

Impact of Marine Woodborers *Dicyathifer manni*, *Sphaeroma terebrans* and *Cirolana* sp. on the Mangroves of the Kenyan Coast

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ABSTRACT

Three species of woodborers from marine mangrove plants at Mida Creek, Tudor Creek and Gazi Bay along the Kenyan coast are identified. The relationship between the borers and the host mangrove plants (*Avicennia*, *Sonneratia*, *Rhizophora*) are described. Observation of infested plants showed that woodborers - *Dicyathifer manni* (Wright, 1866), *Sphaeroma terebrans* (Bate, 1866) and *Cirolana* sp. occur on submerged parts of roots (prop-roots and pneumatophores), stems and branches. All the observed species appear to have a host preference. Among the two most frequent marine woodborers, *S. terebrans* occurs only on *Avicennia* sp. whereas *D. manni* occurs mostly on *Rhizophora* plants, but also on *Sonneratia*. The marine woodborers deteriorate the vegetation. Therefore, the present study is relevant to the restoration, conservation and management of mangroves.

Key Words: Woodborers; Mangrove Plants; Host Preference; Bio-deterioration

INTRODUCTION

The ability to consume wood as food (xylotrophy) is unusual among animals. In terrestrial environments, termites and other xylotrophic insects are the principle wood consumers while in marine environments wood-boring bivalves and isopods fulfil this role.

The role played by woodborers along the Kenyan coast has not been studied, especially the bio-deterioration of vegetation by various marine woodborers. They are also capable of extending their destructive activities to drift wood and wooden boats. Besides, there is need to monitor woodborer distribution and abundance in the mangroves because of their vulnerability in the event the sea level rises. In case the forests recede or migrate inland, benchmarks can be established against which such changes can be measured keeping these organisms as indicator species. Therefore, the woodborers of mangroves along the coast in Kenya were investigated.

STUDY AREA

Mangrove forests are very interesting intertidal ecosystems, appearing as islands of different shapes, separated from each other by sandy or rocky shores of various sizes. On the Kenyan coast, mangroves are distributed discontinuously, often limited to very narrow creeks and bays (Figure 1).

Adult woodborers were collected from wood in mangroves of Mida Creek (North coast), Jomvu Kuu within Tudor Creek (Island) and Gazi Bay (South coast) in the intertidal region along the Kenyan coast.

Mida Creek or Watamu Marine National Reserve (3°20' S, 40°00' E) is situated 100 km north of Mombasa in Kilifi district. The reserve, established in 1968, contains natural elements such as mangroves, coral reefs, and mud flats and is a sanctuary for shorebird populations. Seven of the 9 mangrove species described in Kenya are found in Mida Creek and they occupy a total

area of 1746 hectares. The dominant species are *Rhizophora mucronata* Lamk. (Rhizophoraceae), *Ceriops tagal* (Perr.) C.B. Robinson (Rhizophoraceae) and *Avicennia marina* (Forsk.) Vierh. (Avicenniaceae). There is no obvious zonation that is displayed by the dominant mangrove species in Mida Creek. *A. marina*

and *Lumnitzera racemosa* Willd. occupy the landward zone, whereas mostly *C. tagal* and *R. mucronata* mosaic covers the middle zone. Wherever present, *Sonneratia alba* Sm. (Sonneratiaceae) occupies the seaward margin, but is replaced by tall *A. marina* and *R. mucronata* along small creeks (Kairo et al. 2002).

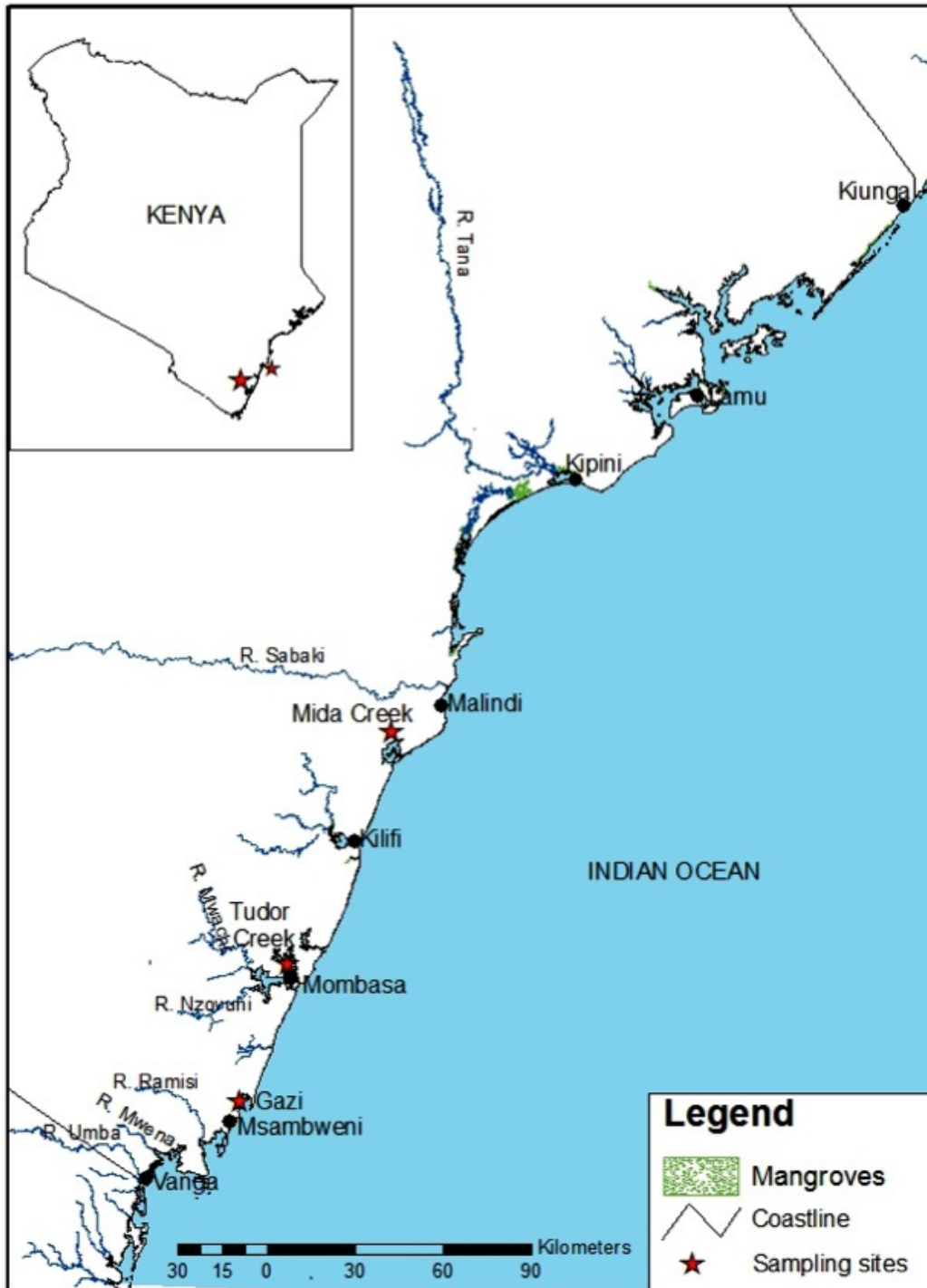


Figure 1. Map of The Kenyan Coast Showing Sampling Sites

Jomvu Kuu is a section of Tudor Creek (4° 01' 48" S; 39° 40' 12" E). Tudor Creek bounds Mombasa island on the northwest and extends some 10 km inland. The creek has two main seasonal rivers, Kombeni and Tsalu, draining an area of 550 km² (450 and 100 km² respectively) with average freshwater discharge estimated at 0.9 m³ s⁻¹ during the inter-monsoon long rains. It is a well-developed mangrove forest mainly composed of *R. mucronata*, *A. marina* and *S. alba*. The basin has an area of 6.37 km² at low water spring and 22.35 km² at high water spring. Mangrove forests occupy 8 km² of the creek. Like Mida Creek, there is no obvious zonation displayed by the dominant mangrove species in Tudor Creek. *A. marina* and *L. racemosa* occupy the landward zone, whereas mostly *C. tagal* and *R. mucronata* mosaic covers the middle zone and occupies the seaward margin whenever present. Along small creeks, *S. alba* is replaced by *A. marina* and *R. mucronata* (Mohamed et al. 2009).

Gazi Bay (4° 25' S, 39° 30' E) lies 50 km south of Mombasa. The bay is 18 km² sheltered from the Indian Ocean by a peninsula to the east and a fringing coral reef to the south. Mangrove vegetation, penetrated by two tidal creeks, covers 6.61 km² of the bay. The western creek has an inland continuation as the seasonal river Kidogweni. The eastern creek has no such freshwater input. Eight mangrove species are found in Gazi Bay. *R. mucronata* and *C. tagal* account for over 60% of the vegetation cover. Other species are *S. alba*, *Bruguiera gymnorhiza* (L.), *Avicennia nzarirza* (Forssk.) Vierh., *L. racemosa*, *Xylocarpus granatum* Koen. and *Heritiera littoralis* Dryand ex H. Ait. The mangrove species occur in clear zonation patterns, with belts parallel to the low-water line. In general, *S. alba* forms the outermost zone towards the open water, followed by pure stands of *R. mucronata* or mixed stands of *R. mucronata* and *B. gymnorhiza*, and in turn these stands are followed by pure or mixed stands of *C. tagal* and *A. marina*. Along the river Kidogweni and other creeks, *A. marina* usually replaces *S. alba*. On the seaward side, the mangrove forest is bordered by intertidal and subtidal areas covered with seagrasses (Slim et al. 1996, Bosire et al. 2003).

Collection and Preservation of Marine Woodborers

Collection Strategy

Woodborers were collected from the three sampling sites during the years 2011 and 2013. The collection efforts at each site lasted from 1–3.5 h, with two to three people observing and noting the number of attacked plants, and

collecting sample specimens of woodborers encountered as they walked haphazardly through each site (Gilbert et al. 2008). Infested mangrove wood including those of decaying logs were identified to species level by experts. The woodborers were collected from *R. mucronata*, *S. alba* and either *A. marina* or *A. nzarirza* mangrove wood in the 3 sampling sites. The borers were collected from submerged parts of roots (proproots, pneumatophores), stems and branches in the mangrove ecosystem. They were immediately transported alive to the Kenya Marine and Fisheries Research Institute laboratory where specimens for morphological and molecular identification were obtained.

The mollusc borers were extracted by carefully cutting open the damaged wood samples and best specimens were obtained when the borers were dissected out as soon as the wood was removed from the water. For later extraction, the destroyed wood pieces were submerged in 70% alcohol and then transported to the laboratory wrapped in cotton and cloth soaked in alcohol. Specimens removed from the wood were preserved in a mixture of four parts 70% alcohol and one part glycerine. This way the periostracal margins of the pallets were kept soft and pliable and in case the alcohol evaporated, the glycerine kept the pallets moist for some more time.

The crustacean borers on the other hand were collected by keeping the infested wood samples in a trough of diluted seawater or by adding a little formalin. The change in the milieu and the traces of formalin forced the borers out of their burrows and specimens were easily collected with a fine brush or forceps. The crustacean borers were also preserved in alcohol. No formalin was used in preservation as it slowly dissolves the tubercles on the dorsal surface of the animals, rendering species identification difficult. Specimens for molecular identification were preserved in absolute ethanol until DNA extraction, which was carried out from thoracic legs (pereopods) for isopods and the muscle below the head in bivalves.

The woodborers were identified based on macroscopic and microscopic morphological characteristics through comparison with appropriate literature (Kuhne 1971, Harrison and Holdich 1984). This was confirmed by molecular identification.

Morphological Identification of Woodborers

Shipworms (Teredinidae) morphological identification was based entirely on shells and pallets. Characters of systematics value for species identification were nature

of the shell valves, tubes (internal lining of the borrows which sometimes gets thickened as a tube particularly at the posterior end), pallets (a pair of calcareous organ situated at the posterior end of the animal which is used to plug the entry hole during adverse conditions or when the borer is disturbed) and siphons. Of these, the morphological variations exhibited by the pallets are remarkable and almost all the species can be identified from their pallets (Turner 1966, 1971).

Piddocks (Pholadidae) identification was based on the shape of shell valves, nature and arrangement of accessory plates (protoplax, mesoplax, metaplax and hypoplax), presence or absence of callum in the adult stage, presence or absence of apophysis, and on the morphology of the siphons. In some members (Sub-family Martesiinae), the young and adult are different morphologically, the former having an anteriorly beaked and widely gaping shell and the latter having this gape closed by a calcareous deposit, the callum (Turner 1971). The nature of the chitinous lamellae on the posterior slope of the shell, when present, also helps in species separation.

For pill-bugs (Sphaeromatidae and Cirolanidae), characters of taxonomic value were the number and disposition of large tubercles on the dorsal posterior part of body, posterior part of the telson and shape of the epistome. Of these, the arrangement of the large tubercles is strikingly different and showed variations characteristic of each species (Pillai 1961, Kuhne 1971, Harrison and Holdich 1984).

In addition to morphological characteristics, burrows produced by each of the above three types of borers are also characteristic of its occupant. Shipworms bore deep into the wood making long tunnels almost parallel to the grain whereas burrows of pholads are pear shaped, superficial and nearly at right angle to the grain. Pill bugs produce burrows cylindrical on the wood surface at right angle to the grain with juvenile burrows working from the main parent tunnel leaving side branches.

Molecular Identification of Woodborers

DNA was extracted using Quick-gDNA™ Miniprep extraction kit (Zymo Research) according the manufacturer's instruction. The obtained DNA was stored at -20 °C. The mitochondrial cytochrome c oxidase subunit I gene (COI) (Baratti et al. 2005) was amplified using Phusion High-Fidelity DNA polymerase (Thermo Scientific) following the manufacturer's instructions with slight modifications, using the following forward

and reverse primers; 5'-gggtcaacaatcataaagatattgg-3' and 5'-taaacttcagggtgaccaaataatca-3' respectively.

The polymerase chain reaction (PCR) was performed in a total volume of 50 µL using 28.95 µL distilled water, 1.0 µL dNTPs (10 mM), 10 µL 5x GC phusion buffer, 2 µL MgCl (25 mM), 2.4 µL forward primer (10 mM), 2.4 µL reverse primer (10 mM), 0.75 µL dimethylsulphoxide (DMSO), 0.5 µL phusion DNA polymerase (0.02U µL⁻¹) and 4.0 µL DNA template. The PCR profile included initial denaturation step at 95 °C for 3 minutes followed by 45 cycles of 95 °C (30 seconds) denaturation, 50 °C (30 seconds) annealing, 72 °C (1min) extension and a final 72 °C (7 min) extension.

The PCR product was analysed on 1% agarose gel electrophoresis containing 0.5 mg mL⁻¹ ethidium bromide. The amplification patterns were viewed with DNA gelviewer (Bio-Rad) and cleaned using QIAquick PCR purification kit according to manufacturer's instruction. The purified DNA was quantified with a nanodrop spectrophotometer. The clean PCR product was sequenced with an ABI sequencing kit (Big Dye Terminator Cycle Sequencing, Applied Biosystems).

Preparation reaction for big dye PCR consisted of Big dye (0.5 µL), 5X seq buffer (1.75 µL), 10mM primer (1.0 µL), PCR product (4.0 µL) and water (2.75 µL). The PCR profile was 96°C for 1 min followed by 25 cycles of 96 °C for 30 seconds, 50 °C for 30 seconds and 60 °C for 4 minutes. Clean up (ethanol/ sodium acetate precipitation of extended sequencing products) consisted of nuclease free water (24.5 µL), 3M sodium acetate pH 5.2 (3.0 µL) and absolute ethanol (62.5 µL). To analyse the gene, 10 µL of HI-DI formamide was added and samples denatured at 96 °C for 3 min. The samples were electrophoresed on 3130xl Genetic Analyzer (Applied Biosystems®).

DNA Sequence Analysis

The electropherograms were viewed using Finch TV Ver. 1.5 and aligned using DNA Baser software Ver. 3.5 to correct apparent anomalies. To search for available matches with published sequences of other invertebrates, we aligned the sequences on BLAST at the NCBI web site.

Species Specificity Analysis

Species preference data was analyzed by non-parametric statistic chi-square test using SPSS Ver. 16.0 to calculate the probability that each wood borer would be found on a particular host at the observed frequency or less, or the

observed frequency or more, in a host tree species abundant habitat. The expected distribution of collections among the three host species, assuming no host preference, was estimated from combined counts of infested living and dead trees in the three sampling sites.

RESULTS

Morphological and Molecular Identification of Marine Woodborers

Combining DNA barcoding and anatomotyping, 3 taxa of woodborers were identified from the three sampling sites. They were: *Dicyathifer (Teredo) mannii*, *Sphaeroma terebrans* and *Cirolana* sp. (Figure 2).

Morphological features of *D. mannii* included long cylindrical bodies with two small anterior valves and a paired posterior pallet. They were enclosed in tunnels with calcareous lining. Cytochrome c oxidase gene 1 (CO1) homology of *D. mannii* confirmed the morphological identification. The highest nucleotide similarity score was with *Dicyathifer mannii*, partial sequence, 99% identity, NCBI accession No. JF899180.1. *S. terebrans* morphological features included an almost spherical body and round epistome, with prominent tubercles on the dorsal posterior part of the body and posterior part of the telson. *Cirolana* sp. were swift moving, with a body different from that of *S. terebrans* as it was slender and had no prominent tubercles, with a tapering epistome.



Figure 2. Woodborer Morphology and Burrows : A: *Sphaeroma terebrans* (X10) Phylum Arthropoda, Class Crustacea, Order Isopoda. B: *Cirolana* sp. (X10) Phylum Arthropoda, Class Crustacea, Order Isopoda. C: *Sphaeroma terebrans* and *Cirolana* sp. burrows. D: *Dicyathifer (Teredo) mannii* (Phylum Mollusca, Class Bivalvia, Family Teredinidae). E: *Dicyathifer mannii* burrows.

We obtained good PCR product for the three wood-borer species but it was not possible to obtain good sequences for *S.terebrans* and *Cirolana* sp. despite repeated attempts with various reaction conditions.

The burrows produced by the above three species differed (Figure 2). Those produced by *D. mannii* were long tunnels parallel to the grain. *S. terebrans* and *Cirolana* sp. (whenever present) co-existed in the same burrows. These were cylindrical on the wood surface with branches in the wood giving a honeycomb appearance.

We observed 50 infested tree host species in the three sampling sites of mangrove forest. *D. mannii* infested 25, *S. terebrans* 16 and *Cirolana* sp. 9 tree host species respectively (Table 1).

Table 1. Woodborers collected from mangrove wood

Wood borer	Host plant	No. of attacked plants			Total No. Observed
		Gazi Bay	Tudor Creek	Mida Creek	
<i>D. mannii</i>	<i>R.mucronata</i>	8	7	5	20
	<i>Sonneratia alba</i>	2	1	1	4
	<i>Avicennia</i> sp.	1	0	0	1
	Total	11	8	6	25
<i>S. terebrans</i>	<i>R.mucronata</i>	0	0	0	0
	<i>Sonneratia alba</i>	0	0	0	0
	<i>Avicennia</i> sp.	7	5	4	16
	Total	7	5	4	16
<i>Cirolana</i> sp.	<i>R.mucronata</i>	0	0	0	0
	<i>Sonneratia alba</i>	0	0	0	0
	<i>Avicennia</i> sp.	5	4	0	9
	Total	5	4	0	9
	Grand total	23	17	10	50

The woodborer species were found multiple times showing strong host preferences, each being found exclusively (or nearly so) on only one of the three mangrove host species (Table 2).

Most of the specimens identified were extracted from dead wood; however, *D. mannii* was also found in living roots of *Rhizophora*. Generally they had entered dead wood then invaded living tissue. Comparison of the woodborers found in the three mangrove habitats showed few strong differences. *Cirolana* sp. was not encountered at Mida Creek, *S. terebrans* and *Cirolana* sp. were found exclusively on *Avicennia* sp., whereas *D. mannii* was found mainly on *R. mucronata* but also on *S. alba* (Table 1).

Mangrove Wood Deterioration

The mangrove woodborers were found at temperatures ranging from 24°C to 28.6°C, salinity of between 35‰ and 36‰. They could withstand abrupt changes in water conditions, particularly temperature and salinity. Severe damage of mangrove wood by the woodborers was observed (Figure 3).

DISCUSSION AND CONCLUSIONS

This study identified three mangrove woodborer taxa in the mangrove forests along the Kenyan coast. It also demonstrates that there is a strong host-preferring tendency in the woodborers along the Kenyan coast. The host specific *S. terebrans* and *Cirolana* occur only on *Avicennia* sp. *D. mannii* occurs mostly on *Rhizophora* plants, but also on *Sonneratia*. *Cirolana* sp. appears to be uncommon occurring only in 9 out of 50 samples. The

Table 2. Host preference of various species of mangrove woodborers

Woodborers	Gazi Bay			<i>R. mucronata</i>	Tudor Creek		<i>R. macronata</i>	Mida Creek	
	<i>R. mucronata</i>	<i>S. alba</i>	<i>Avicennia</i> sp.		<i>S. alba</i>	<i>Avicennia</i> sp.		<i>S. alba</i>	<i>Avicennia</i> sp.
<i>D. mannii</i>	8	2	1	7	1	0	5	1	0
<i>S. terebrans</i>	0	0	7	0	0	5	0	0	4
<i>Cirolana</i> sp	0	0	5	0	0	4	-	-	-
Total	8	2	13	7	1	9	5	1	4
χ^2 (df)	19.30(4)			17.00(4)		10.00 (2)			
p-value	0.0007			0.0019		0.0067			

P-values are for within study sites tests and indicate the probability of observing a sample statistic as extreme as the test statistic (significant at $\alpha=0.05$). Emboldened values indicate that a species is significantly more common on that host than expected.



Figure 3. Mangrove wood deterioration by borers. A: Heavily fragmented piece of wood with vacant tunnels created by extensive terenid boring activity. B, C and D, tree trunks severely damaged by isopod borers.

two most abundant woodborer species were *D. mannii*, and *S. terebrans*, each found in 50% and 32% of the observed infested mangrove host plants respectively.

The observed specificity is not a function of local host density, as the studied mangrove forests were host abundant. Host specificity of borers can probably be attributed to the interrelationship that may exist between microorganisms and marine borers. The microflora that colonise wood substratum are known to influence and facilitate larval settlement of sedentary marine organisms (Mitchell and Kirchman 1984). The woodborers might require certain specific types of microflora, especially fungi, to induce settlement, attachment and metamorphosis (Santhakumaran and Sawant 1998). Besides, mangrove plant species that showed no attack could be having natural immune mechanisms or stress induced responses that prevented attack. This need to be investi-

gated as it may provide opportunities for evolving methods for controlling wood-boring organisms and curbing the problem of bio-deterioration.

Our choice of study sites is a representative of the Kenyan coast: Mida Creek (North coast), Tudor Creek (Island) and Gazi Bay (South coast). The three study sites are separated by 50-100 km but the difference in wood-borers in mangrove habitat was small. Except for *Cirolana* sp., there was overlap with woodborers collected from mangroves of the three study sites.

Sphaeromatidae and Teredinidae species that we collected have been described elsewhere in the world on multiple hosts. *Sphaeroma terebrans* has been reported to live mainly in aerial roots of the mangrove *Rhizophora mangle* in tropical and subtropical regions. *Sphaeroma* taxa have also been reported to be present in single holes bored in the pneumatophores of another mangrove tree,

Sonneratia alba (Harrison and Holdich 1984, Villalobos et al. 1985, Barrati et al. 2011). In this study however, *S. terebrans* was exclusively observed in *A. marina*. According to Cragg (1993, 2007) teredinids inhabit a *Rhizophora*-dominated forest in Papua New Guinea. In addition, Brearly et al. (2003) reported that *D. mannii* was found in *Rhizophora*, *Avicennia* and *Bruguiera* mangrove plants in Australia but was most abundant in *Rhizophora*. This is in agreement with our study as *D. mannii* was observed mainly on *Rhizophora* sp.

In all our observations and collections, *Cirolana* sp. was not found in burrows on their own. They were always found in association with *S. terebrans*. Cirolanidae have not been reported to be woodborers. Since they are found in *S. terebrans* burrows, they could be mistaken to be woodborers while in essence, they could be scavengers. This could mean that they are *S. terebrans* natural enemies. Keable (2001) describes scavenging species of *Cirolana* from the Australian coast. Bowman et al. (1981) also reported that an exotic *Cirolana* sp., *C. arcuata*, was found in the company of *Sphaeroma quoyana* in the San Francisco bay, North America.

Our study has two limitations: first, without experimental manipulations we are unable to distinguish between preferences for a particular host or site and aversion to others. Second, we were unable to determine relative densities of all roots (prop roots, pneumatophores), stems and branches of potential host plants, whether or not they had woodborers. Using overall relative abundances is the preferred approach for testing host specificity (Gilbert and Sousa 2002, Gilbert et al. 2007). But by observing all the infested tree host species we encountered, and noting which woodborer was found within them, we were testing against the relative frequencies of host species that supported the particular woodborers, and our results should be robust. Besides, the collection was spread over a long period of time and therefore was not affected by seasonal patterns.

By their tunnelling, woodborers eventually cause the wood in which they live and on which they feed to disintegrate (Cragg et al. 2009). Bores and tunnels produced by the woodborers greatly increase the surface area available for fungal and bacterial decay process, which, in turn, accelerate the disintegration of wood into smaller pieces. The service rendered by woodborer community in the process of wood disintegration thereby cleansing the mangrove ecosystem from unwanted trash wood is very significant, but when this phenomenon occurs on living vegetation, their role tends to be negative. They are then viewed as pests that warrant

control measures. Therefore, the present investigation might be useful in mangrove ecosystem restoration, environmental conservation and management.

This study recommends monitoring of woodborer community to give an overall idea of the intensity of the problem and enable removal of the source of infestation at the very onset. It also recommends study of natural bio-resistance of the mangrove plants that show no attack as well as investigations on effect of fungal metabolite on larval settlement in the fungi-borer interrelationships and specificity theory. Lastly, it will be rewarding if further studies were done to establish the possibility of the *Cirolana* sp. being natural enemies of *S. terebrans* as they can be used in the natural control of wood-boring pests.

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