting as a proxy for other physical factors. The upper boundary of the *Dotilla* zone was defined by the total water content of the sediment. The physical structure of sandy beaches depends on the interaction between sand, tide and waves (Short 1996). An alteration in one of these factors will result in a change to the slope of the beach. During storms for example, the increased wave height and tidal range will act to flatten and widen beaches, whilst calm conditions have the opposite effect (McLachlan & Brown 2006). Thus the absolute height of the upper limit of the *Dotilla* zone is not constant and will move if the beach slope changes.

Dotilla intermedia were only found in sediments with water content of 15 % or higher. One of the distinguishing features of *Dotilla* crabs is the presence of a band of setae on the fourth abdominal segment which function in water uptake. The abdomen can be lowered, pressing the setae into the sand allowing the uptake of interstitial water (Hartnoll 1973). The presence of such an adaptation indicates the evolutionary importance of water to *Dotilla*. Both *Scopimera* spp. and *Dotilla* spp. possess membranous disks, known as 'tympana' or gas windows, on the meral segments of their legs. Tympana are respiratory surfaces that function in aerial gas exchange (Maitland 1986). The tympana in *Scopimera* are thicker than in *Dotilla*, allowing *Dotilla* to have relatively greater respiratory efficiency but at the cost of poorer water/ion retention (Matsumasa *et al.* 2001). *Dotilla* may be restricted to sediments with greater water content to offset the increased rate of water loss through the tympana, whereas *Scopimera* are better adapted to survive higher up the beach where the sediment water content is lower. The increased respiratory efficiency of *Dotilla*, combined with the presence of the water uptake setae to balance water loss, may allow the genus to spend more time on the surface without returning to their burrows compared to *Scopimera*. This would maximise the time available between emersion and immersion by the tide, thus permitting *Dotilla* to occupy areas lower down the beach than *Scopimera*. Examining a single species like *Dotilla intermedia* highlights the importance of species physiology on community structure, which may be lost when examining sandy beach ecology only at the community level.

Mean surface sediment organic content decreased further up the shore, although there was no significant difference between the organic content found in areas occupied by *Dotilla intermedia* and those measured in the area above the *Dotilla* zone. The presence of *Scopimera* sp., which feed and burrow in an identical manner to *Dotilla* (Zimmer-Faust 1987), inhabiting the high shore above the *Dotilla* zone supports the suggestion that the surface organic content of the sediment is not a factor controlling the upper limit to the *D. intermedia* distribution.

The upper limit to the *Dotilla* zone could potentially be defined by competition with *Scopimera*. However, no direct interactions were observed between the two species during the course of this investigation (Allen pers. obs.). The very low densities that both *Scopimera* sp. and *D. intermedia* occurred at on the high shore make competition for space or resources unlikely. Nevertheless, further work involving exclusion pens and transplanting *D. intermedia* into the *Scopimera* zone and vice versa would clarify the role competition plays, if any, in delimiting the upper boundary of the *Dotilla* zone.

The lower boundary of the *Dotilla* zone was governed by the tide. The activities that *D. intermedia* can perform are limited by the time between each successive immersion by the tide. *D. fenestrata* has been shown to occupy an area on the beach that is exposed between successive tidal immersions for a sufficient time period to allow for feeding (Hartnoll 1973; Fishelson 1983), in addition to engaging in other behaviours such as burrow maintenance, mating and territorial displays (Gherardi *et al.* 1999). It is likely that similar limitations apply to *D. intermedia*, requiring individuals to live a certain distance from the low water mark. Using the tide tables for the region, the vertical height from the lower edge of the *Dotilla* zone to the low water mark was calculated for each transect, and compared to the length of time between tidal immersions. The lower limit of the *Dotilla* zone was exposed for approximately 4-5 hours between successive immersions. This suggests that the lower edge of the *Dotilla* zone will shift according to the spring-neap tidal cycle. Such semi-lunar rhythms have been reported previously in both molluscs and crustaceans (McLachlan 1987).

Bradshaw & Scoffin (1999) concluded that the main factor governing the distribution of *Dotilla myctiroides* in Tang Khen Bay, Phuket, was the duration of the daytime exposure (i.e. the amount of time the crabs were emersed between tides). This agrees with the hypothesis that the length of emersion controlled the low shore boundary of *D. intermedia*, but not that the high shore boundary was controlled by the water content of the sediment. However, *D. myctiroides* occupied the top of sand ripples in the bay, with pools of water present on either side of each ripple. This potentially kept the water content of the sediment sufficiently high that it did not become a limiting factor, leaving the period of tidal emersion as the driving factor in the species distribution.

Inhabiting an area too close to the low water mark imposes other physical environmental restrictions for *Dotilla intermedia*. The development of suction *s* (i.e. negative pore water pressure relative to atmospheric pressure) was an essential threshold condition for the creation of burrows by *Scopimera globosa*, with burrowing performance depending strongly on the state of *s* in association with the groundwater level (Sassa & Watabe 2008). In low-shore areas, the value of *s* was not favourable for the construction of the semi-permanent cylindrical burrows found higher on the shore, thus resulting in the formation of 'igloo' style burrows instead. A number of *Dotilla* species have been reported to construct two different forms of burrow – simple vertical cylindrical tubes, and 'igloos'. An igloo is formed by a crab rotating in the sand beneath the surface and forming a wall of sand pellets around and over itself, and is built in semi-fluid unstable sand (Takeda *et al.* 1996). A higher percentage of igloos were seen on the low shore than higher on the beach (Allen pers. obs.).

It is not known whether the zonation patterns demonstrated by other species of *Dotilla* are constrained by the same factors identified during this study for *D. intermedia.* It would be interesting to repeat the methods employed during the ecological study of *D. intermedia* for other *Dotilla* species to examine whether beach slope, sediment water content and the length of exposure are equally important controls on sandy shore zonation throughout the genus.

3.4.2. SEDIMENT GRAIN SIZE AND SORTING

Sediment grain size and sorting were not responsible for controlling where *Dotilla intermedia* were found on the beach. There was a large range in the measured average particle size at each area within the *Dotilla* zone. Prapas beach was characterised by moderately sorted sediments and a wide range of grain sizes. In comparison Bang Ben had well-sorted sediments with finer average grain size. The difference in the sediment characteristics of the two beaches suggests that Bang Ben was more sheltered than Prapas.

Sediment particle size was not a critical factor in defining the zonation of *D. myctiroides* from Porto Novo in India, with individuals found in sediment with the silt-clay fraction ranging from 0.3 % to 30 % (McIntyre 1968). However it is likely that sediment grain size would be the ultimate limiting factor in dictating which beaches would be suitable for *D. intermedia* to inhabit. In estuarine areas of the Laem Son National Park where sediment grain size was smaller, *D. intermedia* was replaced by *D. myctiroides.* Sediment grains need to be of a certain size in order to be sifted through the mouthparts of *Dotilla* spp., and different species favour different sized particles depending on the nature of their spoon-tipped setae (Vogel 1984). The division of habitats by sediment grain size has also been demonstrated in *Uca* (e.g. Robertson & Newell 1982; Lim 2005). Particles that are too big cannot be fed upon. In addition, beaches need to have sediment types suitable for the construction of burrows (Ansell 1988). To define the limits of sediment particle sizes inhabited by *D. intermedia* further investigation would be required, covering more beaches with a wider range of grain sizes.

Surveys were made of other beaches in the region and around Phuket. Those that had coarse sand were not occupied by any *Dotilla* crabs, although those with fine sand were inhabited by *Dotilla myctiroides* (CJ Allen & GLJ Paterson pers. obs.). These observations would match the predictions of current hypotheses concerning how the morphodynamic state of the beach affects species distributions. As beaches move from dissipative in character (i.e. fine sand, flat slopes, low substrate penetrability and high water content) to reflective (i.e. coarse sand, short swash periods, steep slopes, high substrate penetrability, low organic matter and low sediment water content) species are excluded due to the harsher swash climate (Swash Exclusion Hypothesis; McArdle & McLachlan 1991, 1992) and the coarser sands making the environment more inhospitable (Habitat Harshness Hypothesis; Defeo *et al*. 2001, 2003).

3.4.3. DENSITY PATTERNS IN *DOTILLA INTERMEDIA*

The highest observed densities of *Dotilla intermedia* from this study were from Prapas beach in 2007, which ranged from 12 to 424 individuals m^{-2}, with an average of 102 individuals m^{-2}. These density counts are lower than those reported for other species, with *D. fenestrata* having densities of 48 to 564 individuals m^{-2} (Hartnoll 1973) and *D. myctiroides* 178 - 501 individuals m^{-2} (Halis & Yaziz 1982). The only recorded densities for *D. intermedia* are from McIntyre (1968), who reported an average of two crabs per three m^{2} at Porto Novo in India. These values are very low compared to those found in this study, and probably do not represent a well established population of *D. intermedia*. Although there is no information on exact densities, Kemp (1919) was able to examine a collection of more than 300 individuals of *D. intermedia* from Chandipur in India, suggesting that the species occurred in relatively high numbers at this location.

The size of the feeding area around each burrow was closely related to crab size and the density of the population. McIntyre (1968) observed that the length of the longest feeding track from the burrow in *Dotilla myctiroides* was crucially important in determining the density of the population. As crab size increased, the size of the feeding area also increased, meaning that large crabs lived at lower densities than small crabs. This would lead to intraspecific competition for space within the *Dotilla* zone. The random to regular distribution indicated by the nearest neighbour analysis suggests that individuals were fitting their feeding areas around one another, probably mediated by territorial interactions between individuals (see Schöne (1968) for descriptions of agonistic displays in *Dotilla*). The data shows that the distance crabs travelled from their burrow when feeding was a function of crab size rather than being a set distance consistent in all individuals, which might be expected if there was a mechanical limitation on the distance travelled from the burrow, such as necessity for water or threat from predation.

Dotilla intermedia fed in roughly circular zones. Assuming their feeding territories do not overlap, as crab density increases it would be expected that the nearest neighbour values would begin to demonstrate a trend towards a more regular distribution, as the circular feeding zones arrange around one another to maximise the available space. However, this was not shown by the data. The range of crab sizes that were found at all densities may mask this trend, with a variety of different sized circular feeding areas increasing the number of possible arrangements in a given space, leading to a variation in nearest neighbour distances, resulting in a more random than regular spatial distribution. At low densities, there was an absence of many crabs that would force a more regular arrangement of feeding areas. Thus individuals were less restricted in the spatial distribution of their burrows, leading to the large range in the *R*-values observed at low densities.

Nutrients in the sediment are replenished by deposition of organic material by the tide, which varies in an unpredictable manner both temporally and spatially, creating patchiness in sediment organic richness (Gherardi *et al.* 1999). Variation in the organic content of the sediment did not appear to have any effect on the distribution of *Dotilla intermedia* on the shore. The nearest neighbour data indicated that crabs tended towards either a random or regular distribution across the shore (depending on the density of individuals within the quadrat). If food patches were small (i.e. smaller than the size of the quadrat), then it might be expected that the crabs would demonstrate an aggregated distribution, with individuals grouping in the same area to exploit a high organic content food patch. However, if food patches are large (i.e. greater than the size of the quadrat), then the spatial distribution of the crabs within a quadrat would not be expected to be altered.

3.4.4. SIZE SEGREGATION

There was a difference in the pattern of distribution between large and small crabs within the *Dotilla* zone. The area close to the low water was dominated by smaller crabs, with larger crabs occurring mainly from the mid-shore upwards. This was more pronounced on Bang Ben than on Prapas beach. Size segregation may possibly act as a process to partition space and avoid intraspecific competition for food and space, with each population component having a differential capacity to select a desirable microhabitat (Defeo & McLachlan 2005). The Habitat Favourability Hypothesis (Caddy & Defeo 2003) suggests that under optimal conditions, the centre of a species' range may be occupied by larger and more dominant intraspecific competitors for food and space, whereas small individuals (recruits) may be displaced towards suboptimal conditions on one or both extremes of the species distribution range. *Dotilla intermedia* appears to follow the description of the Habitat Favourability Hypothesis, with the large crabs occupying the central and high shore areas of the species distribution, and the smaller crabs displaced towards one extreme, the low shore. The differences in the size-frequency distributions observed between Prapas in 2007 and 2008 could have resulted from the growing on of some individuals over time, leading to the higher percentages of the larger size classes in 2008. The flatter beach profile of Bang Ben led to a wider *Dotilla* zone, providing more space for crabs to feed and attain larger body sizes than on Prapas.

The low water area may be suboptimal for larger crabs as the short exposure time would result in less time to feed. Assuming crabs feed at the same rate regardless of size, larger crabs would need to feed in a larger area, requiring more time. This may not be an issue for smaller crabs, who may be able to gather enough energy from feeding for their metabolic requirements from the 4-5 hours before low shore immersion. In order to establish whether crabs feed at the same rate irrespective of size, it would be necessary to conduct further experiments examining the amount of sediment fed on by different-sized crabs over a set length of time.

The geophysical conditions of the low shore may make the construction and maintenance of semi-permanent vertical burrows more difficult. The vertical burrows of *Dotilla myctiroides* are used in their mating behaviour, with male crabs capturing females and pushing them into their burrows (Tweedie 1950). The inability to construct vertical burrows in the low shore may prevent mating from occurring there, thus restricting mature crabs to areas higher up the beach. Further work using histology to microscopically assess the gonad development of small crabs from the low shore would reveal whether mating was occurring in this area of the beach or not.

The size distribution patterns with respect to shore height observed in this study for *Dotilla intermedia* have also been observed in other species of *Dotilla* (Hartnoll 1973; Dray & Paula 1998; Flores *et al.* 2005), as well as other crustacean species. *Uca tangeri* has been shown to prefer the higher tidal zone with the increased exposure time allowing for the construction of deeper burrows that also provide better predation protection (Klaassen & Ens 1993). Larger individuals have also been shown to have a tendency to occupy higher tidal levels in *Scopimera* *inflata* (Fielder 1971), *Sesarma ricordi,* and *Pachygrapsus gracilis* (Warner 1969). Fielder (1971) suggested that the reliance of *Scopimera inflata* on groundwater may be responsible for this size distribution. As the tide recedes, the water table falls, and the crabs are required to deepen their burrows to follow the water table. This effect is greater further up the shore and Fielder hypothesised that only the largest crabs would be physically capable of deepening their holes at the highest parts of the shore. A similar physical limitation may also contribute to the distribution patterns seen in *D. intermedia*, although the water uptake setae on the abdomen of *Dotilla* may negate the necessity of burrowing to great depths. Hartnoll (1973) found that the burrows of *D. fenestrata* did not penetrate below the water table and thus did not provide a source of free water to the crab, suggesting that the crabs instead relied on interstitial water collected using their water uptake setae.

Hartnoll (1973) suggested that the adult *Dotilla fenestrata* preferred lower stations where particle size was smaller, with associated higher organic and water content. There was very little variation in the observed sediment grain size from the present study, perhaps eliminating this effect in structuring the distribution of the larger adult crabs on the beach. Although the surface organic content did increase towards the low water mark, it was highly variable across the whole *Dotilla* zone.

The presence of small crabs in all areas of the beach may be due to juveniles settling into any patches available on the shore. Larger crabs avoid the lower shore, which may result in high numbers of small crabs settling there. Although small crabs were observed across the entire *Dotilla* zone on Prapas, there tended to be fewer small crabs in the high shore region. The survival of the small crabs on different parts of the beach is unknown, although it is hypothesised to be lower further up the beach, due to their reduced ability to cope with the longer levels of exposure in this region (Pellegrino 1984). Warner (1969) correlated the presence of larger individuals of *Sesarma ricordi* and *Pachygrapsus gracilis* at higher tidal levels to the fact that larger crabs are not subject to the same degree of desiccation as smaller crabs. If time had been available in the present study, small crabs could have been transplanted into the high shore area, and monitored over a period of time. The fitness of the crabs would be assessed using a test such as self-righting time to see whether there were any detrimental effects from living in a high shore area as opposed to lower down the beach.

3.4.5. POPULATION SIZE STRUCTURE AND SEX RATIO

The population size-frequency distributions were generally unimodal (apart from Prapas 2008) and not normally distributed. Poisson-like distributions have been found in fiddler crabs due to seasonal mortality pulses in harsh environmental conditions (Thurman 1985). The absences of larger individuals in the populations were shown by the right-skewed distributions, which generally indicate a higher mortality in the larger size classes (Litulo *et al.* 2005). Unimodal distributions are common in tropical crustaceans, suggesting stable populations with continuous recruitment and constant mortality rates (Diaz & Conde 1989).

The population size structure for *Dotilla intermedia* differed on Prapas beach between November 2007 and April 2008. There was a reduction in the number of small crabs (2 - 2.6 mm *CW*), which could have resulted from selective predation or a disturbance event that eliminated crabs of this size. The higher percentages of juvenile crabs (<2mm *CW*) found in 2008 might indicate a recruitment event between the two sampling periods, with juveniles occupying the spaces that would have been occupied by the small crabs. Slightly higher percentages of larger size classed crabs in 2008 could indicate the growing on of individuals from 2007.

The presence of larger size classes on Bang Ben compared to Prapas beach in 2008 suggested that Bang Ben was a more favourable place for *Dotilla intermedia*. The beach slope was flatter on Bang Ben, a characteristic typical of more sheltered dissipative shores (Short 1996). The sediment data indicates that Bang Ben had well sorted fine sediment, as opposed to the moderately sorted sediment of a variety of grain sizes found at Prapas, which supports the idea than Bang Ben was the more sheltered of the two beaches. The Habitat Harshness Hypothesis (Defeo *et al.* 2001, 2003) postulates that the harsh environment of reflective beaches forces organisms to divert more energy towards maintenance, resulting in lower fecundity, growth, mass and survival. The observed differences in crab sizes between Prapas and Bang Ben offers some support to this hypothesis, with the crabs at Prapas reaching lower sizes due to the harsher environment there compared to Bang Ben. A sheltered shore would be a more physically stable environment, decreasing mortality from disturbance (although the densities of the crabs at Bang Ben were generally lower than on Prapas, the bigger crabs on Bang Ben fed in larger feeding areas). This would leave fewer gaps for juvenile crabs to settle into, which could explain the lower percentages of juvenile crabs at Bang Ben compared to Prapas. The maximum *CW* measured from museum collection material for *D. intermedia* was almost 2 mm greater than the maximum *CW* measured for *D. intermedia* in the Laem Son. The predictions of the Habitat Harshness Hypothesis suggest that the environmental harshness of the beaches in the Laem Son could be limiting the maximum size reached by individuals of *D. intermedia* in this locality. If populations of *Dotilla intermedia* can be found on beaches that are more reflective or more dissipative in character, then the impact of beach morphology on *D. intermedia* can be investigated in more detail.

Deviations from a 1:1 sex ratio are widespread in marine crustaceans, resulting from sex reversal or differential migration, mortality and growth rate between the sexes (Wenner 1972). However, the overall sex ratio for *Dotilla intermedia* did not differ significantly from 1:1. This agrees with data collected on other species of *Dotilla*. Although *D. myctiroides* was originally reported to show a sex ratio that deviated from 1:1 (Halis & Yaziz 1982), no statistics were performed on these data. A retrospective Chi-squared test with Yates Correction for Continuity was undertaken on their data, and there was no significant difference from a 1:1 ratio (χ^2 = 0.663; ns). The sex ratio in *D. fenestrata* also does not differ significantly from 1:1 (Litulo *et al*. 2005).

Male crabs showed a larger average body size than female crabs in *Dotilla intermedia*. Similar results have been shown for *D. fenestrata*, with females more abundant at smaller size classes, and males outnumbering females in the largest classes (Hartnoll 1973; Litulo *et al*. 2005). An absence of large female crabs has also been shown in *D. myctiroides* (Halis & Yaziz 1982). Sexual dimorphism is common in brachyuran crabs (e.g. Spivak *et al*. 1996; Henmi 2000), and can develop for a number of reasons. Females may concentrate a relatively large part of their energetic budget into ovary development, which may lead to lower somatic growth when compared to males. Males with larger body sizes have increased chances of winning intra-specific fights and obtaining females for copulation (Christy & Salmon 1984; Dray & Paula 1998).

3.4.6. SANDY BEACH ZONATION SCHEMES

In a wider context, the data provides support for Dahl’s (1952) and Salvat's (1964) generalised schemes of sandy shore zonation. Dahl divided the shore into three zones based on the distribution of characteristic crustacean species, whilst Salvat split the shore into four regions based on changes in sand moisture content. The boundaries of some of Dahl’s and Salvat’s zones roughly coincide, showing a correspondence between physical and biological zones on sandy beaches, as demonstrated by the large influence sediment water content has on the distribution of *Dotilla intermedia.*

Although moisture zones do generally coincide with faunal zones, faunal zones are dynamic and vary temporally. The physical boundary of a species distribution depends on the swash and moisture zones on the shore and not on absolute tide level. Therefore it is better to define such flexible zones on the basis of centres of distribution of characteristic taxa, rather than on sharp boundaries defined by Salvat’s sand moisture levels (McLachlan & Jaramillo 1995). The change in the area occupied by *Dotilla intermedia* on Prapas beach between 2007 and 2008 demonstrates this variability, and why the use of characteristic species is a more robust method of defining beach zones than sand moisture content.

3.5. SUMMARY

The boundaries of the zone inhabited by *Dotilla intermedia* were related to the physical factors imposed by the beach gradient. The high shore limit was defined by the total water content of the sediment, with *D. intermedia* absent from sediment with less than 15 % total water content. The low shore boundary of the zone was set by the temporal constraints of the tidal cycle, with crabs requiring a minimum amount of time (4-5 hours) between tidal immersions to feed. Within the *Dotilla* zone, there were patterns of distribution associated with the size of the crabs: larger crabs lived higher up the beach than smaller crabs. *D. intermedia* showed a 1:1 sex ratio, and displayed sexual asymmetry, with male crabs larger on average than female crabs. The unimodal population size structure suggests that *D. intermedia* had continuous year round recruitment.

The observed distribution of *Dotilla intermedia* is consistent with the predictions of several non-contradictory hypotheses concerning patterns observed on sandy beaches. Size segregation across the beach follows the Habitat Favourability Hypothesis, whilst the absence of any populations on more reflective beaches matches the predictions of the Swash Exclusion Hypothesis and Habitat Harshness Hypothesis. There were differences in crab sizes between the two study beaches. Larger crabs were found on Bang Ben than on Prapas beach. The differences in the sediment grain size, sorting and beach slope indicate than Bang Ben is more dissipative shore than Prapas. One prediction of the Habitat Harshness Hypothesis suggests that as beaches become more reflective, the harsh environment leads to smaller organisms, which appears to be supported by the differences between crab sizes at Bang Ben and Prapas beach. This investigation demonstrates that the study of a single key species can identify some of the physical processes that drive zonation patterns on sandy shores, and assuming that the general fauna show similar patterns to *D. intermedia*, may help to elucidate what underlying factors influence community structure in sandy beach ecosystems.

4. GENETIC VARIATION IN POST-TSUNAMI POPULATIONS OF *DOTILLA INTERMEDIA*

4.1. INTRODUCTION

The tsunami of 26th December 2004 devastated the soft sediment marine communities in south-east Thailand, and the populations of *Dotilla intermedia* in Ranong Province were no exception. Prior to the tsunami, *D. intermedia* occurred in immense numbers, with over 20 million individuals observed on a 5 km stretch of beach (GLJ Paterson pers. comm.). The tsunami overturned large quantities of beach sediment, which probably would have been fatal to the fragile crabs. However, the rate of survival of *Dotilla intermedia* on the tsunami impacted beaches is unknown. By April 2005, individuals were already present on the shore, although at much lower abundances than previously seen in 2000. The small individual size of these crabs suggested that they may have represented new colonisers, as opposed to being survivors of the wave (Kendall *et al.* 2006). Regardless of whether the crabs present in April 2005 represented survivors of the tsunami or post-tsunami colonisers, the populations of *D. intermedia* in the Laem Son had undergone a very heavy mortality (possibly extinction) event that would have acted to create a bottleneck in the genetic diversity of the populations.

The recolonisation of the tsunami-impacted beaches by *Dotilla intermedia* can be used as a case study to assess the impact of an extinction/recolonisation event on the genetic variation of a population. Although the impact of a bottleneck event on the genetic diversity of populations has been extensively modelled mathematically (e.g. Nei *et al.* 1975; Chakraborty & Nei 1977; Sirkkomma 1983; Maruyama & Fuerst 1985), there have been very few temporal studies based on natural populations with which to test the predictions of these models. Nei *et al.* (1975) were the first to model the effects of bottlenecks on population genetic variability. Their model predicted that populations suffer a reduction in genetic variation following a bottleneck event, with the decline in variability linked to the magnitude of the bottlenecking event. Once genetic variability reached a certain minimum level, it then started to increase again. Population growth rates were shown to have a large influence on the effect of the bottleneck. At high population growth rates, the amount of variability lost within a population was less compared to a population with a lower growth rate, even if the magnitude of the bottlenecking event was extreme.

Within the Laem Son National Park, *Dotilla intermedia* are only found on the exposed oceanic sandy beaches that were all heavily impacted by the tsunami. Populations of *D. intermedia* from different beaches would have undergone a similar genetic bottlenecking event, which could potentially lead to an increase in genetic variability between these spatially separate populations over time. Wright (1977) predicted that the process of extinction and recolonisation could enhance the rate of genetic differentiation between local demes. Conversely, extinction and recolonisation may actually be an important source of gene flow between populations that could lower the rate of genetic differentiation compared to a static case with no extinctions (Slatkin 1977, 1985, 1987). Wade & McCauley (1988) mathematically investigated both Wright and Slatkin's interpretations of the effects of extinction and recolonisation on the genetic differentiation of populations. They found that although under certain ecological conditions it was possible for the local population dynamics to diminish genetic differentiation by enhancing gene flow, the general effect of extinction and recolonisation lead to an increase in genetic differentiation. The amount of genetic connectivity between geographically distant populations of *D. intermedia* is not known. The examination of the genetic structure of two populations from different beaches could reveal the effect of the tsunami on the genetic differentiation between these two populations.

The collection and genetic analysis of specimens of *Dotilla intermedia* from Prapas beach at over four years provided a data set with which to test the predictions of the model of Nei *et al*. (1975). Comparisons of specimens collected from Bang Ben and Prapas beach in the same year allowed an examination into the geographical genetic variability between spatially distant populations of *D. intermedia*, which provided insights into the genetic connectivity between the two beaches.

4.2. METHODS

4.2.1. SELECTION OF TECHNIQUES FOR POPULATION GENETICS

There are several different molecular techniques available for use in population genetics. Three of the most widely used techniques are DNA polymorphism, amplified fragment length polymorphisms (AFLPs) and microsatellites.

The AFLP technique is based on the detection of genomic restriction fragments by PCR amplification, and can be used for DNA of any origin and complexity. Fingerprints can be produced without any prior gene sequence knowledge, and the number of fragments detected in a single reaction can be ‘tuned’ by selection of specific primer sets (Vos *et al.* 1995). AFLPs have very low error levels, high replicability, can be performed using minimal amounts of DNA, and can be generated at high speed, making them a time-efficient method (Mueller & Wolfenbarger 1999). AFLPs were probably the most suitable marker for this investigation, but unfortunately the costs associated with using this method were prohibitive.

Microsatellites, also known as simple sequence repeats (SSRs) or tandem repeats, are repeating sequences of 1–6 base pairs of DNA. Microsatellites can be found in both coding and non-coding regions of the DNA genome. The locus containing the microsatellite is often polymorphic because of variation in the number of repeats. Microsatellites are amplified via the polymerase chain reaction (PCR), with the unique sequences flanking the SSRs used to design primers. The use of microsatellites requires either prior knowledge of an organism’s genome, or the construction of a genome library, to allow for screening of SSR regions that can be used to identify suitable areas for PCR amplification (Li *et al*. 2002). There is no genome sequence information currently available for the *Dotilla*, so the construction of a genomic library would be required in order to utilise microsatellites for this study, in addition to identifying and refining suitable primers. Thus the use of microsatellites was deemed to be too time-intensive for this study.

DNA polymorphism uses the PCR reaction to amplify highly variable target regions within the genome. The limitation of DNA polymorphism is that it uses only a small section of an organism’s genome, and that in order to reveal any information about the genetic structure of a population the targeted region needs to be variable. DNA polymorphism has been widely used for crustacean population genetic studies, which have revealed several variable markers suitable for use in this study. DNA polymorphism was the technique chosen to examine the population genetic structure of *Dotilla intermedia* in the Laem Son.

Two mitochondrial genes (COI, 16S) were selected for use in the present study as they have been successfully used in a number of other crustacean population genetic studies (e.g. Sarver *et al.* 1998; Schubart *et al.* 2000; Petersen 2007; Cook *et al.* 2008; Palero *et al.* 2008). With regards to the Ocypodidae, there have been some population level studies using COI within this family of Crustacea (e.g. Kawane *et al.* 2008). ITS-1 has been demonstrated to be a variable nuclear marker suitable for crustacean intraspecific studies (Chu *et al.* 2001), and thus was chosen as an alternative marker in case the mtDNA markers proved to be insufficiently polymorphic within populations of *Dotilla*.

4.2.2. DNA EXTRACTION AND QUANTIFICATION

A number of specimens of *Dotilla intermedia* were selected from material that had been collected from Prapas beach and Bang Ben on four different occasions (Table 4.1). Whole crabs were immediately preserved in 100% molecular grade ethanol until DNA extraction in the UK.

Table 4.1. Summary of *Dotilla intermedia* specimens analysed during the population genetic study

Time Period	Beach	Number specimens analysed
April 2005	Prapas	20
Nov 2006	Prapas	15
Nov 2007	Prapas	15
April 2008	Prapas	19
	Bang Ben	20

DNA extraction was carried out using DNeasy Blood and Tissue Kit (Qiagen), as per the manufacturer’s instructions. The protocol is detailed in full below. All centrifugation steps were carried out at room temperature (15-25 ºC).

- Muscle tissue was removed from the chelipeds and pereiopods of individual crabs, and placed into a 1.5 ml microcentrifuge tube.

- 180 µl Buffer ATL was added to the tube, followed by 20 µl Proteinase K. The tube was vortexed briefly to mix thoroughly, and placed in a shaking incubator at 56 ºC for 12 hours to lyse cell walls.
- After incubation, 200 µl of Buffer AL was added to the microcentrifuge tube and vortexed for 10 seconds, followed by the addition of 200 µl 100% molecular grade ethanol before vortexing the tube again for 10 seconds. The mixture was then pipetted into a DNeasy Mini Spin Column in a 2 ml collection tube and centrifuged at 6,000 x *g* (8,000 rpm) for 1 minute.
- The spin column was removed and placed into a fresh 2 ml collection tube. 500 µl of Buffer AW1 was pipetted into the top of the spin column, and centrifuged at 6,000 x *g* (8,000 rpm) for 1 minute.
- The spin column was removed again and placed into another fresh 2 ml collection tube and 500 µl Buffer AW2 added, and then centrifuged at 20,000 x *g* (14,000 rpm) for 3 minutes.
- The spin column was removed and placed into a final fresh 2 ml collection tube. 100 µl Buffer AE was pipetted directly onto the DNeasy membrane in the spin column, and left to incubate at room temperature for 1 minute, before being centrifuged at 8000 rpm for 1 minute. A further 100 µl of Buffer AE was added to the spin column, left to incubate for 1 minute at room temperature, and centrifuged at 8000 rpm for 1 minute.
- The eluate was removed and put into a 1.5 ml microcentrifuge tube which was then capped and labelled according to the specimen. Extracted DNA was stored in a freezer at -10 ºC.

The extracted template DNA was quantified using a Thermo Fisher Scientific NanoDrop pedestal. The NanoDrop can measure the UV-vis spectra for a range of samples including DNA, RNA, dyes, peptides and cell cultures. The absorbance of a sample at 260 nm represents nucleic acids, whilst absorbance at 280 nm represents proteins, phenol and EDTA. The ratio of the optical density between 260 nm and 280 nm provides a measure of the purity of a sample, with DNA samples having a 260/280 ratio of 1.8 and RNA a ratio of 2.0.

1.5 µl of ultrapure water was used to initialise the NanoDrop, before 1.5 µl of buffer AE was used to blank the instrument. 1.5 µl of template DNA was then loaded on the pedestal and measured. After the measurement was complete, the pedestal was wiped clean ready for the measurement of subsequent samples (see Gallagher & Desjardins (2006) for a full description of NanoDrop protocols).

4.2.3. PCR AMPLIFICATION AND SEQUENCING OF DNA

Three potentially informative markers were chosen for this study - mitochondrial cytochrome *c* oxidase I (COI), mitochondrial large ribosomal subunit (16S) and internal transcribed spacer region 1 (ITS1). Table 4.2 lists the forward and reverse primers used during the polymerase chain reaction (PCR) to amplify each gene region.

For each PCR reaction a volume of template DNA equivalent to 10 ng was added to a 0.2 ml PCR tube containing 'Ready to Go' PCR bead (Amersham Pharmacia). 1 µl of forward and 1 µl of reverse primer (at concentrations of 10 pmol) were also added to the tube, and the total volume was bought up to 25 µl with ultrapure water. Thermocycling reactions were then carried out on a Bio-Rad DNA Engine. Table 4.3 lists the PCR temperature profile cycles used for each marker investigated. Amplified products were size fractionated on a 1 % agarose gel in TAE buffer and stained with 10mg/ml ethidium bromide. Gels were visualised on a UV transilluminator to determine the size of the amplified fragments, and whether they matched the expected amplicon size for each marker (see Table 2.1). Successful PCR products were sent to the DNA sequencing facility at the Natural History Museum, London, for purification and sequencing. The PCR products were purified using a QIAquick PCR purification kit (Qiagen). Sequencing reactions were carried out on the purified products using Fluorescent Dye Terminator Sequencing Kits (Applied Biosystems) run on an Applied Biosystems 377 automated sequencer. Chromatograms of overlapping sequences were assembled and edited using the software Sequencher ver. 4.5. Alignments of the sequences for each marker were manually performed using the software BioEdit v. 7 (Hall 1999).

Table 4.2. Primer pairs used for PCR amplification of DNA, with primer melting temperatures (Tm; ºC) and expected size of amplified fragments

Marker	Primer	Nucleotide Bases (5' - 3')	Tm (ºC)	Amplicon Size	Reference
COI	HCO2198r	TAACTTCAGGGTGACCAAAAAATCA	66.1	700 bp	Pfeiler *et al.* (2005)
	LCO1490f	GGTCAAGAAATCATAAAGATATTGG	60.4		
16S	16Sar	CGCCTGTTTATCAAAAACAT	59.1	600 bp	Schubart *et al.* (2006)
	16Sbr	CCGGTCTGAACTCAGATCACACGT	67.1		
ITS1	SP-1-5'138	CACACCGCCCGTCGCTACTA	69.7	1000 bp	Chu *et al.* (2001)
	SP-1-3'	ATTTAGCTGCGGTCTTCATC	60.8		

Table 4.3. Thermocycling profiles for PCR reactions; all reactions were carried out on a Bio-Rad DNA Engine thermocycler

	COI	16S	ITS1
Initial denaturation	1 min at 94ºC	3 min at 94ºC	90 sec at 94ºC
Amplification Cycle	30 cycles -	40 cycles -	33 cycles -
	1 min at 94ºC	45 sec at 94ºC	20 sec at 94ºC
	1 min at 48ºC	1 min at 48ºC	30 sec at 56.8ºC
	1 min at 72ºC	1 min at 72ºC	30 sec at 72ºC
Final Extension	10 min at 72ºC	10 min at 72ºC	5 min at 72ºC
Reference	Pfeiler *et al.* (2005)	Schubart *et al.* (2006)	Chu *et al.* (2001)

4.2.4. ANAYSIS OF DNA POLYMORPHISMS

A number of different methods were employed to analyse the genetic variation shown by each marker within the population at Prapas beach in 2005, 2006, 2007 & 2008., and between populations sampled from Prapas and Bang Ben in 2008. First, the haplotype (*h*) and nucleotide (π) diversity were calculated using the software DnaSP ver.5 (Librado & Rozas 2009). The *h* statistic (Nei 1987) estimates the probability of randomly drawing two different haplotypes from a sample, whilst π measures the mean number of nucleotide differences between all pairs of haplotypes in a sample (Tajima 1993).

Tajima's *D* (Tajima 1989) was calculated in DnaSP in order to assess whether each population showed any significant deviation from the neutral model of evolution (i.e. the majority of mutations result from random drift rather than active selection; Kimura 1968). The significance of the *D* statistic was established by a simulation method implemented by the programme, where simulation statistics are recomputed from randomly generated samples within a population at equilibrium and repeated for 10000 simulations to obtain the null distribution of the test statistic and its *P* value. Tajima's test uses the infinite-site model without recombination (i.e. all new mutations are novel and different from pre-existing ones), making it appropriate for use with short DNA sequences, and is based on the differences between the number of segregating sites and π. In addition to assessing whether a population differs significantly from the neutral model of evolution, Tajima's *D* can also reveal information about the genetic structure of a population. mtDNA is normally considered selectively neutral, so significant shifts in the values of *D* may be used to infer the effects of historical demographic changes that may still be evident among the haplotypes found within a population. A negative value for *D* indicates an excess of recently derived low frequency mutations, whilst a positive number shows an excess of older intermediate frequency mutations. Demographic expansion following a founder or bottleneck event may result in the accumulation of derived haplotypes of low frequency in a population, resulting in a negative *D* value. Conversely, strong positive values of *D* may result from a reduction of low-frequency mutations caused by either balancing selection for older haplotypes or the admixture of previously separate populations.

Analysis of Molecular Variance (AMOVA) (Excoffier et al. 1992) was implemented using Arlequin ver. 3.1 (Excoffier *et al.* 2005) to test for any significant variations in the genetic composition of populations of *Dotilla* at Prapas beach over time for each of three markers investigated. An AMOVA was also used to examine the amount of variation between *Dotilla* populations collected from Prapas beach in 2008 and Bang Ben in 2008.

Haplotype networks were used to display the temporal changes in the haplotype distribution of the population at Prapas beach. Haplotype networks were constructed for each marker using the statistical parsimony procedure as implemented in TCS ver. 1.21 (Clement *et al.* 2000). A haplotype network comprises of a number of linked circles. Each circle within the network represents a distinct haplotype, and the diameter of the circle is proportional to the frequency at which that haplotype occurs within the population. Bars on the lines represent the number of single-base substitutions between haplotypes. A haplotype network was also constructed to illustrate any haplotype differences between the populations of *Dotilla intermedia* from Prapas beach and Bang Ben in 2008.

4.3. RESULTS

4.3.1. TEMPORAL GENETIC VARIATION AT PRAPAS BEACH

Table 4.4 summarises the genetic diversity found in the population of *Dotilla intermedia* on Prapas beach at each time point sampled for all of the markers investigated. AMOVA tests (Table 4.5) showed a significant proportion of the overall genetic variability was accounted for by the among-year component (COI - $F_{ST} = 0.023$, $P < 0.05$; 16S - $F_{ST} = 0.056$, $P < 0.001$; ITS1 - $F_{ST} = 0.039$, $P < 0.05$). Pairwise comparisons (Table 4.6) show that there was a significant difference between all of time periods for 16S, but for COI the only significant differences were between 2005 & 2008, and 2006 & 2008. ITS1 showed significant differences between 2005 & 2006, 2005 & 2008, and 2006 & 2008.

The haplotype networks show a similar pattern between all of markers, with a central dominant haplotype that represented the majority of the individuals within the population in all years, and a small number of less frequently occurring haplotypes (Fig. 4.1). These rare haplotypes were very closely related to the dominant central haplotype, which was reflected in the low nucleotide diversity values (π) from each time period. For both COI and 16S there were a total of 13 different haplotypes present in the population over time, and 10 different haplotypes for ITS1. Both the mtDNA markers show several low-frequency haplotypes that are only present in 2005 and that are absent from later time periods. ITS1 additionally has two moderately frequent shared haplotypes occurring in all years, unlike the mtDNA markers.

Haplotype diversity (h) for each gene was plotted against the time after the tsunami that the population was sampled (Fig. 4.2). Both mtDNA genes (COI & 16S) showed h decreasing with time until about 38 months, after which point it began to increase again. However, the pairwise comparisons from the AMOVA suggest that this rise in the genetic variation of the population between 2007 and 2008 was only significant for 16S. 16S was generally a more variable genetic marker than COI, with higher haplotype diversity present in the population at each time period. The nuclear gene marker (ITS1) showed a different trend to the mitochondrial markers, with h increasing steadily over time.

Tajima's *D* values were all negative except for ITS1 in Prapas 2008, although none of the values were significant. This demonstrates that there was no deviation from mutation drift equilibrium in the population. Negative *D* values are often used to support the presence of an excess of low frequency haplotypes, but have also been suggested to provide evidence for recent population growth (Aris-Brosous & Excoffier 1996).

Table 4.4. Genetic diversity in populations of *Dotilla intermedia* on Prapas beach for COI, 16S and ITS1 markers. n = number individuals; H = number of haplotypes; *h* = haplotype diversity; π = nucleotide diversity

COI	**n**	**H**	***h***	**π**	**Tajima's D**
Prapas 2005	20	6	0.516	0.00159	-0.52778 n.s
Prapas 2006	15	5	0.476	0.00130	-1.66013 n.s
Prapas 2007	15	3	0.257	0.00045	-1.49051 n.s
Prapas 2008	19	3	0.292	0.00051	-1.11995 n.s
16S					
Prapas 2005	20	8	0.700	0.00277	-0.58021 n.s
Prapas 2006	15	4	0.467	0.00148	-1.00949 n.s
Prapas 2007	15	3	0.257	0.00065	-1.49051 n.s
Prapas 2008	19	5	0.386	0.00128	-1.96578 n.s
ITS1					
Prapas 2005	20	3	0.416	0.00053	-0.52778 n.s
Prapas 2006	15	4	0.543	0.00074	-1.00949 n.s
Prapas 2007	15	5	0.629	0.00099	-1.07138 n.s
Prapas 2008	19	6	0.778	0.00179	0.11915 n.s

Table 4.5. AMOVA results table examining the differences among and between the genetic variation of the population of *Dotilla intermedia* on Prapas beach from 2005 to 2008

Source Variation	**Df**	**SS**	**Variance Components**	**Variation (%)**
COI				
Among years	3	1.242	0.00699	2.32
Within year	65	19.120	0.29416	97.68
Total	68	20.362	0.30115	
	F_{ST} = 0.0232		$P < 0.05$	
16S				
Among years	3	2.019	0.01976	5.58
Within year	65	21.720	0.33416	94.42
Total	68	23.739	0.35391	
	F_{ST} = 0.0558		$P < 0.001$	
ITS1				
Among years	3	2.460	0.02332	5.26
Within year	65	27.308	0.42012	94.74
Total	68	29.768	0.44344	
	F_{ST} = 0.0391		$P < 0.05$	

Table 4.6. Pairwise F_{ST} *P* values from AMOVA analysis of *Dotilla intermedia* populations on Prapas beach from 2005 to 2008 for COI, 16S and ITS1 markers. * = $P < 0.05$; ** $P < 0.001$; ns = no significant difference between population

COI	Prapas 2005	Prapas 2006	Prapas 2007	Prapas 2008
Prapas 2005	-	-	-	-
Prapas 2006	0.450 ns	-	-	-
Prapas 2007	0.144 ns	0.730 ns	-	-
Prapas 2008	0.036 *	0.018 *	0.288 ns	-
16S				
Prapas 2005	-	-	-	-
Prapas 2006	<0.001 **	-	-	-
Prapas 2007	<0.001 **	<0.001 **	-	-
Prapas 2008	<0.001 **	<0.001 **	<0.001 **	-
ITS1				
Prapas 2005	-	-	-	-
Prapas 2006	0.036 *	-	-	-
Prapas 2007	0.099 ns	0.991 ns	-	-
Prapas 2008	0.045 *	0.036 *	0.243 ns	-

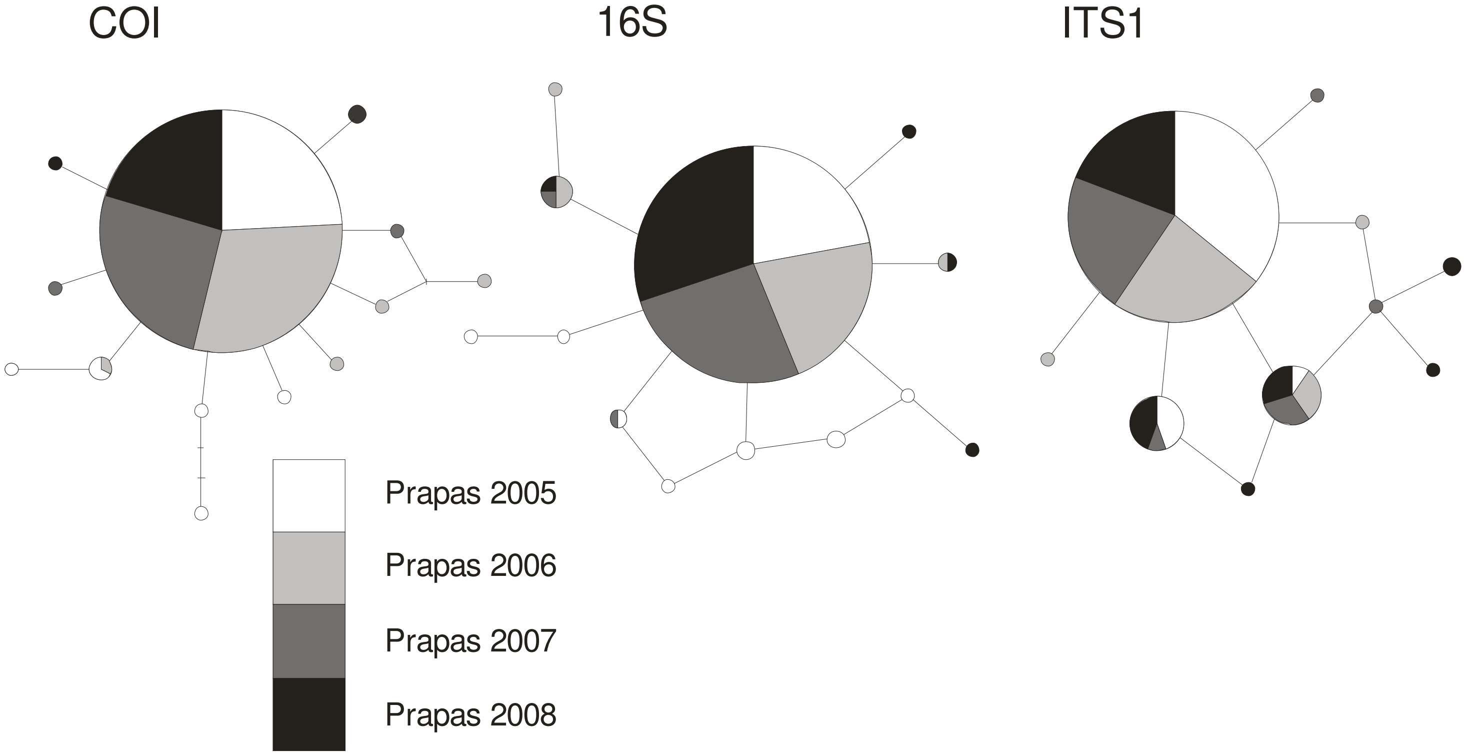


Fig 4.1. Statistical parsimony haplotype networks for *Dotilla intermedia* on Prapas beach. Each circle represents a haplotype and its diameter is proportional to the frequency of the haplotype in the population. The different coloured wedges indicate the proportion of individuals from each time point within a given haplotype. Bars represent the number of nucleotide substitutions between haplotypes

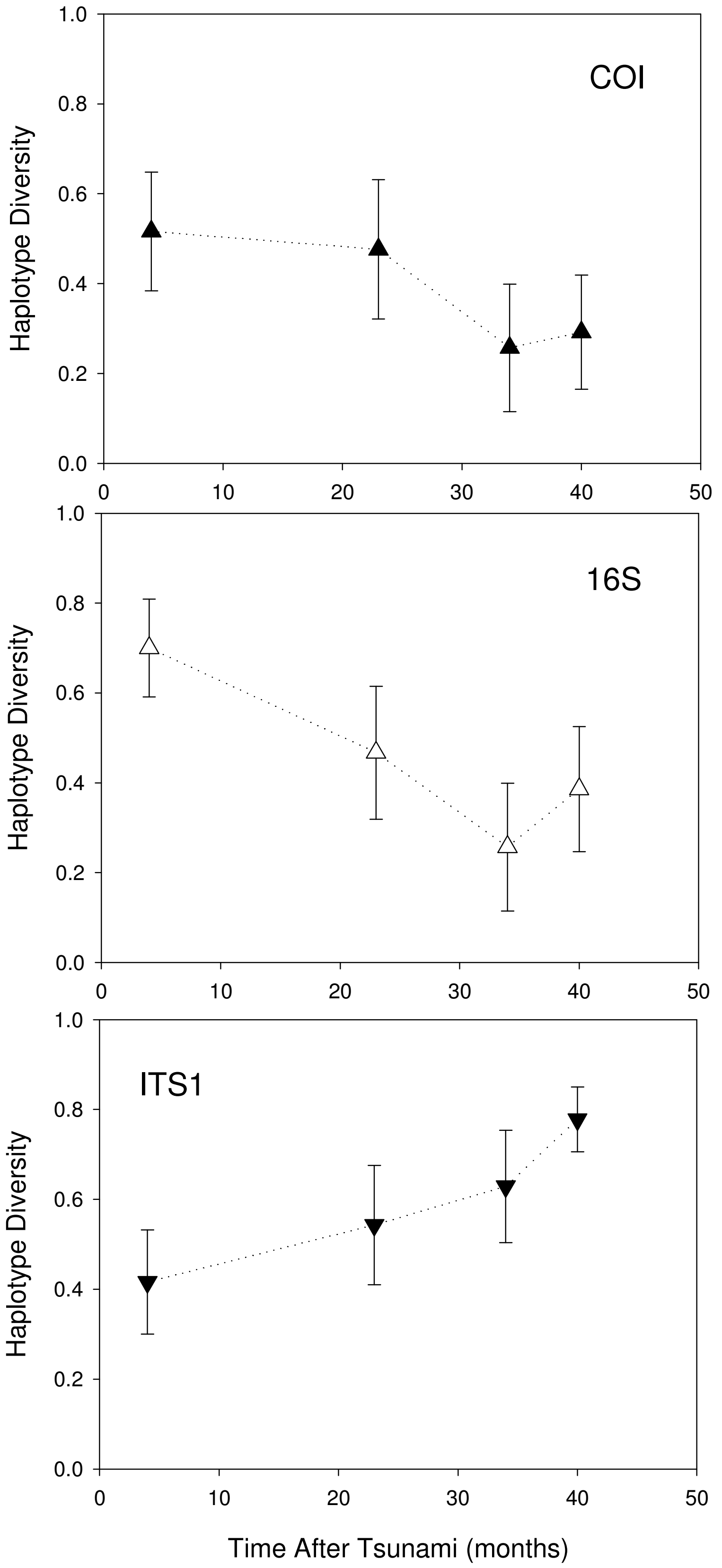


Fig. 4.2. Haplotype diversity of *Dotilla intermedia* on Prapas beach plotted against time after the tsunami for COI, 16S and ITS1. Error bars ± 1 SD

4.3.2. GEOGRAPHICAL VARIATION

The genetic diversity of *Dotilla intermedia* populations from Prapas beach and Bang Ben in 2008 are summarised in Table 4.7. There was very little variability in *h* for the mitochondrial markers (16S & COI) between the two beaches, although there was a more distinct difference in the number of haplotypes found on the two beaches for ITS1, with 6 identified on Prapas and only 3 on Bang Ben. The patterns shown by the haplotype networks were quite consistent between the markers (Fig. 4.3). The haplotype networks showed that the populations shared the most dominant haplotype for each marker. There were low numbers of other haplotypes that were very closely related to the central dominant haplotype, most of which were not shared between the two beaches. Apart from the dominant central haplotype, there was only one shared haplotype for 16S and one for ITS1, with no shared haplotypes present for COI. The shape of the haplotype networks were reflected by the low π values for each marker. Low π values indicate a low mutation rate, and an uneven spread of haplotypes, with each haplotype only having small, single-based nucleotide differences.

AMOVAs showed that there was no significant differences in the genetic variability of the populations at Prapas and Bang Ben in 2008 for COI ($F_{ST} = 0.029$, ns), 16S ($F_{ST} = 0.031$, ns) and ITS1 ($F_{ST} = 0.045$, ns) (Table 4.8). Values for Tajima's *D* were not significant for any of the gene regions, indicating that neither of the populations deviated from mutation-drift equilibrium.

Table 4.7. Genetic diversity in populations of *Dotilla intermedia* from Prapas beach and Bang Ben in 2008 for COI, 16S and ITS1 markers. n = number individuals; H = number of haplotypes; *h* = haplotype diversity; π = nucleotide diversity

COI	**n**	**H**	***h***	**π**	**Tajima's D**
Bang Ben 2008	20	4	0.363	0.00065	-1.44071 n.s
Prapas 2008	19	3	0.292	0.00051	-1.11995 n.s
16S					
Bang Ben 2008	20	4	0.432	0.00128	-0.15565 n.s
Prapas 2008	19	5	0.386	0.00128	-1.96578 n.s
ITS1					
Bang Ben 2008	20	3	0.511	0.00070	0.06325 n.s
Prapas 2008	19	6	0.778	0.00179	0.11915 n.s

Table 4.8. AMOVA results table examining the differences among and between populations of *Dotilla intermedia* from Prapas beach and Bang Ben in 2008

Source Variation	**Df**	**SS**	**Variance Components**	**Variation (%)**
COI				
Among pop.	1	0.281	0.00550	3.06
Within pop.	37	6.437	0.17397	96.94
Total	38	6.718	0.17947	
	$F_{ST} = 0.02913$		$P > 0.05$ ns	
16S				
Among pop.	1	0.417	0.00789	2.91
Within pop.	37	9.737	0.26316	97.09
Total	38	10.154	0.27105	
	$F_{ST} = 0.03063$		$P > 0.05$ ns	
ITS1				
Among pop.	1	0.978	0.02400	4.50
Within pop.	37	18.868	0.50996	95.50
Total	38	19.846	0.53396	
	$F_{ST} = 0.04496$		$P > 0.05$ ns	

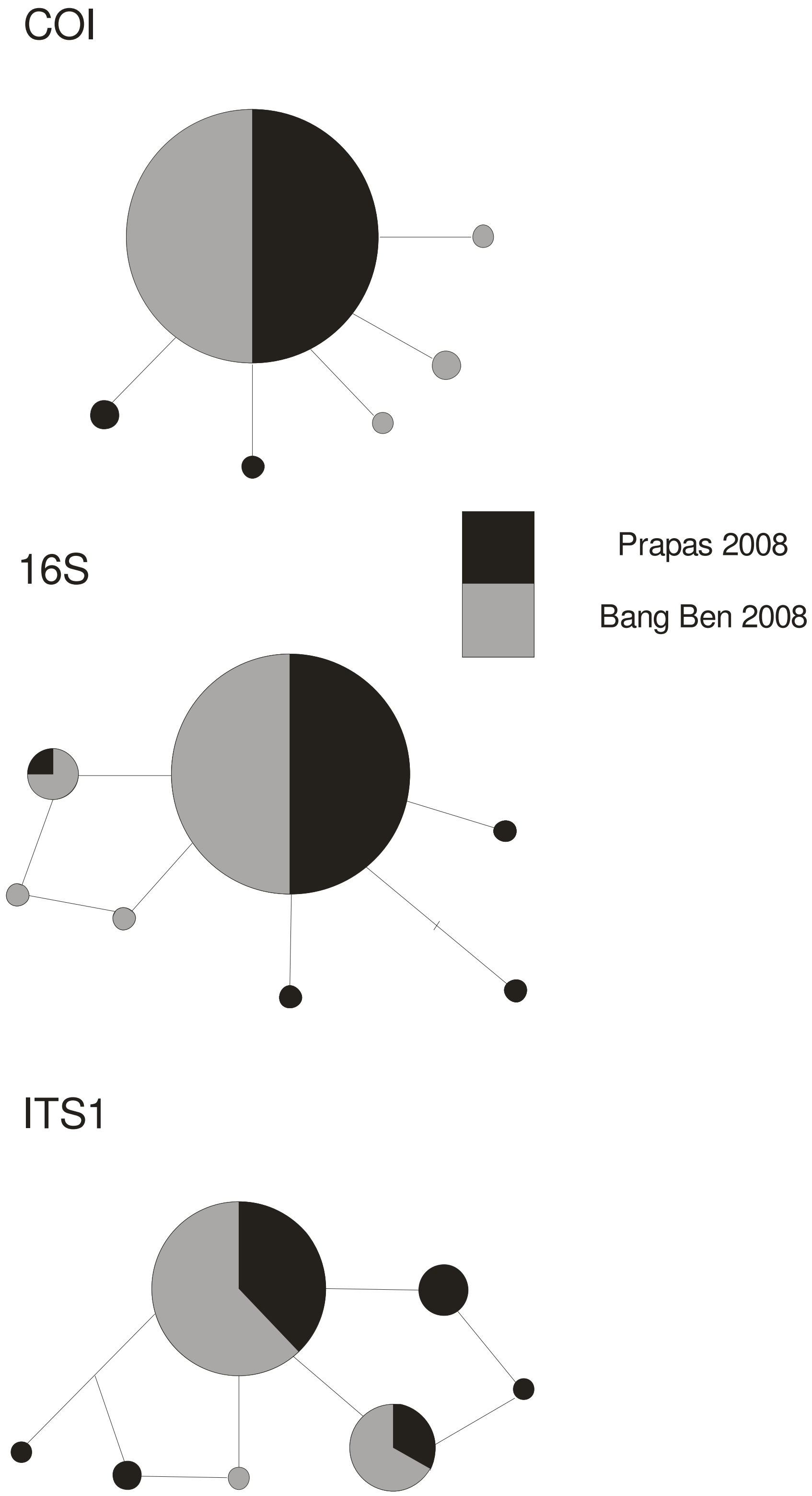


Fig 4.3. Statistical parsimony haplotype networks for *Dotilla intermedia* from Prapas beach and Bang Ben in 2008. Each circle represents a haplotype and its diameter is proportional to the frequency of the haplotype in the population. The different coloured wedges indicate the proportion of individuals from each time locality within a given haplotype. Bars represent the number of nucleotide substitutions between haplotypes

4.4. DISCUSSION

4.4.1. GENETIC POPULATION STRUCTURE IN *DOTILLA INTERMEDIA*

For each of the three molecular markers investigated, there was very little overall variability. The haplotype networks all showed a star-like phylogeny. Each of the genes were characterised by one central haplotype that occurred at very high frequencies, representing at least 60 % of the sampled individuals, regardless of the time or location that the population was sampled, indicating a high degree of genetic homogeneity. The remaining unique haplotypes were very closely related to the common central haplotype, and occurred at much lower frequencies. The high frequencies of the central common haplotypes and the low number of closely related haplotypes were reflected in the low π values and the low h values.

We can compare the values of h and π for *Dotilla intermedia* obtained using COI as a marker to those measured for other decapod species (Table 4.9), which may highlight which ecological processes have shaped the genetic structure of the population of *D. intermedia*. Whilst the low π was common to all species, *D. intermedia* showed much lower values for h than any of the species in the table, except for two populations of *Gaetice depressus* where the low h values ($h = 0.233$ & 0.511) were attributed to population bottlenecks or founder events (Kawane *et al.* 2008). This supports the suggestion that the tsunami has acted as a genetic bottlenecking event on the populations of *D. intermedia*.

A star-shaped haplotype network with one dominant central haplotype and a high number of other low-frequency, closely related haplotypes is typical of species that have suffered a bottleneck and a subsequent expansion. These star-shaped haplotype networks are characterised by high h and low π values (e.g. *Palinurus gilchristi,* Tolley *et al* 2005; *Palinurus mauritanicus,* Palero *et al.* 2008). “Bush-like” networks where several haplotypes are equally dominant are characteristic of populations that maintain large stable population sizes (e.g. *Chiromantes dehaani,* Kawane *et al.* 2008). The haplotype networks for *Dotilla intermedia* were more similar to the star-shaped networks that indicate a population that has undergone a bottleneck followed by a recent expansion, except that values for h are low, indicating a very uneven mix of haplotypes within the *D. intermedia* population. The star-shaped networks have a large number of low frequency haplotypes that help to raise the haplotype evenness within the population, leading to high *h* values. This suggests that the population of *D. intermedia* on Prapas beach has yet to fully expand since the tsunami, although negative Tajima's *D* values, indicating recent population growth (Aris-Brosous & Excoffier 1996), hint that the population may be in the process of expansion. This population growth may lead to an increase in new mutations that would in turn increase the amount of different haplotypes within the population, raising the value of *h*.

Table 4.9. Values of haplotype diversity (*h*) and nucleotide diversity (π) for several different crustacean species, with author comments describing why these values were obtained. Unless given as a range, *h* and π are mean values calculated from a group of populations

Author	**Species**	**Marker**	***h***	**π**	**Author comments**
Tolley *et al.* (2005)	*Palinurus gilchristi*	12S rRNA	0.858	0.0042	Large number low frequency haplotypes closely related to a common central haplotype; evidence for recent population expansion.
Palero *et al.* (2008)	*Palinurus elephas*	COI	0.588	0.0016	One extremely abundant haplotype, many low frequency closely related haplotypes. *P. mauritanicus* lives in deeper waters, so sea level or temperature changes have less effect compared to *P.elephans*, hence differing values for *h*.
	Palinurus mauritanicus		0.905	0.0045	
Gopurenko & Hughes (2002)	*Scylla serrata*	COI	0.091–0.672		Low diversity values found in recently established populations, whilst higher values found in more historically prevalent populations.
Kawane *et al.* (2008)	*Chiromantes dehaani*	COI	0.841	0.0050	"Bush like" haplotype networks suggest *C. dehaani* maintained large stable population sizes. Low *h* (0.233 & 0.511) and low π values in some populations of *G. depressus* indicate bottleneck or founder effects.
	Deiratonotus japonicus		0.793	0.0034	
	Gaetice depressus		0.744	0.0026	
	Ocypode ceratophthalma		0.862	0.0056	
Petersen (2007)	*Hemigrapus oregonesis*	COI	0.632-0.994	0.0055-0.0125	No significant differences between *h* and π values between populations, suggesting unified expansion rather than one older population expanding first.
Cassone & Boulding (2006)	*Pachygrapsus crassipes*	COI	0.923	0.0090	Large number low frequency haplotypes suggest extremely large effective female population size. High *h* and close genetic similarities suggest large numbers of haplotypes arise in populations and are lost after accumulating a few nucleotide differences and/or recent population expansion.

4.4.2. TEMPORAL CHANGES IN POPULATION GENETIC DIVERSITY AT PRAPAS BEACH

The tsunami had a large impact on the genetic structure of the population of *Dotilla intermedia* on Prapas beach. The tsunami wave is hypothesised to have acted as an extreme bottlenecking event, causing a severe decline in population numbers that resulted in a loss of genetic variability within the population as low frequency haplotypes were eliminated. This hypothesis was supported in the two mitochondrial markers investigated. Both COI and 16S showed a decrease in h with time after the impact of the tsunami wave. This trend matches the model predictions of Nei *et al.* (1975), which showed a sharp decline in genetic variability in subsequent generations of a population following a bottleneck event. The rise in h shown by the mtDNA data at the end of time series runs slightly contrary to the predictions of the model, although this increase in genetic variability was only significant for 16S. The model predicts that genetic variability should eventually reach a minimum level following the initial decrease. After a period of time at this minimum level, there should be a slow increase in variability as new mutations are established in the population. Increasing the amount of different haplotypes would act to decrease the unevenness of haplotypes within the population. Compared to the model predictions, an increase in h for COI and 16S around 38 months after the wave impact would represent extremely fast recovery for the population of *D. intermedia*. A more likely explanation is that the specimens collected on Prapas in 2008 represented a more genetically diverse subsection of the whole population. Examination of specimens collected from a more recent time period would reveal whether the increase seen in 2008 is the start of an upward trend in genetic diversity, or if a plateau in diversity had been reached.

Whilst some of the haplotypes for each genetic marker persist over time, there are other haplotypes that are unique to only one time period. This may be a consequence of environmental pressures eliminating some low frequency haplotypes and mutations producing new haplotypes. However, it could also be a function of an inadequate sample size failing to capture a representative section of the population, missing these low frequency haplotypes. Kalinowski (2005) investigated the effects of increasing the sample size of a population on the coefficient of variation for estimates of genetic distances for polymorphic loci. When the genetic distance between populations is low, then a large number of samples should be examined (i.e. if $F_{ST} < 0.01$, 100 or more individuals per population should be sampled), whilst if the genetic distance is high, then a smaller number of samples can be used (i.e. if $F_{ST} > 0.05$, sampling 20 individuals per population should be sufficient). The F_{ST} values calculated during the AMOVAs suggest that amount of specimens analysed during this study were insufficient to capture the majority of variation present in the population at Prapas beach.

There was a distinct difference in the temporal changes of *h* between the mtDNA markers and the nuclear marker (ITS1). Rather than decreasing after the tsunami like the mtDNA markers, *h* steadily increased with time for ITS1. The patterns of genetic diversity observed between nuclear and mtDNA are not expected to be the same, as the two genomes respond differently to both stochastic and population processes (Birky *et al.* 1989). The effective population size of mtDNA is much smaller than nuclear DNA, and therefore the loss of mtDNA diversity is expected to be more rapid than nuclear DNA in newly colonised populations. Populations that have been founded by closely related kin or by a small number of females would have a large reduction in their mitochondrial genetic diversity (Wade *et al.* 1994). If the beaches in the Laem Son were recolonised by a small number of *Dotilla intermedia,* either from individuals that had survived the wave or new recruits settling onto the beaches from an offshore larval pool, then we would expect the mtDNA variability to decline in a pattern as observed. Although the differences between the two genomes can explain why nuclear diversity was higher than that measured for mtDNA, it does not offer an explanation as to why the nuclear and mtDNA markers show opposite temporal trends. This difference may stem from the fact that ITS1 is a non-coding region of the nuclear genome, and therefore is theoretically free to mutate to a high degree. This study highlights the importance of studying multiple molecular markers, as varying selection pressures for different types of markers may result in contrasting results.

The genetic diversity in the pre-tsunami population of *Dotilla intermedia* on Prapas beach is unfortunately unknown. There may have been much more genetic variation for both the mtDNA and nuclear markers, with values of *h* much higher than measured in 2005. The value of *h* may have drastically declined for both nuclear non-coding and mtDNA markers as an immediate consequence of the tsunami eliminating rare haplotypes from the population, down to the values measured in

2005. The nuclear marker may then have begun to increase as new mutations occurred, possibly helped by the fact ITS1 is non-coding region. The nature of the recolonisation event could have led to a further reduction in mtDNA diversity over time as offspring produced by a small number of females began to dominate the population. Fig. 4.4 outlines these theoretical patterns, with pre-tsunami values for *h* estimated using data from Table 4.7.

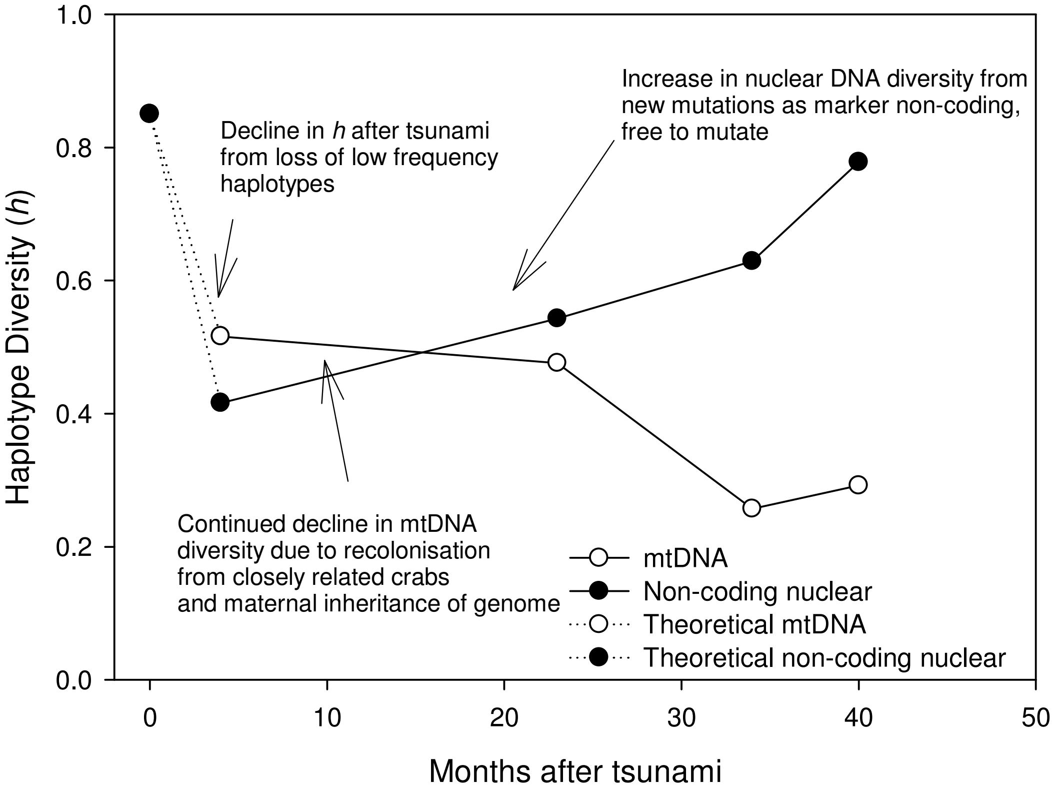


Fig. 4.4. Theoretical graph showing hypothetical changes in haplotype diversity (*h*) over time for *Dotilla intermedia* on Prapas beach. Pre-tsunami estimates for *h* (time = 0) are based on values from Table 5.7; mtDNA values are those measured for COI; nuclear non-coding values are those measured for ITS1

Increasing the numbers of specimens analysed from the collected material for each time period would clarify whether the observed patterns were an artefact created by failing to adequately sample a representative subsection of the whole population. In addition, continuing the sampling on Prapas beach for an extended time period would provide a more accurate view on the exact trends in the genetic diversity of the population, allowing for more definitive conclusions regarding the hypothesised population genetic bottleneck caused by the tsunami.

4.4.3. GEOGRAPHIC VARIATIONS IN GENETIC STRUCTURE

On Prapas beach, mtDNA genes showed a significant reduction in genetic diversity, with *h* in 2008 half that recorded for 2005. The similarities between mtDNA genetic variability for Prapas and Bang Ben in 2008 suggest that, like Prapas, Bang Ben had also suffered a large reduction in genetic diversity following the impact of the tsunami, and it is easy to envisage this pattern of loss in diversity repeated in other populations of *Dotilla intermedia* all along the coastline affected by the tsunami. It is hypothesised that the extinction and recolonisation experienced by populations of *D. intermedia* could have enhanced the genetic differentiation between local demes, as many of the low frequency haplotypes shared between populations may have been eliminated by the impact of the tsunami (Wright 1977; Wade & McCauley 1988).

Apart from the dominant central haplotype, there were very few shared haplotypes in the investigated mtDNA markers between Prapas and Bang Ben. Although the AMOVA showed there were no significant differences between the genetic structures of the two populations, there were several unique haplotypes present on each beach. However, without pre-tsunami data from the beaches, it was not possible to assess whether the tsunami has altered the genetic differentiation between the two beaches. The pre-dominant haplotype was shared between Bang Ben and Prapas beach, indicating that there may be some gene flow between the populations on the two beaches, and the numerical analysis of the data does not show that the tsunami had acted to enhance the genetic variability between the two populations. Continued sampling of *D. intermedia* on both beaches over a long time scale would reveal whether the populations are becoming more genetically differentiated as they recover and expand over time, with new mutations occurring independently within each population.

4.5. SUMMARY

The investigation of the population genetics of *Dotilla intermedia* has allowed an insight to the impacts of a large scale disturbance event on the genetic structure of a population. The haplotype networks, combined with the generally low haplotype and nucleotide diversities, and negative Taijima's *D* values, offer some support for the hypothesis that the impact of the tsunami may have created a genetic bottleneck in the populations of *D. intermedia* from both Prapas beach and Bang Ben, and that the population may be in the early stages of expansion.

The reduction in the number of *D. intermedia* on Prapas beach had resulted in significant differences in the genetic variability of the population with time after the tsunami, with mtDNA genes showing a decline in haplotype diversity down to a minimum level. The patterns shown by the mtDNA markers followed the predictions of Nei *et al.*'s (1975) model concerning the effects of bottleneck events on population genetic variability. However, the nuclear marker (ITS1) demonstrated an opposite trend, with haplotype diversity increasing with time. The different modes of inheritance between the mitochondrial and nuclear genomes are unlikely to have resulted in opposite trends between the two types of markers. The lack of pre-tsunami samples means that is not possible to confirm whether genetic variability was higher for both nuclear and mtDNA before the tsunami, and then suffered a dramatic decline in variability after the impact of the tsunami. Whilst diversity in the ITS1 marker may have then begun to increase as the population of *D. intermedia* was re-established on the beach, the mtDNA markers may have shown a continual decline with time due to the process of beach recolonisation involving offspring from a small number of females. As ITS1 is a non-coding region it exhibits a much higher mutation rate than a coding region, and thus may increase haplotype diversity at a much greater rate than the mtDNA markers.

The small sample sizes analysed from each time point make definitive conclusions about the effect of tsunami on *D. intermedia* hard to draw. Analysing additional specimens collected from each time point to increase the sample size, along with continuing to sample the population on Prapas beach for further time periods, may help to elucidate the trends in the data and resolve some of these alternative hypotheses.

Comparisons between two populations of *Dotilla intermedia* from Prapas beach and Bang Ben in 2008 suggest that the two populations had experienced similar changes to their genetic structure, with genetic variability within both populations declining as a result of the bottlenecking effect of the tsunami. There was no support for the hypothesis that the tsunami may have enhanced the genetic variability between the two beaches. Although the majority of the less frequent haplotypes were found to be unique for one beach or the other, both populations shared a common dominant haplotype for each marker. The interpopulation variability between beaches may have already existed pre-tsunami, and there was no numerical evidence that the impact of the tsunami may have enhanced the genetic variability between the populations. Continuing to sample both populations over time would reveal whether there is a further increase in genetic variability between the two beaches.

5. GENERAL DISCUSSION

5.1. OVERVIEW OF RESULTS

This study involved an examination of a species of crab from the genus *Dotilla* found on the exposed oceanic sandy beaches in the Laem Son National Park, Thailand. Despite observation on the numerical dominance of *Dotilla* within the sandy shore fauna, no detailed work had previously been undertaken on this species of crab. There were three main aims to this thesis. These have been listed below, each with a synopsis of the relevant results from this study.

- **What was the identity of the *Dotilla* species present on the exposed oceanic sandy beaches of the Laem Son?**

Specimens collected from the Laem Son were identified as *Dotilla intermedia* using the key to *Dotilla* (Kemp 1919). Further morphological analysis of specimens, involving comparisons to museum collection material, supported this diagnosis. A molecular phylogeny confirmed that the unknown species was neither of the *Dotilla* species previously reported from Thailand (*D. myctiroides* and *D. wichmanni*). This represented the first time *D. intermedia* had been recorded from Thailand. Morphological analysis of collection material confirmed that Kemp's (1919) key was robust for identification of *Dotilla.* For cases where the carapace sculpting of specimens was not clear, which would make Kemp's key difficult to successfully use, additional diagnostic characters have been outlined that can be easily used to distinguish between species of *Dotilla*, such as the shape of the first male pleopod. This thesis represents the first time that the first male pleopods have been figured for all *Dotilla* species in one study; this is also the case for the figures of the *Dotilla* male abdomens. A biogeographical map showing the ranges of the different *Dotilla* species was also constructed from literature records and museum collection material. Some *Dotilla* species share mutual affinities with each other with regards to certain morphological characters; several of these mutual affinities have been outlined.

- **What can be determined about the ecology of *Dotilla intermedia* on the sandy beaches in the Laem Son?**

There was a close relationship between the beach gradient and the vertical height of the *Dotilla* zone on the beaches studied. The slope of the beach determined the sediment water content and the length of tidal exposure at a given point on the beach. The upper boundary to the *Dotilla* zone was governed by the total water content of the sediment, with *D. intermedia* absent from sediment with less than 15 % water content. The amount of sediment water content required in an area inhabited by *D. intermedia* is probably defined by the physiological adaptations of the species. The lower shore boundary of the *Dotilla* zone was controlled by the length of tidal exposure, with crabs requiring a minimum of 4-5 hours of emersion from the sea. This amount of exposure is necessary for the species to be able to feed sufficiently between successive tidal immersions. Sediment particle size and organic content were not significant factors controlling the distribution of *D. intermedia*. Within the *Dotilla* zone, size segregation could be seen, with adult crabs favouring the high shore, and small crabs more common on the low shore. This size segregation may have been related to size-dependant physiological tolerances, or driven by intraspecific competition forcing smaller crabs onto a less favourable part of the beach, or a combination of these two factors. The observations on the zonation of *D. intermedia* offer some support to several hypotheses concerning patterns seen in sandy shore macrofauna, such as the Habitat Favourability Hypothesis and the Habitat Harshness Hypothesis. *D. intermedia* was shown to have a 1:1 sex ratio, and demonstrated sexual asymmetry, with male crabs having a larger average body size and reaching a higher maximum body sizes compared to female crabs. There was a unimodal population size structure, which suggested that *D. intermedia* had a continuous reproductive cycle.

- **How did the tsunami affect the population genetic structure of *Dotilla intermedia* in the Laem Son?**

It is hypothesised that the tsunami acted as a bottle necking event, reducing the population genetic variability within a population of *Dotilla intermedia* on Prapas beach. The data demonstrated a reduction in the mtDNA diversity of the population over time, which reached a plateau around 38 months after the tsunami. The reduction in mtDNA diversity matched model predictions (Nei *et al.* 1975) concerning the influence of a bottlenecking event on the genetic variability within a population. The re-establishment of the *D. intermedia* population on Prapas beach would have involved a small number of females or closely related kin, thus resulting in a large reduction in mtDNA diversity over time. The genetic structure of the population was similar to that of other crustacean species that had recently undergone an extinction event followed by a subsequent population expansion. The non-coding nuclear marker showed an opposite trend to the mtDNA markers, with genetic diversity increasing with time. This could be due to differences in the selection pressures between the mtDNA markers and the non-coding nuclear marker. However, only small sample sizes were used from each time point, making it hard to draw definitive conclusions regarding the hypothesis that the tsunami may have created a bottleneck in the genetic variability of the population. Although there were some differences in the population genetic structure between Prapas beach and Bang Ben in 2008, analysis of the data did not support the hypothesis that the tsunami may have enhanced the genetic differentiation between the two populations.

5.2 ECOLOGICAL IMPACTS OF THE TSUNAMI ON *DOTILLA INTERMEDIA*

There have been several studies that have described changes to the topology of beaches following the impact of the tsunami in Thailand (Lander *et al.* 2003; Kendall *et al.* 2006; Wilkinson *et al.* 2006). Distinct changes to the slope of beaches were also reported along the Chennai coast in India (Altaff *et al.* 2005). The ecological work undertaken in this thesis demonstrated that the location of the area inhabited by *Dotilla intermedia* has a very close relationship to the gradient of the beach. One direct consequence of sediment accumulation or removal caused by the tsunami would be to alter the beach gradient, and thus shift the position of the *Dotilla* zone on the shore.

In addition to altering the topology of beaches, the tsunami created a large change in the patterns of grain size distribution on exposed beaches throughout the

Laem Son. There was a general coarsening of sediment resulting from the overturning of the beaches by the tsunami wave, and the sediment on the tsunami-impacted beaches had yet to reach pre-tsunami conditions by April 2005 (Kendall *et al.* 2006). Changes in sediment grain size created by the tsunami would be expected to have a large impact on *Dotilla intermedia*. Although sediment grain size was not found to be significant in controlling the zonation of *D. intermedia* on beaches within the Laem Son, it is hypothesised that sediment grain size dictates which beaches are available for habitation by *D. intermedia*. Sediment of a particular size range is necessary to sift through the mouthparts of the crabs when feeding (e.g. Robertson & Newell 1982; Lim 2005). However, the granulometric analysis of sediments by Kendall *et al.* (2006) was based on measuring the overall granulometry of a sediment volume, and did not focus on the thin surface layer processed by the crabs. Sections through the sediment showed strong patterns of layering consistent with considerable resuspension and settlement, with the coarse material overlain by finer material (Kendall *et al.* 2006). Therefore the grain size of the surface sediment may not have been outside the size range required by *D. intermedia* to feed upon (Fig. 6.2).

Fig. 5.1. Cross section of sediment from Prapas beach, April 2005. Fine sediment can be seen at the surface layer overlying coarser sediment (Photograph courtesy of G. Paterson)

The coarsening of the sediment may have had more impact on the ability of *Dotilla intermedia* to burrow through the sediment. There have been several studies on effect of differing sediment types on the burrowing of the mud crab *Helice crassa*. The depth to which *H. crassa* burrows is related to the relative proportion of the silt-clay fraction in the sediment (Takeda & Kurihara 1987), with the crab burrowing deeper in muddier sediments than sandier sediments (Nye 1977). The tsunami-related coarsening of the sediment may have resulted in *D. intermedia* only being able to construct shallower burrows, which in turn may have had important implications for the effectiveness of the burrow as a refuge from desiccation and predation (Warren 1990). However, considering the speed at which the populations of *D. intermedia* recovered to their pre-tsunami numbers in the Laem Son, it may be that there was little or no impact on the burrowing ability of the crabs.

The changes in the sediment granulometry and beach topology created by the tsunami would slowly begin to revert to pre-tsunami conditions over time by sediment movement caused by tidal action. Sediment resuspension and resettlement would slowly move coarse particles deeper into the sediment, whilst sediment removal or accumulation would shape the slope of beaches. When compared to other large-scale marine disturbances such as tropical storms and cyclones, tsunami-generated disturbance can be seen to have a similar level of impact. In both storms and tsunamis, the majority of disturbance on the near-shore marine environment is physically derived from wave action. Along the Andaman coast of Thailand most of these storm events occur in the winter months, with a peak in November, meaning that the 2004 tsunami occurred during a period when large scale disturbances would be predicted. As a result of the historical precedence of winter storms, the exposed shore fauna that occupy the Andaman coast would be expected to have evolutionary adaptations to overcome disturbance during this time period, so the overall impact of the tsunami may have been moderated by virtue of its timing (Paterson *et al.* In press). Bearing this in mind, it is perhaps not surprising that the populations of *Dotilla intermedia* were able to recover relatively quickly after the impact of the tsunami.

5.3. REPRODUCTION IN *DOTILLA INTERMEDIA* AND POST-TSUNAMI RECOLONISATION

Previous studies on *Dotilla* have shown that continuous reproduction occurs in both *D. myctiroides* (Halis & Yaziz 1982) and *D. fenestrata* (Litulo *et al.* 2005). Ecological data presented in Chapter *3* demonstrated unimodal population size frequency distributions for *D. intermedia* on Prapas beach, which are typical of crustacean species that display continuous year-round reproductive patterns. Although no formal data has been presented in this thesis, small numbers of ovigerous females were found on Prapas beach at every time period sampled, supporting the suggestion that *D. intermedia* exhibits continuous reproduction.

The beaches along the coast of the Laem Son National Park are infrequently impacted by storm surges generated by cyclones, which disturb the sediment of the sandy beaches. Year-round reproduction may be an adaptation to allow *Dotilla intermedia* to survive disturbance by cyclones. Such a reproduction pattern would result in larvae continuously present in an offshore pool. New recruits would be drawn from this larval pool to replace any losses caused by cyclones and other disturbance events, regardless of when in the year these events may occur. However, the impact of the yearly monsoon is probably more important in the population dynamics of *D. intermedia* than unpredictable storm events. There may be peaks in reproduction for *D. intermedia* associated with the end of the monsoon. The monsoon creates dynamic and unstable conditions for fauna on sandy beaches in India, with severe wave action leading to a high degree of sediment erosion that can wash away high shore macrofauna (e.g. Panikkar 1970; Dwivedi *et al.* 1973). A large post-monsoon influx of new recruits would be required to balance losses in a population. Both *D. fenestrata* (Litulo *et al.* 2005) and *D. myctiroides* (Halis & Yaziz 1982) show peaks in reproductive activity associated with the monsoon. *D. myctiroides* demonstrated high reproductive activity between November and February in Malaysia (Halis & Yaziz 1982), which has a similar monsoon season to Thailand. However, when examining over 300 crabs to examine the sex ratio of *D.intermedia* in November 2007, only two ovigerous females were found. If there was a post-monsoon reproductive peak then it could be expected that more ovigerous females would have been encountered.

This discrepancy could have resulted from the sampling technique employed; crabs were collected by locating open burrows and excavating the resident individual. Ovigerous females may remain in their burrows whilst brooding, and would be missed by using open burrows to locate crabs. An alterative method to have used when collecting crabs would have been to sift a large volume of sediment through a sieve that would retain the crabs. However, the small size of crabs would mean that a relatively fine sieve would have had to been used, making this a very time consuming method. Copulation can occur both above and underground in the Ocypodidae (e.g. Yamaguchi 1971, 1998; Koga *et al.* 1993). Tweedie (1950) reported that male *Dotilla myctiroides* actively capture females and pull them down into their burrows for mating. After copulation, the females of some ocypodid crabs, such as *Ilyoplax pusillus* (Henmi & Kaneto 1989) *Uca perplexa* (Henmi 2003) and *Scopimera globosa* (Henmi & Kaneto 1989; Koga 1995), remain in plugged burrows whilst brooding, and do not emerge to feed. Other species such as *Macrophthalmus japonicus* (Henmi & Kaneto 1989), *Uca vocans* and *U. dussumieri* (Henmi 2003) activity feed on the surface mud whilst incubating. Ovigerous *Dotilla myctiroides* were seen on the surface during this study (Allen pers. obs.), perhaps suggesting that female *Dotilla* do not remain in their burrows when brooding.

A peak in recruitment would be expected to be seen when examining the population size-frequency distribution of the species, as shown in the deep-sea ophiuroid *Ophiomusium lymani* (Gage & Tyler 1982). A detailed study would be required to fully understand the reproductive cycle of *D. intermedia*. Monthly samples of crabs from a section of beach would have to be captured, measured, sexed and the number of ovigerous females recorded for up to a year. Examining changes in population size structure would reveal if large numbers of new recruits arrive on the beach at certain times of the year, and whether this is in turn related to the monsoon.

The level of survival of *Dotilla intermedia* after the impact of the tsunami wave is unknown. If a substantial number of crabs were able to survive the impact of the wave then there would have been a much lower impact on the genetic variation within the population, with no bottlenecking event occurring. The reduction in mtDNA diversity over time, combined with the star-shape of the haplotype networks and the negative values for Tajima's *D* (Chapter *4*), provide evidence that a population bottleneck did occur after the tsunami, suggesting that survival from the tsunami was limited. The successive waves of the tsunami resulted in a large volume of sediment on beaches being overturned and homogenised. *Dotilla* crabs only burrow down to depths of approximately 10 cm, and are relatively soft-bodied, making their survival of the tsunami highly unlikely.

Knowledge of the reproductive cycle of *Dotilla intermedia* is necessary to fully understand the post-tsunami recolonisation of the beaches in the Laem Son. If *Dotilla intermedia* has a reproductive cycle with a post-monsoon peak in reproductive activity, then this would roughly coincide with the occurrence of the tsunami in December. There would be a large number of larvae ready to recolonise the beach, which may have moderated the long-term impact of tsumani on *D. intermedia* populations. If in contrast *D. intermedia* has a continuous low reproductive cycle, then there would still have been larvae present to resettle the beaches, but in much lower numbers. This could have lead to a greater length of time before the populations were able to fully re-establish themselves on the beach. Also, if the re-established populations were derived from a smaller number of recruits, then there would be a potentially greater effect on the genetic structure of the population, with variation in mtDNA expected to be lower as individuals would be more closely related (Wade *et al.* 1994), leading to a more genetic homogeneity within the population.

Regardless of whether *D. intermedia* has continuous reproduction either with or without seasonal peaks related to the monsoon, there would be larvae present in the water column ready to settle onto the beaches immediately after the impact of the tsunami. *D. intermedia* had already partially recolonised the oceanic sandy beaches in the Laem Son by April 2005, although the densities had yet to reach pre-tsunami levels (Kendall *et al*. 1996). The ecological data presented in this thesis suggests that recruits only settle into gaps that are not disturbed by the feeding activity of other crabs within the *Dotilla* zone. The impact of the tsunami would have created many gaps within the *Dotilla* zone, reducing any density-dependant mortality and therefore allowing a much higher settlement of larvae.

The sizes of the crabs collected in April 2005 on Prapas beach were comparable to those gathered at later time periods (Fig. 3.33; Chapter *3*). Combined with the presence of ovigerous females, this suggests that at least some of the crabs had reached a size suitable for reproduction four months after the tsunami. Little is known about the lifecycle of *Dotilla intermedia*, and taking four months to reach breeding size may not be unusual for the species. However, *D. myctiroides* has been estimated to take between 10 to 13 months to reach maximum adult body size from juvenile stage I, with recruits that settle post-monsoon attaining breeding size ready to reproduce in the post-monsoon period the following year (Halis & Yaziz 1982). This suggests that compared to *D. myctiroides, D. intermedia* either has a much faster growth rate, or that the post-tsunami environmental conditions may have favoured a rapid increase in the size of new recruits. Lower densities of crabs could have reduced intraspecific competition, and the destruction caused by the tsunami wave may have released many more organics into the system, potentially leading to an increase in the available food supply for *D. intermedia*, allowing them to reach maturity and reproduce on a short time scale. Additionally, the tsunami would have undoubtedly had an impact on the *Ocypode* crabs that are the principal predator of *D. intermedia* in the Laem Son, which could have acted to suppress predation pressure, allowing the newly settled *D. intermedia* to thrive.

5.4. FUTURE WORK

- Construct a molecular phylogeny for the genus *Dotilla* based on 16S and/or COI markers. A molecular phylogeny could be compared to the attempted morphological phylogeny to see whether the topologies are similar, and whether they correspond to the biogeographical distributions of the *Dotilla* species.
- Undertake experimental tests to confirm the hypotheses generated from this study concerning how *Dotilla intermedia* was distributed on the shore. Exclusion pens could be used to move crabs outside the *Dotilla* zone and their fitness monitored over time (e.g. by measuring self-righting time). Within the *Dotilla* zone, small crabs could be moved higher up the beach, and the larger crabs moved lower down the shore, and their fitness measured over time.
- Establish the reproductive cycle of *Dotilla intermedia* by running a year-long study with monthly sampling for a representative sample of the population on Prapas beach. Crabs should be measured, sexed and the number of ovigerous females counted to allow examination of changes in population size-frequency distributions over time, and whether there are any peaks in reproductive activity associated with the monsoon

- Expand on the population genetic study by increasing the number of analysed specimens at each time point. In addition, samples collected from more recent time periods could be added to the time series to elucidate the trends in the data. More recent samples from Bang Ben could also be analysed and compared to Prapas beach to see whether the populations are becoming more or less similar with time.

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APPENDIX I

KEY TO THE GENUS *DOTILLA* (FROM KEMP 1919)

1. Carapace as long as broad, except for the lateral grooves practically devoid of sculpture; chelipeds at least three times the length of the carapace. Tympana on all segments of the sternum ***D. myctiroides***

Carapace broader than long, its surface strongly sculptured; chelipeds at the most little more than twice the length of carapace. **2.**

2. Groove parallel to lateral margin of carapace anteriorly bifurcated or Y-shaped **3.**

Groove parallel to lateral margin of carapace simple, not bifurcated anteriorly **6.**

3. Two long parallel Λ-shaped grooves on dorsum of carapace, the lower enclosing a large triangular plane area with base occupying the whole of the posterior margin **4.**

No parallel Λ-shaped grooves on dorsum of carapace; a cardio-intestinal area (much narrower than posterior margin) defined by lateral grooves **5.**

4. Tympana present on second and third segments of sternum; fingers of chela longer than palm, each in the adult male with a large tooth on its inner edge. ***D. fenestrata***

No tympana on sternum; fingers of chela not longer than palm and without large teeth ***D. sulcata***

5. Gastric area triangular; a faint transverse groove near posterior margin; dactylus of last leg not one and half times as long as propodus ***D. pertinax***

Gastric area pentagonal; no posterior transverse groove; dactylus of last leg twice as long as propodus ***D. malabarica***

6. Gastric and cardiac areas entire, not divided by a median longitudinal groove; transverse groove near posterior margin incomplete in the middle; no lobules isolated by grooves on gastric region; adult male with a tooth below orbital angle and a strong compressed tubercules on inner and proximal aspect of carpus of chelipeds; tympana present on all segments of sternum ***D. wichmanni***

A deep mid-dorsal groove extending from front to posterior margin; transverse posterior groove complete; 4 (or 5) small lobules on gastric region isolated by grooves; no tooth below orbital angle and no tubercule on carpus of chelipeds; no tympana on sternum **7.**

7. Only a single oblique groove running from side of the cardiac region towards postero-lateral angle; lower surface of palm not carinate ***D. blanfordi***

Two oblique grooves running from side of cardiac region towards postero-lateral angle; lower surface of palm strongly carinate ***D. intermedia***

APPENDIX II

DIAGRAMMATIC VIEWS OF GENERALISED CRABS USED IN TAXONOMIC DESCRIPTIONS (FROM SHEN 1931).

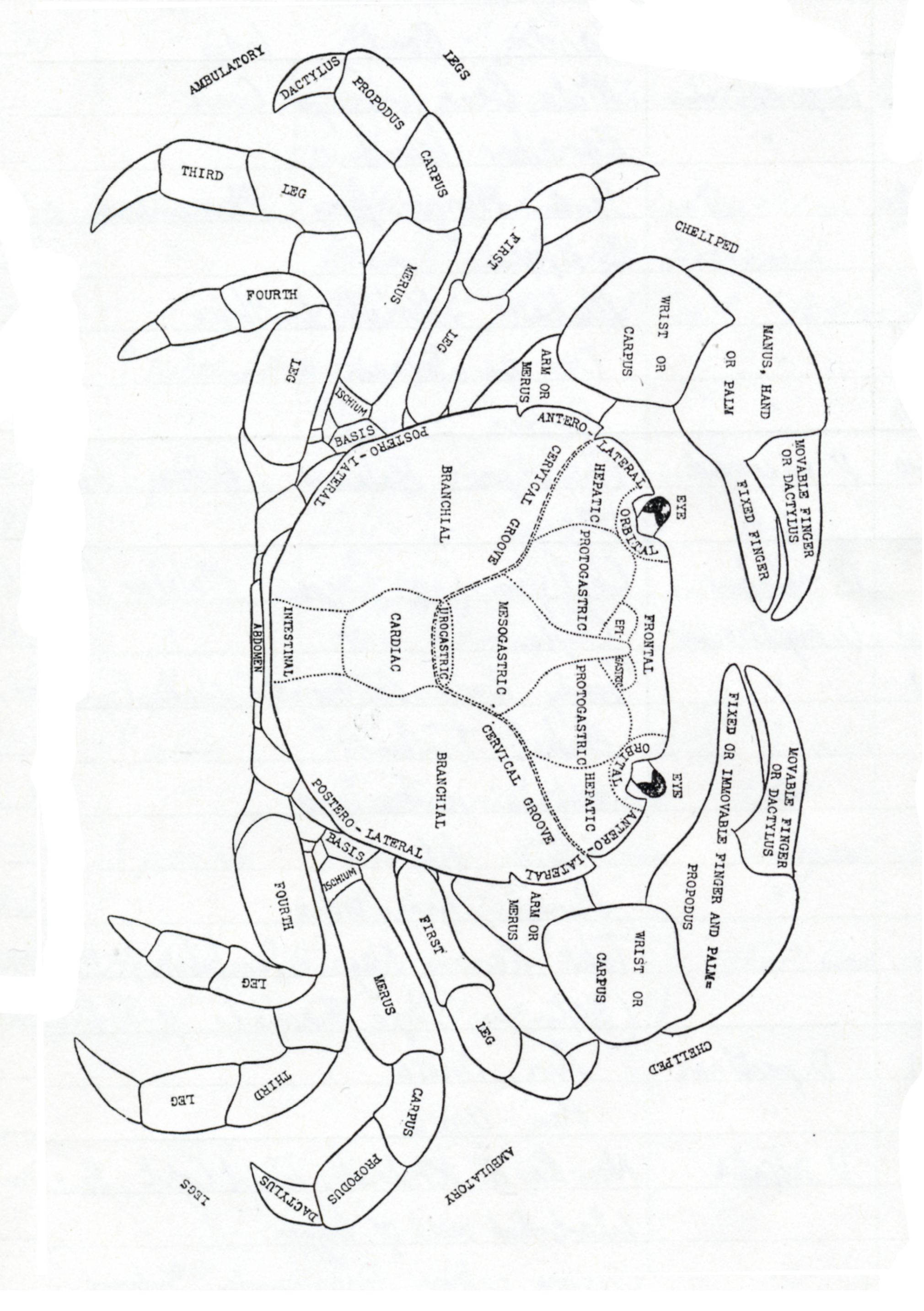

Fig. II.1. Diagrammatic dorsal view of a crab, showing terms used in taxonomic descriptions in Chapter 3.

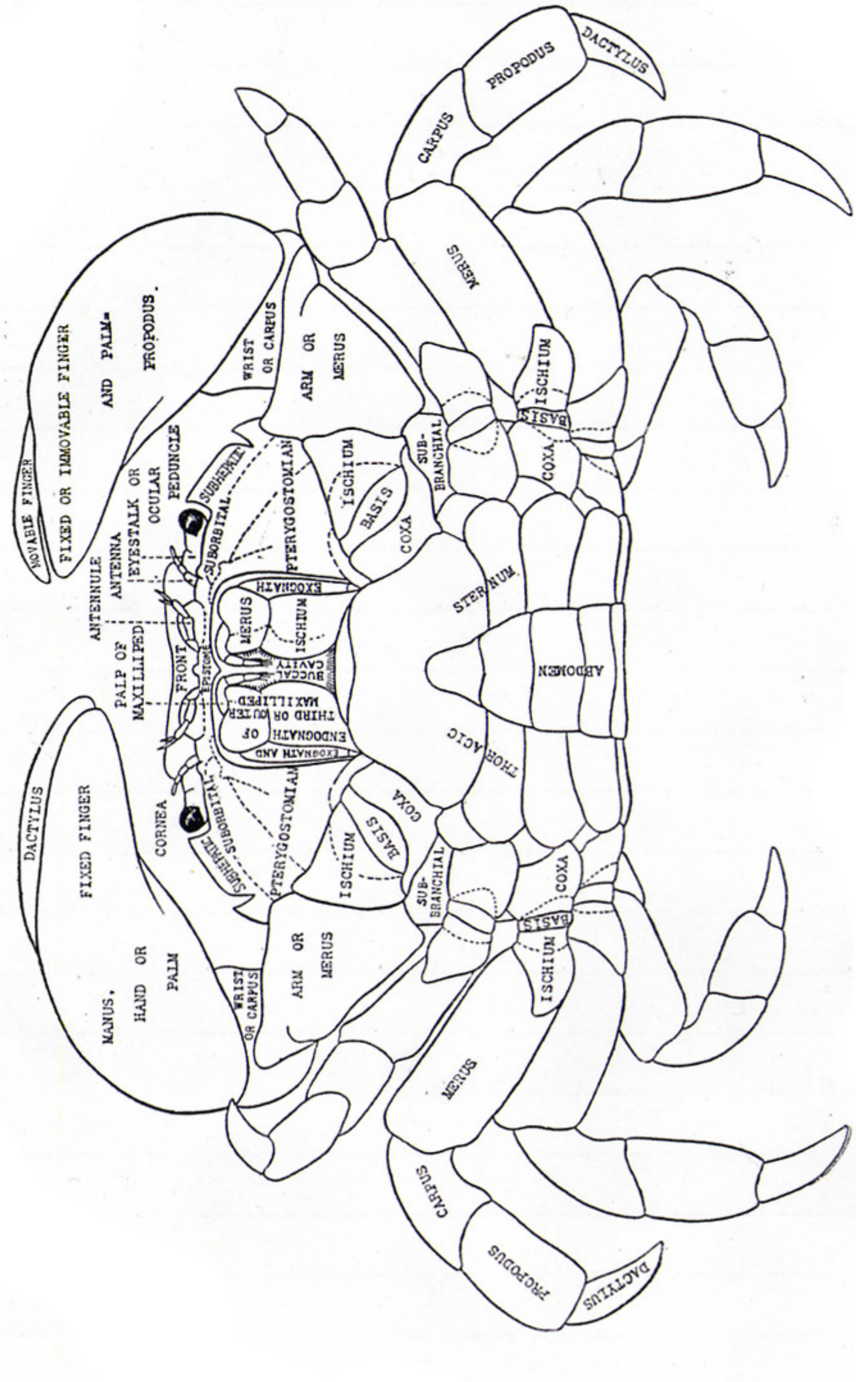

Fig. II.2. Diagrammatic ventral view of a crab, showing terms used in taxonomic descriptions in Chapter 3.

APPENDIX III

MOLECULAR PHYLOGENY FOR *DOTILLA* SPECIES FROM THAILAND

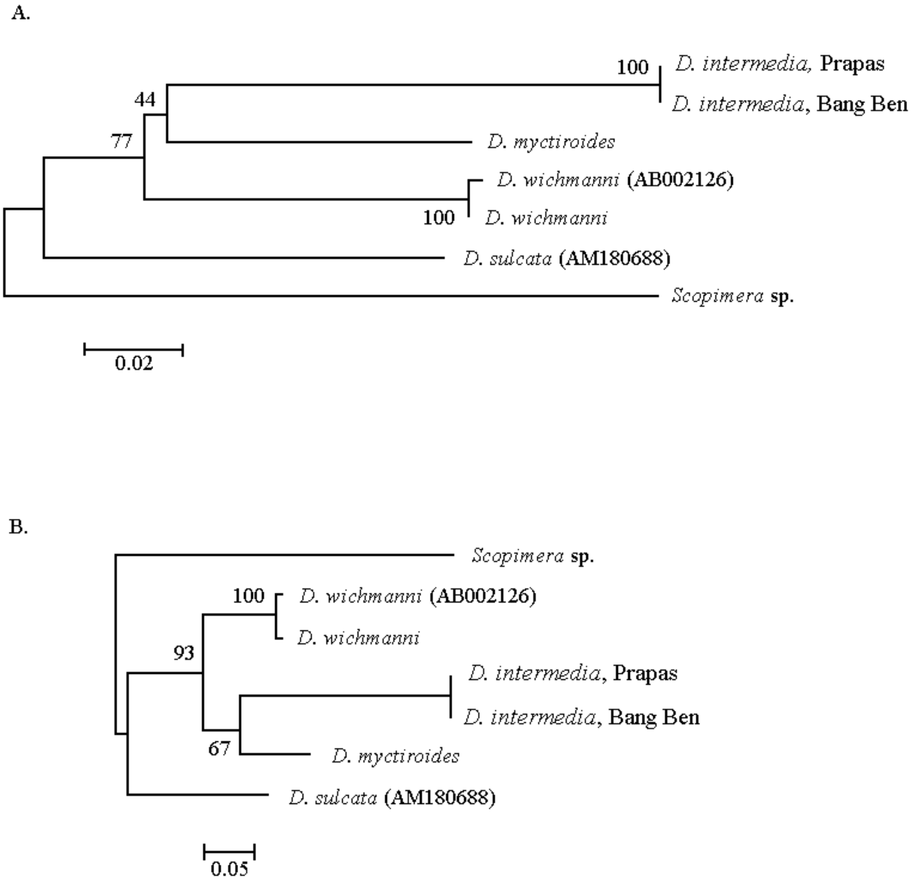

Fig. III.1. Phylogenetic trees for 16S gene, with *Scopimera* sp. as an outgroup.

A. Neighbour-joining tree. Numbers on branches are bootstrap proportions;

B. Bayesian Inference tree. Numbers on branches indicate posterior probability values

APPENDIX IV

LIST OF PUBLICATIONS

The following manuscript stems from the research undertaken during this PhD and is in press at the time of thesis submission. A copy of the manuscript can be found on the following pages.

Allen CJ, Paterson GLJ, Hawkins LE, Hauton C, Clark PF, Aryuthaka C (2010) Zonation on sandy tropical beaches: a case study using *Dotilla intermedia* (Brachyura: Ocypodidae). Marine Ecology Progress Series 408: 97-107

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Allen CJ, Paterson GLJ, Hawkins LE, Hauton C, Clark PF, Aryuthaka C

Zonation on sandy tropical beaches: a case study using *Dotilla intermedia* (Brachyura: Ocypodidae)

Christopher J. Allen[1, 2, *], Gordon L. J. Paterson[2], Lawrence E. Hawkins[1], Chris Hauton[1], Paul F. Clark[2], Chittima Aryuthaka[3]

[1]School of Ocean and Earth Science, University of Southampton, National Oceanography Centre, Southampton SO14 3ZH, UK
[2]Department of Zoology, The Natural History Museum, London SW7 5BD, UK
[3]Department of Marine Science, Kasetsart University, Band Khen, Bangkok 10900, Thailand

ABSTRACT: The factors underlying intertidal zonation patterns of fauna on exposed sandy beaches were investigated and focussed on the crab *Dotilla intermedia.* Dotillids form dense populations on sandy beaches across the Indo-Pacific region and play an important role in processing superficial sediment. Analyses from 2 study sites indicate that the gradient of the beach slope was correlated to the height from the high water mark at which the boundaries of the crab zones occurred, with physical factors associated with the beach gradient influencing the distribution of *D. intermedia* on the beach. The upper limit of the dotillid zone was controlled by the total water content of the sediment, with *D. intermedia* absent in areas with total water content less than 15%. Tidal influences defined the lower boundary of the dotillid zone, with crabs requiring an area with a minimum exposure time between tidal immersions (4 to 5 h) to feed on the sediment. Within the dotillid zone, the mid and low shore regions were the most densely populated. There were differences in the distribution of *D. intermedia* according to crab size, with the low shore area dominated by small crabs and larger crabs occurring higher up the beach. In terms of the time constraints between successive tidal immersions, the high shore was a more preferential area for large crabs to live in, giving them greater time to feed, construct burrows and engage in other activities, leaving low shore areas open for the settlement of juveniles.

KEY WORDS: Zonation · *Dotilla intermedia* · Sandy beach ecology · Autoecology

INTRODUCTION

Exposed sandy beaches are amongst the harshest aquatic ecosystems on Earth (McLachlan et al. 1993). In these physically controlled environments biological interactions are minimal and communities are structured by the independent responses of individual species to the physical environment, as suggested by the autoecological hypothesis (Noy-Meir 1979, McLachlan 1990). Experimental studies have shown an increase in community level descriptors from harsh to more benign beaches (e.g. McLachlan et al. 1981, 1993, Jaramillo et al. 1995), matching the predictions of this hypothesis. The review by McLachlan & Dorvlo (2005) indicates that the most recent studies of sandy beach ecology have correlated the physical shore attributes with biotic community level indices, such as species diversity, total abundance and biomass. However, these biotic measures reflect the summation of the responses of individual populations. The investigation of the relationship between the distribution of key species and their physical environment can provide an insight into the processes that structure community level patterns. The present study departs from the usual community level approach to beach ecology by investigating the autoecology of a key macrofaunal species of a tropical sandy shore.

The present study examines the autoecological zonation of a littoral crab species, *Dotilla intermedia* De Man 1888, on 2 exposed intermediate beaches from

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*Email: chris.allen@noc.ac.uk

the Laem Son National Park in Ranong Province, Thailand. Crabs of the genus *Dotilla* are ecologically important members of tropical sandy shore intertidal communities. Dotillid crabs are found on tropical shores and mudflats from East Africa and the Red Sea eastwards to Japan (Alcock 1900). They live at high densities on beaches, with reports of numbers reaching over 500 individuals m^{-2} (Hartnoll 1973, Hails & Yaziz 1982). *Dotilla* spp. construct simple burrows in which they remain whilst submerged by the tide. The burrow acts as an important refuge from predators and environmental exposure such as heat irradiation and desiccation (Warner 1977). After the tide has receded the crabs emerge to feed on organic detritus from the surface layer of the sediment (Tweedie 1950). Crabs feed in a radial pattern centred on the burrow, always leaving a path clear of feeding pellets to allow retreat back into their burrow (Luschi et al. 1997). The burrowing and feeding activities of the crabs at low tide result in the rapid turnover of the sediment (Fishelson 1983, Bradshaw & Scoffin 1999), which has a significant effect on the meiofaunal community by preventing colonisation of species not adapted to intense surface disturbance and lowering numbers of harpacticoid copepods (Olafsson & Ndaro 1997). Whilst several dotillid species, such as *Dotilla myctiroides* (e.g. Tweedie 1950, Hails & Yaziz 1982, Takeda et al. 1996, Bradshaw & Scoffin 1999) and *D. fenestrata* (e.g. MacNae & Kalk 1962, Hartnoll 1973, Gherardi et al. 1999), have been extensively examined, there have been very few studies on *D. intermedia*.

In this study, we investigated which physical factors control the boundaries of the zone inhabited by *Dotilla intermedia*. We examined the relationship between the beach gradient and the extent and position of the dotillid zone and looked at which physical factors associated with the beach gradient may be responsible for the observed patterns. We also examined whether there was any evidence of size segregation within the dotillid zone and whether this in turn is related to available foraging time (which is governed by tidal immersion and exposure), population density or sediment particle size and organic content.

MATERIALS AND METHODS

Study area. Two exposed oceanic intermediate sandy beaches in the Laem Son National Park, Ranong Province, Thailand, were examined: Prapas Beach (9° 21′ 57 N, 98° 23′ 41 E) and Bang Ben (9° 36′ 11 N, 98° 27′ 56 E). Both beaches consist of moderately sorted fine sand, with Prapas Beach possessing a steeper slope than Bang Ben (3.69 and 2.26%, respectively). The macrofaunal assemblages on these beaches were characterised by the presence of the crabs *Scopimera* sp. and *Ocypode* sp. in addition to *Dotilla intermedia*, together with the tubes of the polychaete *Diopatra* sp. On each beach, the dotillid zone runs from approximately the mean high water neap to the mean low water neap, with a zone of *Scopimera* sp. occurring higher on the shore.

Data collection and analysis. Transects were used to construct beach profiles and delimit species boundaries. Transects were established perpendicular to the shoreline, running from the extreme high water spring (EHWS) mark to low water. Starting at EHWS, the vertical drop in height (cm) was measured every metre. The heights at which the boundaries of the *Scopimera* sp. zone and the dotillid zone occurred were recorded for each transect. Transects were spaced 200 m apart to capture changes in topology and heterogeneity across the beach. To ensure the same level of exposure, all transects on a beach were measured at low water during a single spring tide. Five transects were measured on Prapas Beach in November 2007 and were repeated in April 2008. Only 3 transects were measured on Bang Ben in April 2008, as fewer transects could be completed over one tidal cycle on the wider beach. The sampling periods were timed to avoid the monsoon season. Dotillid crabs retreat down their burrows during rainfall, which also acts to destroy their feeding pellets, thus leaving no trace as to which area on the beach the crabs occupy.

Quadrats (0.25 m^2) were placed at approximately 2 m intervals across the dotillid zone on each transect. The numbers of crabs present in each quadrat were counted and the quadrat was photographed with a Canon EOS 400D digital SLR camera. These photographs were then analysed using MapInfo Professional v. 9. For each burrow in a quadrat, the burrow diameter and the area in which each resident crab fed were measured, along with the distance to the next nearest burrow.

Ocypodidae crab carapace width (CW) is closely related to burrow diameter (BD) (Zwarts 1985, Dray & Paula 1998, Lee & Lim 2004), with the exact relationship varying amongst species. A preliminary study was conducted to establish the relationship between CW and BD for *Dotilla intermedia.* The diameters of 30 burrows, covering a range of sizes, were measured to the nearest 0.01 mm with a pair of digital vernier calipers, along with the CW of the resident crab. BD was regressed against CW. There was a highly significant linear relationship between crab CW and BD (r^2 = 0.934, $p < 0.0001$); therefore, BD was used as a proxy for CW in analysing body size (Fig. 1).

The dotillid zone on each beach (Prapas in 2007 and 2008 and Bang Ben in 2008) was split into 3 equal areas (upper, middle and low) and crab size frequency distri-

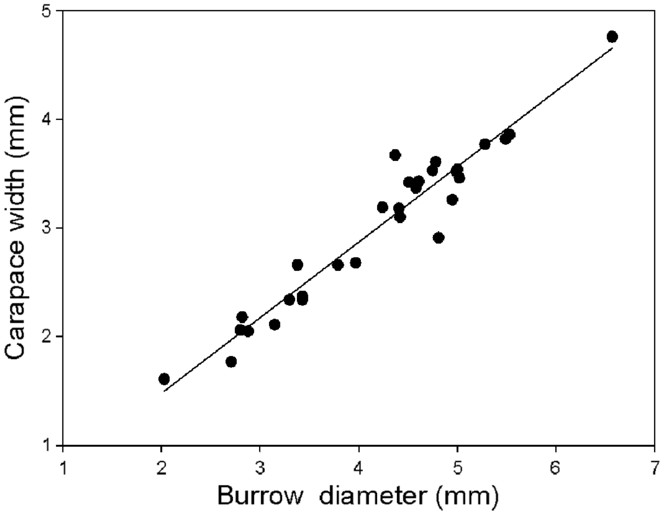

Fig. 1. *Dotilla intermedia.* Linear regression of burrow diameter (mm) plotted against resident crab carapace width (mm). Carapace width = 0.6952 × burrow diameter + 0.0878, r^2 = 0.934, n = 30

butions were constructed from the quadrat data from each area. Kolmogorov-Smirnov tests (Sokal & Rohlf 1995) were used to compare the size frequency distribution between each of the areas on each beach and to compare distributions of each area with its respective area on the other beaches.

Mean crab CW and the mean feeding area within each quadrat were log transformed and correlated using Pearson's correlation coefficient. The nearest neighbour distances were used to analyse the spatial patterns within each quadrat using an established method (Clark & Evans 1954), generating an R-value for each quadrat. The R-value takes a range between 0 and 2.1, and describes the spatial pattern within an area. An R-value of 0 indicates an aggregated distribution, whilst an R-value of 1 shows a random distribution, and a value of 2.1 demonstrates a regular distribution. The R-value was compared with the density within each quadrat.

Sediment samples were collected in April 2008 from both Prapas Beach and Bang Ben. Cores of 5 cm diameter were taken to a depth of 10 cm at 5 points along each transect: above the dotillid zone (1 m above the upper edge of the dotillid zone); high dotillid zone (1 m inside the high shore edge); mid-dotillid zone; low dotillid zone (1 m inside the low shore edge); and below the dotillid zone (1 m outside the low shore edge). Samples were weighed and oven dried at 60°C to a constant weight. The total water content of the sediment was measured as the percentage weight loss of the sample. The dried samples were then sieved through a sieve stack (2 down to 0.064 mm mesh sizes) and each fraction weighed separately. The Gradistat program (Blott & Pye 2001) was used to calculate the average sediment grain size (MDΦ) and the sediment sorting (QDΦ) from each sample. Surface sediment scrapes of 3 mm depth, representing the depth of sediment that *Dotilla* spp. processes when feeding (Luschi et al. 1997), were taken to analyse organic content. Each sample was oven dried at 60°C to a constant weight before being placed into a muffle furnace at 450°C for 6 h. Samples were then reweighed, with the percentage weight loss representing the organic content of the sediment. Data were arcsine transformed, and nested ANOVA tests were used to see whether there were any significant differences in mean sediment total water content and mean sediment surface organic content between the different areas of the dotillid zone and between the 2 different beaches.

Principal component analysis (PCA) (Pearson 1901) was used to examine which environmental variables were most important in controlling the distribution of *Dotilla intermedia* on the beaches, with sediment data from Prapas Beach and Bang Ben collected in 2008 combined together. Four variables were examined: water content of the sediment, surface sediment organic content, MDΦ and QDΦ, with each variable taken from the above, high, middle, low and below areas of the dotillid zone on each transect.

RESULTS

Boundaries of the dotillid zone

The gradient of the beach calculated from each transect had a significant relationship with the height of the upper edge of the dotillid zone ($p < 0.001$) from Prapas Beach in November 2007 and April 2008, and from Bang Ben April 2008. The lower boundary of the dotillid zone was also significantly related to the beach gradient ($p < 0.005$). The r^2 values from the linear relationship show that the beach gradient is more closely related to the height of the high shore boundary of the dotillid zone than to that of the low shore boundary (Fig. 2).

Sediment water and organic content

The mean total water content of the sediment increased from above the high water boundary of the dotillid zone to the low water boundary (Fig. 3). Nested ANOVA showed that there was a significant difference between the water content of the 5 areas of the dotillid zone ($F_{4,5}$ = 42.079, $p < 0.001$) and that there was no significant difference between Prapas Beach and Bang Ben within each beach area ($F_{5,30}$ = 0.820, not significant). Holm-Sidak pairwise comparisons (Sidak 1967)

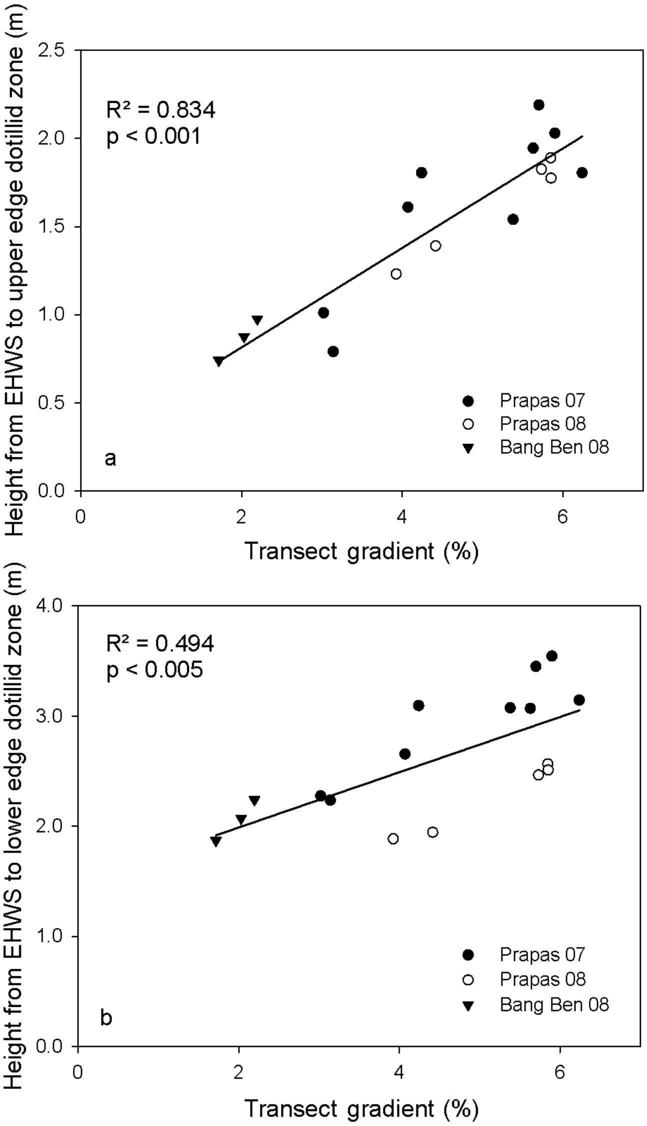

Fig. 2. Transect gradient plotted against the height from extreme high water spring (EHWS) of the boundaries of the dotillid zone. (a) Upper dotillid boundary. (b) Lower dotillid boundary. Height of upper edge dotillid zone = 0.2822 × transect gradient + 0.2501. Height of lower edge dotillid zone = 0.2508 × transect gradient + 1.4867

showed that there was no significant difference between the mean water content from the low and below areas of the dotillid zone (~22.5%). *Dotilla intermedia* was only present in areas where total water content of the sediment was in excess of 15%.

Mean total surface organic content of the sediment was also significantly different between the 5 areas of the dotillid zone ($F_{4,5}$ = 37.578, p < 0.001), with no significant difference found between Prapas Beach and Bang Ben within each area of the dotillid zone ($F_{5,30}$ = 1.545, not significant). However, when looking at the pairwise comparison, the only significant difference occurred between the above zone and below zone samples. The mean surface organic content increased from the high shore to the low shore, although the surface organic content was highly variable across the dotillid zone (Fig. 4).

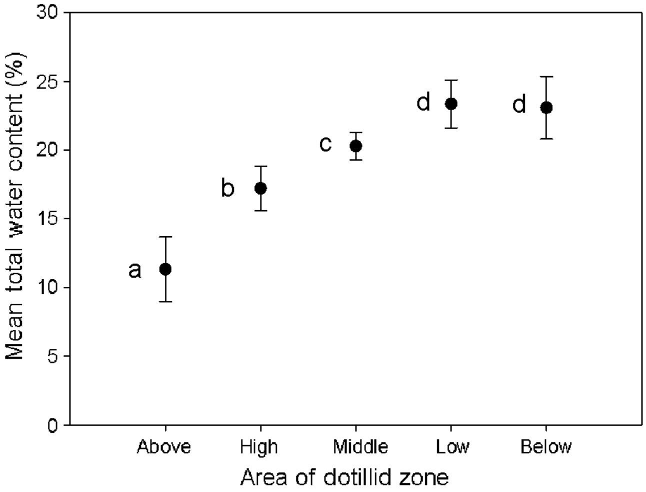

Fig. 3. Sediment mean total water content from different areas of the dotillid zone from Prapas Beach and Bang Ben 2008. Error bars represent ±1 SD, n = 8. Different lower case letters denote statistically significant groups

Principal component analysis

The first 2 principal components (PC) were responsible for controlling the majority of the variation between the samples, with PC1 and PC2 representing 82.7% and 16.9% of the variation, respectively. PC1

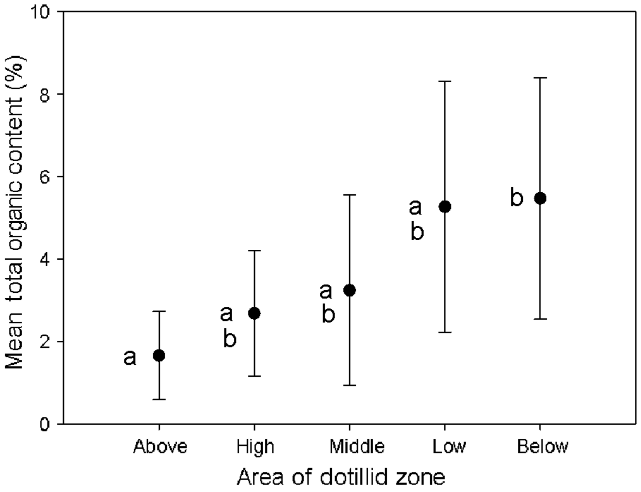

Fig. 4. Sediment mean total surface organic content from different areas of the dotillid zone from Prapas Beach and Bang Ben 2008. Error bars represent ±1 SD, n = 8. Different lower case letters denote statistically significant groups

was dominated by the influence of the total water content of the sediment, whilst the total organic content of the surface sediment was the most important vector in PC2. On a plot of PC1 against PC2 (Fig. 5) the samples from above the dotillid zone clustered together, and there was some grouping with the high and middle zone samples, although they were not as clearly defined as the above zone samples. The low and below zone samples appeared to be poorly separated from each other, suggesting that a variable not examined in the PCA was responsible for defining the lower limit of the dotillid zone.

The total water content of the sediment dominated the separation of the samples by their relative position within the dotillid zone (i.e. above, high, middle, low and below). There was a large amount of variation in the total surface organic content of the sediment, which differentiated between samples from within the same section of the dotillid zone, rather than between the different areas of the dotillid zone. Sediment grain size and sorting represented very little of the observed variation in the zonation of *Dotilla intermedia*.

Size segregation

Beach profiles (mean profile of all transects from each beach) for Prapas 2007, Prapas 2008 and Bang Ben 2008, along with crab CW size-frequency distributions for each area of the dotillid zone (upper, middle and low) are shown in Fig. 6. Kolmogorov-Smirnov tests showed significant differences ($p < 0.001$) between the size-frequency distributions of *Dotilla intermedia* from the upper, middle and low shore areas on each beach. The size distribution in the upper area was skewed towards crabs with a large CW, whilst the low shore area was characterised by a distribution skewed towards smaller CW. The middle area demonstrated a normal distribution in crab CW size-classes. The mean and median CW for each area of the dotillid zone decreased from the upper shore towards the low shore on each beach (Table 1).

There were also significant differences ($p < 0.001$) between the size-frequency distributions of the same shore areas from the different beaches. There was a higher percentage of larger size-classes at all of the shore heights in Prapas 2007 compared with Prapas 2008, whilst Bang Ben was occupied by even larger crabs.

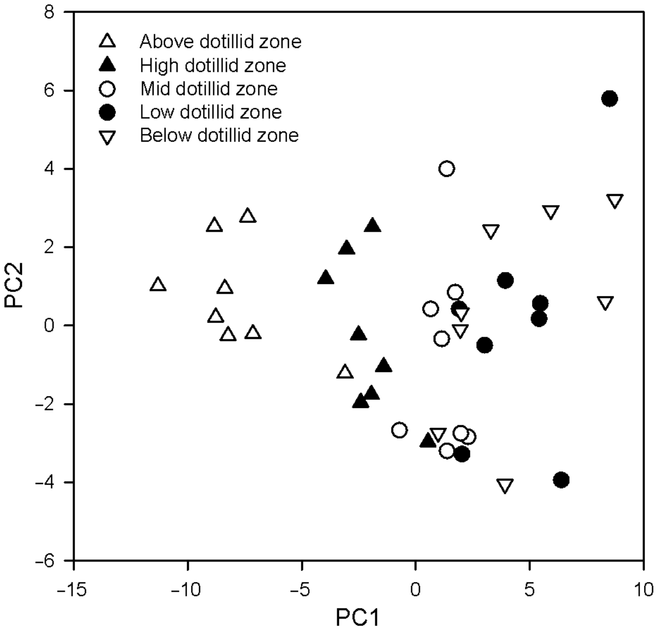

Fig. 5. Principal component 1 (PC1) versus PC2 from Prapas Beach and Bang Ben April 2008

Population density, crab size and feeding

The density of *Dotilla intermedia* within each 0.25 m^2 quadrat had a close relationship with the mean size of the feeding area of the crabs. As density increased, the size of the mean feeding area decreased (Fig. 7). The upper limit of the mean feeding area followed an inverse power curve that designated a maximum size to the feeding area at a given density of *D. intermedia* (maximum mean feeding area [cm^2] = 425 × density crabs$^{-0.695}$). Thus, at a density of 100 individuals (ind.) 0.25 m^{-2} each crab would have a mean maximum-sized feeding area of 17.3 cm^2, whilst 50 ind. 0.25 m^{-2} would have a maximum mean feeding area of 28 cm^2 each. The values falling under this curve may have resulted from starting the sampling before the crabs had had enough time after tidal emersion to feed sufficiently to reach the maximum extent. There was a significant relationship between the log mean CW and the log mean feeding area within a quadrat (Pearson correlation coefficient = 0.713, $p < 0.0001$). The size of the mean feeding area increased as the mean CW in a quadrat increased (Fig. 8).

Nearest neighbour analysis

At low densities, the R-value showed that crabs had a regular distribution in each quadrat. As density increased, the R-value decreased, with distribution becoming more random. By an abundance of around 10 ind. 0.25 m^{-2} the R-values had stabilised at around 1.3 to 1.5, representing a random distribution with a slight trend towards regular spacing within each quadrat, as opposed to aggregation. These trends were common from both beaches in 2007 and 2008 (Fig. 9).

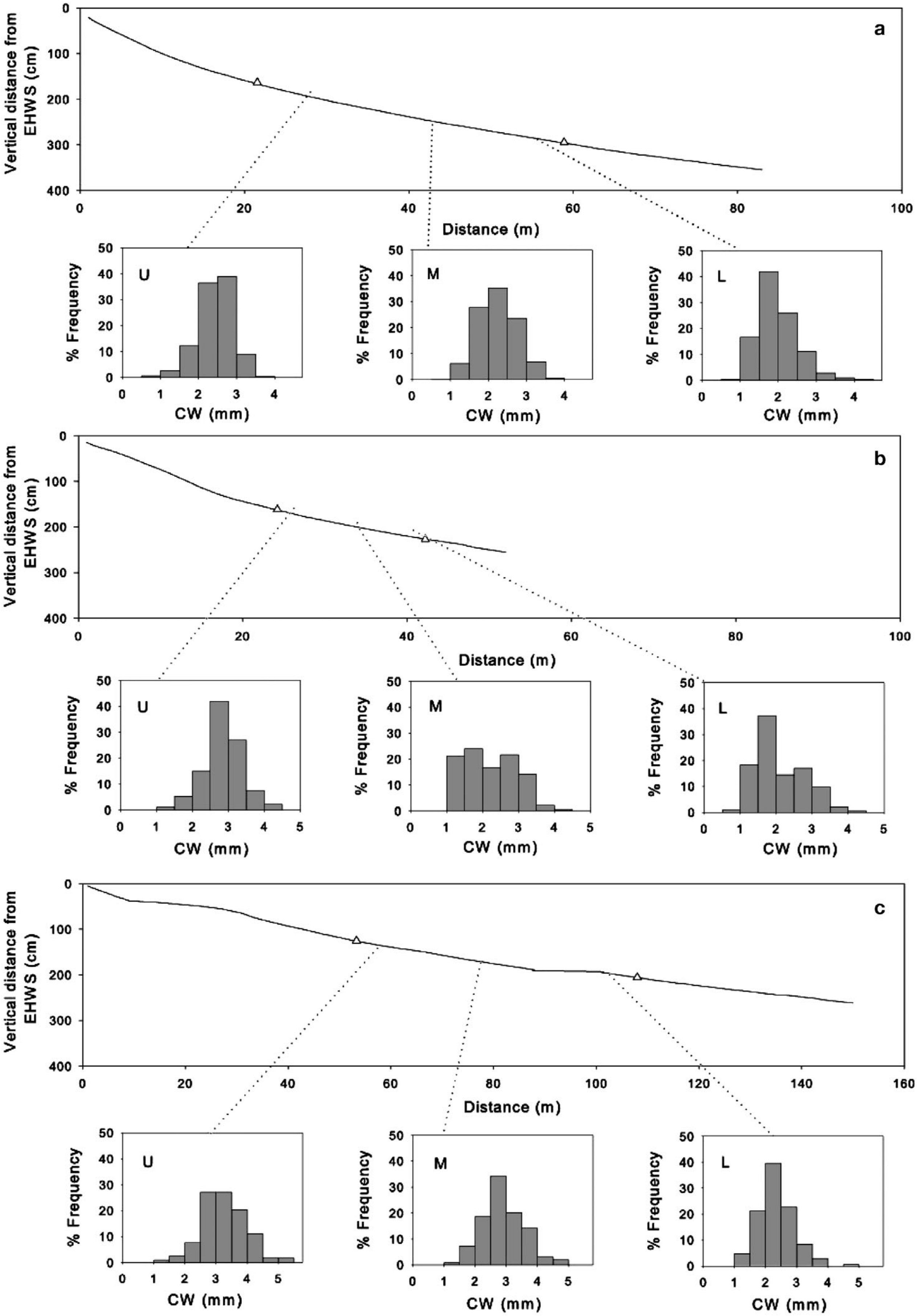
a
b
c
Vertical distance from EHWS (cm)
Distance (m)
0
100
200
300
400
0
20
40
60
80
100
120
140
160
U
M
L
% Frequency
CW (mm)
0
10
20
30
40
50
1
2
3
4
5

Fig. 6. *Dotilla intermedia.* Carapace width (CW) size-class frequency at different beach zones. (a) Prapas Beach 2007; (b) Prapas Beach 2008; (c) Bang Ben 2008. Beach profiles represent the mean profile of all transects for that beach. Triangles denote the boundaries of the dotillid zone on each beach. Histograms show CW distribution in 3 areas of the dotillid zone: upper (U), middle (M) and lower (L)

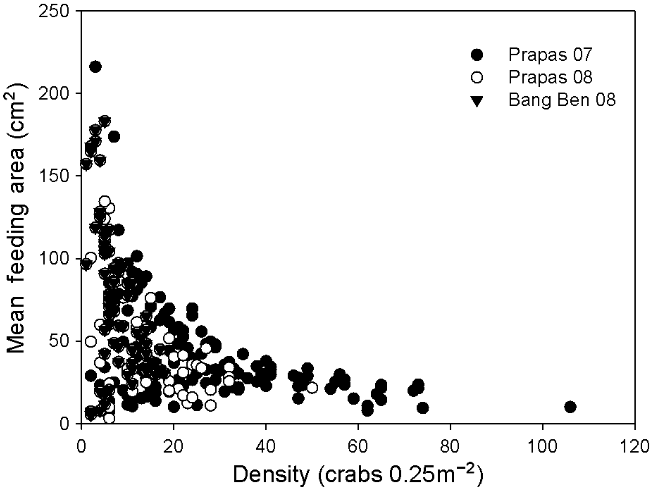

Fig. 7. *Dotilla intermedia.* Density of crabs plotted against mean feeding area within a quadrat (0.25 m^2)

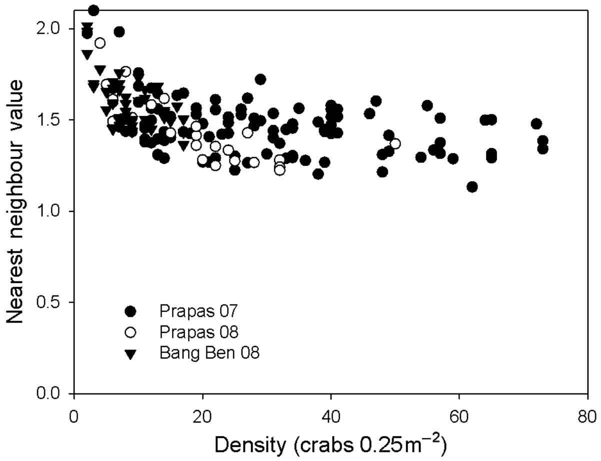

Fig. 9. *Dotilla intermedia.* Density of crabs per quadrat (no. crabs 0.25 m^{-2}) plotted against R-value

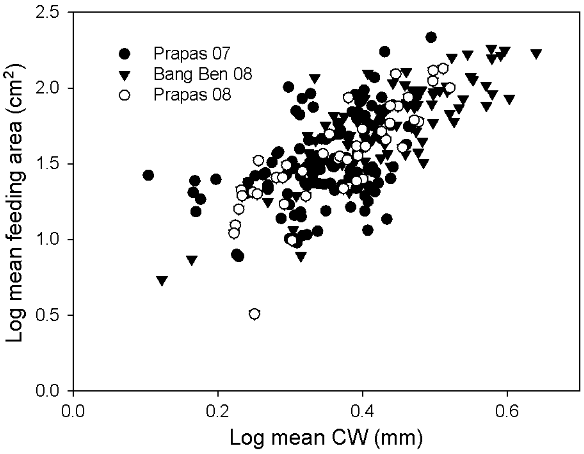

Fig. 8. *Dotilla intermedia.* Log mean crab carapace width (CW) plotted against log mean feeding area within a quadrat (0.25 m^2)

Table 1. *Dotilla intermedia.* Mean and median carapace width (mm) for the upper, middle and lower areas of the dotillid zone on Prapas Beach 2007and 2008 and Bang Ben 2008

Area of dotillid zone	Prapas Beach 2007		Prapas Beach 2008		Bang Ben 2008	
	Mean	Median	Mean	Median	Mean	Median
Upper	2.43	2.46	2.83	2.85	3.25	3.23
Middle	2.23	2.19	2.21	2.17	2.89	2.84
Lower	1.98	1.90	2.06	1.85	2.34	2.31

DISCUSSION

Dotillid zonation

The heights of the upper and lower boundaries of the dotillid zone were closely related to the gradient of the beach and its associated physical factors. The upper boundary of the dotillid zone was defined by the total water content of the sediment. The absolute height of the upper limit of the dotillid zone is not constant and will change over time. Sediment accumulation or removal will alter the beach gradient, which will change the water table height and, in turn, affect the water content of the sediment at a given point on the beach.

Dotilla intermedia were only found in sediments with a water content of 15% or higher. One of the distinguishing features of dotillid crabs is the presence of a band of setae on the 4th abdominal segment that function in water uptake. The abdomen can be lowered, pressing the setae into the sand allowing the uptake of interstitial water (Hartnoll 1973). The presence of such an adaptation indicates an evolutionary importance of water to dotillid crabs. Both *Scopimera* spp. and *Dotilla* spp. possess membranous disks, known as 'tympana' or gas windows, on the meral segments of their legs. Tympana are respiratory surfaces that function in aerial gas exchange (Maitland 1986). The tympana in *Scopimera* are thinner than in *Dotilla*, allowing *Dotilla* to have relatively greater respiratory efficiency, but at the cost of poorer water and ion retention (Matsumasa et al. 2001). *Dotilla* may be restricted to sediments with greater water content to offset the increased rate of water loss through the tympana, whereas *Scopimera* are better adapted to survive higher up the beach where the sediment water content is lower. The increased respiratory efficiency of *Dotilla*, combined with the presence of water uptake setae to balance water loss, may allow members of this genus to spend more time on the surface without returning to their burrows compared with those of *Scopimera*. This would maximise the time available between emersion and immersion by the tide, thus permitting *Dotilla* to occupy areas lower down the beach than *Scopimera*. Examining a single species like *D. intermedia* highlights the importance of species physiology on community structure, which may be lost when examining sandy beach ecology only at the community level.

Mean surface organic content decreased higher up the shore, although there were no significant differences between the values found in the areas occupied by *Dotilla intermedia* and those measured in the area above the dotillid zone. Despite low surface organic content, the high shore area above the dotillid zone was still inhabited by *Scopimera* sp., which feeds in an identical manner to dotillid crabs (Zimmer-Faust 1987). If the organic content of the sediment was sufficient to support *Scopimera* sp., then it would be unlikely that *D. intermedia* could not survive in this area, supporting the hypothesis that surface organic content was not a factor controlling the upper limit to the dotillid distribution.

The upper limit to the dotillid zone could potentially be defined by competition with *Scopimera*. However, no direct interactions were observed between the 2 species during the investigation (C. J. Allen pers. obs.). The very low densities at which both *Scopimera* sp. and *Dotilla intermedia* occurred on the high shore made competition for space or resources unlikely. Nevertheless, further work involving exclusion pens and transplanting dotillid crabs into the *Scopimera* zone and vice versa would clarify the role competition plays, if any, on delimiting the upper boundary of the dotillid zone.

The lower boundary of the dotillid zone was governed by the tide. The activities that *Dotilla intermedia* can perform are limited by the time between each successive immersion by the tide. The area on the shore occupied by *D. intermedia* needs to have sufficient time available between tides for individuals to feed (Hartnoll 1973, Fishelson 1983) in addition to engaging in other behaviours, such as burrow maintenance, mating and territorial displays (Gherardi et al. 1999); *D. intermedia* must therefore live a certain distance from the low water mark. The lower edge of the dotillid zone will shift according to the spring neap tidal cycle. By looking at the vertical height from the lower edge of the dotillid zone to the low water mark and examining the tide tables for the region, we determined that the lower limit of the dotillid zone is exposed for approximately 4 to 5 h between successive immersions.

Bradshaw & Scoffin (1999) concluded that the main factor governing the distribution of *Dotilla myctiroides* in Tang Khen Bay, Phuket, was the duration of the daytime exposure (i.e. the amount of time the crabs were emersed between tides). The crabs occupied the tops of sand ripples in the bay, with pools of water present on either side of each ripple. This potentially kept the water content of the sediment sufficiently high so it did not become a limiting factor in the species distribution, leaving the period of tidal emersion as the driving factor.

Inhabiting an area too close to the low water mark imposes other physical environmental restrictions for *Dotilla intermedia*. The development of suction, *s*, (i.e. negative pore water pressure relative to atmospheric pressure) is an essential threshold condition for the creation of burrows by *Scopimera globosa*, with burrowing performance depending strongly on the state of *s* in association with the groundwater level (Sassa & Watabe 2008). In low water areas, the value of *s* is not favourable for the construction of the semi-permanent cylindrical burrows found higher on the shore, thus resulting in the formation of 'igloo' style burrows instead. A number of *Dotilla* species have been reported to construct 2 different forms of burrow: simple vertical cylindrical tubes, and 'igloo'. An igloos is formed by the crab rotating in the sand beneath the surface and forming a wall of sand pellets around and over itself and is built in semi-fluid unstable sand (Takeda et al. 1996). A higher percentage of igloos were seen in the low shore than higher on the beach (C. J. Allen pers. obs.).

Sediment grain size and sorting was not responsible for controlling where *Dotilla intermedia* were found on the beach. Sediment particle size was not a critical factor in defining the zonation of *D. myctiroides* from Porto Novo in India, with individuals found in sediment with the silt–clay fraction ranging from 0.3 to 30% (McIntyre 1968). However, it is likely that sediment grain size would be the ultimate limiting factor in dictating which beaches would be suitable for *D. intermedia* to inhabit. In estuarine areas of the Laem Son National Park where sediment grain size was smaller, *D. intermedia* was replaced by *D. myctiroides.* Sediment grains need to be of a certain size to be sifted through the mouthparts of *Dotilla* spp., and different species favour different sized particles depending on the nature of their spoon-tipped setae (Vogel 1984). The division of habitats by sediment grain size has also been demonstrated in *Uca* (e.g. Lim 2004). However, sediment particles that are too big cannot be fed upon, and beaches need to have sediment types suitable for the construction of burrows (Ansell 1988). To define the limits of sediment particle sizes inhabited by *D. intermedia* further investigation covering more beaches with a wider range of grain sizes would be required.

Surveys were made of other beaches in the region and around Phuket. Those that had coarse sand were not occupied by any dotillid crabs, although those with fine sand were inhabited by *Dotilla myctiroides* (C. J. Allen & G. L. J. Paterson pers. obs.). These observations would match the predictions of current hypotheses concerning how changing beach morphodynamics affect species distributions. As beaches move from dissipative in character (i.e. fine sand, flat slopes, low substrate penetrability and high water content) to reflective (i.e. coarse sand, short swash periods, steep slopes, high substrate penetrability, low organic matter and low sediment water content) species are excluded due to the harsher swash climate (swash exclusion hypothesis; McArdle & McLachlan 1991, 1992) and the coarser sands making the environment more inhospitable (habitat harshness hypothesis; Defeo et al. 2001, 2003).

Dotillid density patterns

The highest observed densities of *Dotilla intermedia* from the present study were from Prapas Beach in 2007 and ranged from 12 to 424 ind. m^{-2}, with an average of 102 ind. m^{-2}. These density counts are lower than those reported for other species, with *D. fenestrata* having density counts of 48 to 564 ind. m^{-2} (Hartnoll 1973) and *D. myctiroides* 178 to 501 ind. m^{-2} (Hails & Yaziz 1982).

The size of the feeding area around each burrow was closely related to crab size and the density of the population. McIntyre (1968) observed that the length of the longest feeding track from the burrow in *Dotilla myctiroides* was crucially important in determining the density of the population. As crab size increased the size of the feeding area also increased, meaning that large crabs lived at lower densities than did small crabs. This would lead to intraspecific competition for space within the dotillid zone. The random to regular distribution indicated by the nearest neighbour analysis suggests that individuals were fitting their feeding areas around one another, probably mediated by territorial interactions between individuals (see Schöne 1968 for descriptions of agonistic displays in *Dotilla*).

Dotillid crabs feed in roughly circular zones. Assuming their feeding territories do not overlap as crab density increases, it would be expected that the nearest neighbour values would begin to demonstrate a trend towards a more regular distribution, as the circular feeding zones arrange around one another to maximise the available space. However, this was not shown by the data. The range of crab sizes that were found at all densities may mask this trend, with a variety of different-sized circular feeding areas increasing the number of possible arrangements in a given area, leading to a variation in nearest neighbour distances and resulting in a more random than regular spatial distribution.

Nutrient replenishment of the sediment varies between tides following the deposition of organic material. This creates patchiness in organic richness both temporally and spatially. Variation in the organic content of the sediment did not appear to have any effect on the distribution of *Dotilla* on the shore. The nearest neighbour data indicated that crabs tended towards a random distribution across the shore rather than being aggregated together as would be expected if they were grouping in the same area to exploit a food patch with high organic content.

Size segregation

There was a difference in the pattern of distribution between large and small crabs within the dotillid zone. The area close to the low water was dominated by smaller crabs, with larger crabs occurring mainly from the mid-shore upwards. This was more pronounced on Bang Ben than on Prapas Beach. Size segregation may possibly act as an evolutionary process to partition space and avoid intraspecific competition for food and space, with each population component having a differential capacity to select a desirable microhabitat (Defeo & McLachlan 2005). The habitat favourability hypothesis (Caddy & Defeo 2003) suggests that under optimal conditions, the centre of a species range may be occupied by larger and more dominant intraspecific competitors for food and space, whereas small individuals (recruits) may be displaced towards suboptimal conditions on one or both extremes of the species distribution range. The optimal area for the large dotillid crabs appears to be the upper area of the dotillid zone, with smaller crabs displaced towards to the low shore. The differences in the size frequency distributions observed between Prapas Beach in 2007 and 2008 result from some individuals growing over time, leading to the higher percentages of the larger size-classes in 2008. The flatter beach profile of Bang Ben led to a wider dotillid zone, providing more space for crabs to feed and attain larger body sizes than on Prapas Beach.

The low water area may be suboptimal for larger crabs as the short exposure time would result in less time to feed. Assuming crabs feed at the same rate regardless of size, larger crabs would need to feed in a larger area and require more time. This may not be a problem for smaller crabs, which may be able to gather enough energy from feeding for the 4 to 5 h before low shore immersion.

The geophysical conditions of the low shore may make the construction and maintenance of semi-permanent vertical burrows more difficult. The vertical burrows of *Dotilla myctiroides* are used in their mating behaviour, with male crabs capturing females and pushing them into their burrows (Tweedie 1950). The inability to construct vertical burrows in the low shore may prevent mating from occurring there, thus restricting mature crabs to areas higher up the beach. For example, *Uca tangeri* prefers the higher tidal zone (Klaassen & Ens 1993). The increased exposure time allows for the construction of deeper burrows, which also provide better predation protection.

Hartnoll (1973) suggested that the adult *Dotilla fenestrata* preferred lower stations where particle size was smaller, with associated higher organic and water content. Very little variation in sediment grain size was observed in the present study, perhaps eliminating this effect in structuring the distribution of the larger adult crabs on the beach. Although the surface organic content did increase towards the low water, it was highly variable across the whole dotillid zone.

The presence of small crabs in all areas of the beach may be due to juveniles settling into any patches available on the shore. Larger crabs avoid the lower shore, which may result in high numbers of small crabs settling there. Small crabs were observed across the entire dotillid zone on Prapas, but there tended to be fewer small crabs in the high shore region. The survival rate of the small crabs on different parts of the beach is unknown, although it is hypothesised to be lower farther up the beach, due to the crabs' reduced ability to cope with the longer levels of exposure in this region (Pellegrino 1984). This has also been observed in other species of *Dotilla* (Hartnoll 1973, Dray & Paula 1998, Flores et al. 2005).

In a wider context, this study provides support for Dahl's (1952) and Salvat's (1964) generalised schemes of sandy shore zonation. Dahl (1952) divided the shore into 3 zones based on the distribution of characteristic crustacean species, whilst Salvat (1964) split the shore into 4 regions based on changes in sand moisture content. The boundaries of some of Dahl's (1952) and Salvat's (1964) zones roughly coincide, showing a correspondence between physical and biological zones on sandy beaches, as demonstrated by the large influence sediment water content has on the distribution of *Dotilla intermedia*.

Although moisture zones do generally coincide with faunal zones, faunal zones are dynamic and vary temporally. The physical boundary of a species distribution depends on the swash and moisture zones on the shore and not on absolute tide level. Therefore, it is better to define such flexible zones on the basis of centres of distribution of characteristic taxa rather than on sharp boundaries defined by Salvat's (1964) sand moisture levels (McLachlan & Jaramillo 1995). The change in the area occupied by *Dotilla intermedia* on Prapas Beach between 2007 and 2008 demonstrates this variability, and why the use of characteristic species is a more robust method of defining beach zones than is sand moisture content.

CONCLUSIONS

The boundaries of the zone inhabited by dotillid crabs were related to the physical factors imposed by the beach gradient. The high shore limit is defined by the total water content of the sediment, with *Dotilla intermedia* absent from sediment with less than 15% total water content. The low shore boundary of the zone is set by the temporal constraints of the tidal cycle, with crabs requiring a minimum amount of time (4 to 5 h) between tidal immersions to feed. Within the dotillid zone, there are patterns of distribution associated with the size of the crabs; larger crabs live higher up the beach than do smaller crabs. The observed distribution of *D. intermedia* is consistent with the predictions of several non-contradictory hypotheses concerning patterns observed on sandy beaches. Size segregation across the beach follows the habitat favourability hypothesis, whilst the absence of any populations on more reflective beaches matches the predictions of the habitat harshness hypothesis. This study demonstrates that the study of a single key species can identify the physical processes that drive zonation patterns on sandy shores and elucidate what underlying factors influence community structure in sandy beach ecosystems.

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