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First record of the seagrass-boring shipworm *Zachisia* sp. (Bivalve: Teredinidae) in natural and transplanted *Enhalus acoroides* (Hydrocharitaceae) rhizomes in tropical Southwest Pacific

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Abstract. Asriani N, Ambo-Rappe R, Lamuru M. 2019. First record of the seagrass-boring shipworm *Zachisia* sp. (Bivalve: Teredinidae) in natural and transplanted *Enhalus acoroides* (Hydrocharitaceae) rhizomes in tropical Southwest Pacific. *Biodiversitas* 20: 3180-3188. Seagrass ecosystems are under threat worldwide. Seagrass transplantation is one restoration approach that has been tried with uneven success. While transplantation methods and oceanographic parameters have been investigated in attempts to understand the factors affecting transplant survival rate and seagrass transplantation success, little attention has been paid to the possible impact of pests. To our knowledge, we report the first record of the seagrass-boring shipworm *Zachisia* (Teredinidae) in the tropical southwest Pacific. Shipworms were found in rhizomes of the seagrass *Enhalus acoroides* growing in natural beds and in a transplanted area, in the Spermonde Archipelago, eastern Indonesia. In twelve *E. acoroides* rhizome samples (average length 10.5 cm) we found between one and four burrows lined with calcareous tubes 2mm to 5mm in diameter inside the rhizome and six *Zachisia*. Prevalence of *Zachisia* burrows in exposed versus buried rhizomes was 64% vs. 4% in the natural seagrass bed and 100% vs. 0% in the seagrass transplants. Further research is required to determine the species present and its distribution in Indonesian waters as well as the true ecological impact of *Zachisia* sp. on seagrasses.

Keywords: Boring molluscs, seagrass health, seagrass rhizome borer, seagrass transplantation, Teredinidae

INTRODUCTION

Seagrasses are unique among aquatic plants, being the only marine angiosperm group that lives submerged in water (den Hartog 1970; Les and Cleland 1997). Seagrasses have a complex and strong root system that enable seagrass beds to trap sediment (Duarte 2002), while the canopy is thought to reduce wave energy (Gacia et al. 2003). Some seagrass species are directly utilized as the main foods consumed by endangered species including marine turtles and dugongs (Lanyon et al. 1989; Fourqurean et al. 2010). Furthermore, seagrass beds provide habitats for economically important fish and invertebrates (Duffy 2006). In particular, epiphytes and epifauna living on seagrasses provide food for herbivorous and carnivorous animals that live in and around the seagrass beds (Bla Torre-Castro et al. 2008; Lebreton et al. 2009). In the context of global climate change, seagrasses play an important role as they contribute to natural CO₂ capture and sequestration, accounting for approximately 15% of annual "blue carbon" trapped (Fourqurean et al. 2012; Duarte et al. 2013; Lavery et al. 2013). On average seagrass beds sequester almost three times as much per km² as rainforests (Fourqurean et al. 2012).

Regrettably, seagrass meadows face threats worldwide, resulting in a reduction in the area covered (Waycott et al. 2009; Short et al. 2011). The most common causes of

seagrass loss include direct physical damage from anthropogenic activities (e.g. boat anchors and dredging) (Hossain et al. 2018), destructive fishing (Moore et al. 2017), overfishing, land reclamation, and garbage disposal/waste discharge to the sea (Unsworth et al. 2018). The loss of seagrass beds has negative impacts on neighboring ecosystems (Marba et al. 2015). It is therefore important to pay attention to the maintenance of healthy seagrass beds through conservation measures, including restoration activities.

At the global level, seagrass restoration is no longer a novel approach to seagrass conservation. Restoration has been carried out in various temperate and tropical countries. Restoration success and its assessment have been addressed from a variety of aspects. However, one aspect which has received little attention to date is the presence of organisms that could interfere with seagrass health and survival and thus rehabilitation viability (Brearley et al. 2008; Mejaes et al. 2015). In other contexts, studies have found several groups of organisms that can bore into and/or graze on the leaves and/or rhizomes of seagrasses. These organisms include boring polychaete worms that bore into the rhizomes and leaf sheaths of *Posidonia oceanica* in Italy and the Mediterranean Sea (Guidetti 2000; Gambi 2002; Gambi et al. 2005) isopods boring into *Thalassia hemprichii* leaf sheaths in Tanzania and *Posidonia oceanica* in Mediterranean (Guidetti 2000; Gambi and

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MATERIALS AND METHODS

Lorenti 2009); the mollusc *Smaragdia souverbiana* grazing on the leaves of *Halophila ovalis*, *Zostera capricorni* and *Cymodocea serrulata* (Rossini et al. 2014); and molluscs of the genus *Zachsisia* (Bulatoff and Rjabtschikoff 1933) boring into the rhizomes of *Zostera* spp. and *Phyllospadix ivatensis* in Japan and Russia (Turner et al. 1983; Haga 2006; Shipway et al. 2016) and the rhizomes of *Enhalus acoroides* Lf. Royle, 1839 and *Cymodocea rotundata* in Malaysia (Yap et al. 2018). In addition to native pests, introduced (alien) species have also been reported as pests in seagrass meadows (Iannotta et al. 2007; Williams 2007).

It has been suggested that when seagrasses colonized marine waters, they "found a vast new habitat free of terrestrial competitors and insect pests" (Olsen et al. 2016). However, based on the aforementioned studies, it would seem that seagrasses do suffer from a variety of "pests", both native and introduced. Indeed, it is not impossible that seagrasses might suffer a much higher level of infestation than has as yet been realized. In one of region in the tropical southwest Pacific, specifically the Spermonde Archipelago in Indonesia, we found a boring bivalve in an *E. acoroides* rhizome by chance during seagrass transplant monitoring. These seagrass-borers were subsequently found in both natural seagrass beds and transplanted areas. This study aimed to: (i) Document the borer organisms found in natural seagrass beds and seagrass transplants; (ii) Identify the boring organisms found based on morphological characteristics.

Study site

All fieldwork was conducted in the shallow waters south of Barrang Caddi Island, Spermonde Archipelago, in the tropical southwest Pacific region of Indonesia (Figure 1). The observation site was located at approximately 5°05'05" S and 119°19'07" E in natural *E. acoroides* dominated seagrass beds and seagrass transplantation (*E. acoroides*) area, situated approximately 10-50m apart.

Three methods were used at the transplantation site. In method 1, seagrass shoots were attached to a wireframe placed on the substrate; the rhizomes were in contact with the substrate but not buried. In method 2, a shallow trench was dug in the substrate; the rhizomes were laid in the bottom of the trench and fixed firmly in place using metal staples and then covered with the previously removed sediment. The third method also involved digging a trench and burying the rhizomes by replacing the sediment layer; however, instead of stapling the rhizomes in place, sandbags (containing sand mixed with some cement) were placed in a semi-circle around the transplants on the seaward side to provide protection from wave action and currents. The *E. acoroides* transplants were all sourced from the same site in the seagrass meadows around the nearby island of Barrang Lompo (Figure 1). Boring organisms were first observed in the natural seagrass bed on 31 December 2017 and in the seagrass transplants on 17 March 2018.

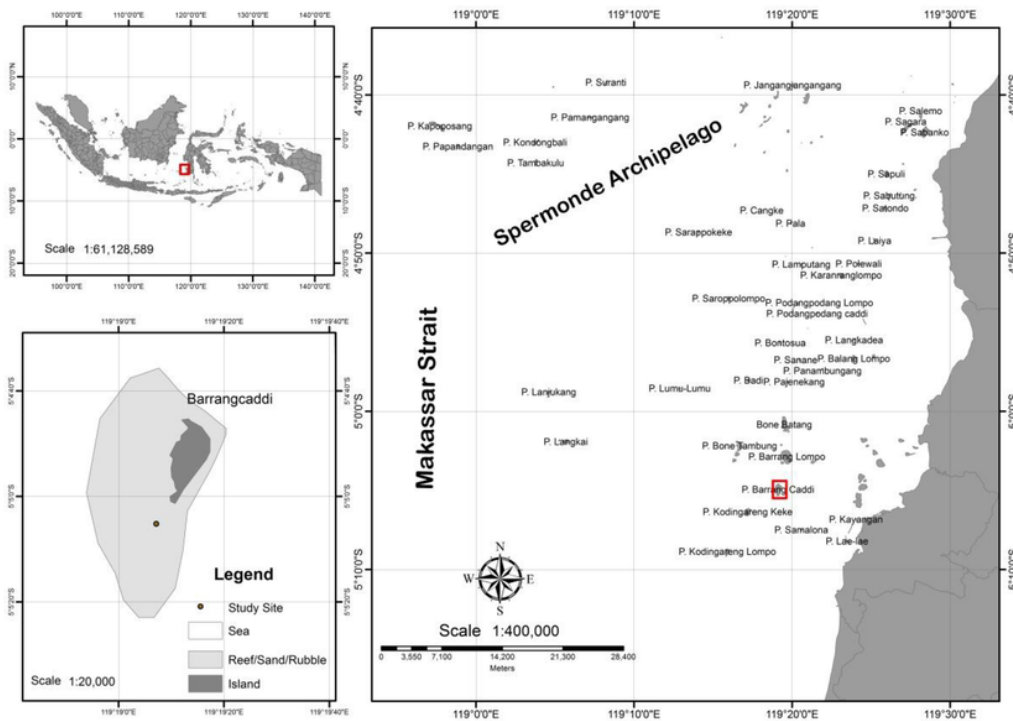


Figure 1. Study sites in Barrang Caddi Island, Spermonde Archipelago, South Sulawesi, Indonesia

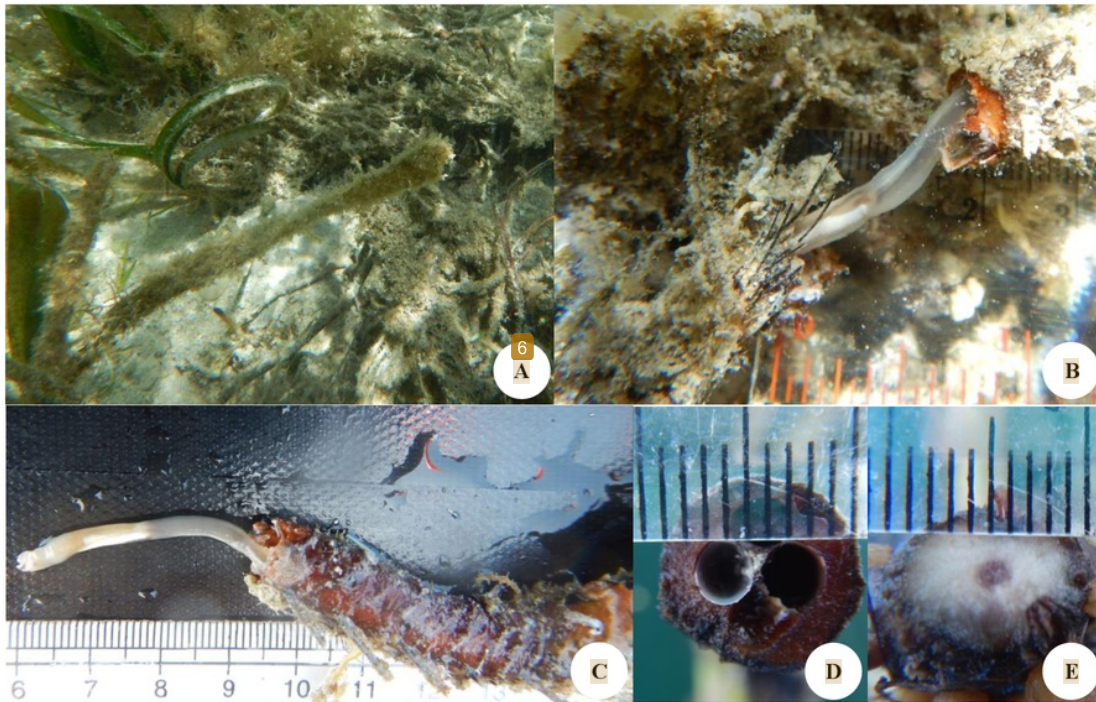


Figure 2. Habitat and specimens of the seagrass-boring mollusc *Zachsia*: A. Exposed *E. acoroides* rhizomes in the natural seagrass bed; B. *Zachsia* visible in an exposed *E. acoroides* rhizome *in situ*; C. *Zachsia* in the rhizome immediately after collection; D. Section of an *E. acoroides* rhizome bored by *Zachsia* with ruler to indicate burrow diameter; E. Section of a healthy *E. acoroides* rhizome.

Sample collection and imaging

The first seagrass rhizome containing a boring mollusc was found by chance in an exposed rhizome of the seagrass *E. acoroides* which was seen during restoration monitoring activities on December 31, 2017. The shoot and exposed rhizome were collected out of curiosity. On examination of this specimen, there were 4 burrow entrances visible in the rhizome; each burrow appeared to have a calcareous lining. The rhizomes were carefully broken apart to enable a more detailed observation, revealing the presence of vermiform soft-bodied organisms within 3 of the calcareous tubes, one of which was a complete specimen. These organisms were carefully removed from the rhizome and then photographed (Nikon AW130 camera) (Figure 1). The photographs were used to identify the organisms based on appropriate references.

It was noticed serendipitously that the presence of these burrows in *E. acoroides* rhizomes could be detected from the difference felt when gently squeezing healthy rhizomes (Figure 2e) and burrowed rhizomes (Figure 2d) between the fore-finger and thumb. This method of detection was validated by the examination of exposed ($n = 100$) and non-exposed ($n = 100$) *E. acoroides* rhizomes in the natural seagrass area (September 2019). The gentle squeeze detection method was 100% accurate on this sample; however, the method could not distinguish occupied burrows from unoccupied burrows. This gentle squeeze

method was then used to determine the prevalence of burrows in the rhizomes of the seagrass transplants.

To support the initial tentative identification and to gain an idea of the prevalence of this boring organism, a further eleven *E. acoroides* shoots with exposed rhizomes were collected at random using a spade on September 17, 2018. Holes (burrow openings or cross-sections similar to Figure 2b or 2d) were present in all eleven rhizomes. The specimens were rinsed in seawater to remove attached sediment. Each sample was then stored in a container filled with seawater to prevent drying. The samples were then taken to the Marine Ecology Laboratory, Faculty of Marine and Fisheries Sciences, Hasanuddin University and the Takalar Brackish-water Aquaculture Laboratory for further observation. The number of burrows in each rhizome was recorded, and the approximate diameter burrow of each burrow was measured using a ruler (precision: 1 mm). Burrow length could not be determined accurately as most burrows had extended beyond the sampled rhizome segment, burrows were not always straight, and the burrowed rhizomes were brittle and broke easily. Breakage occurred when attempting to make longitudinal sections to expose the burrows and the organisms within them; the burrows did not always run the whole length of the rhizome, and the endpoint could not always be identified with certainty.

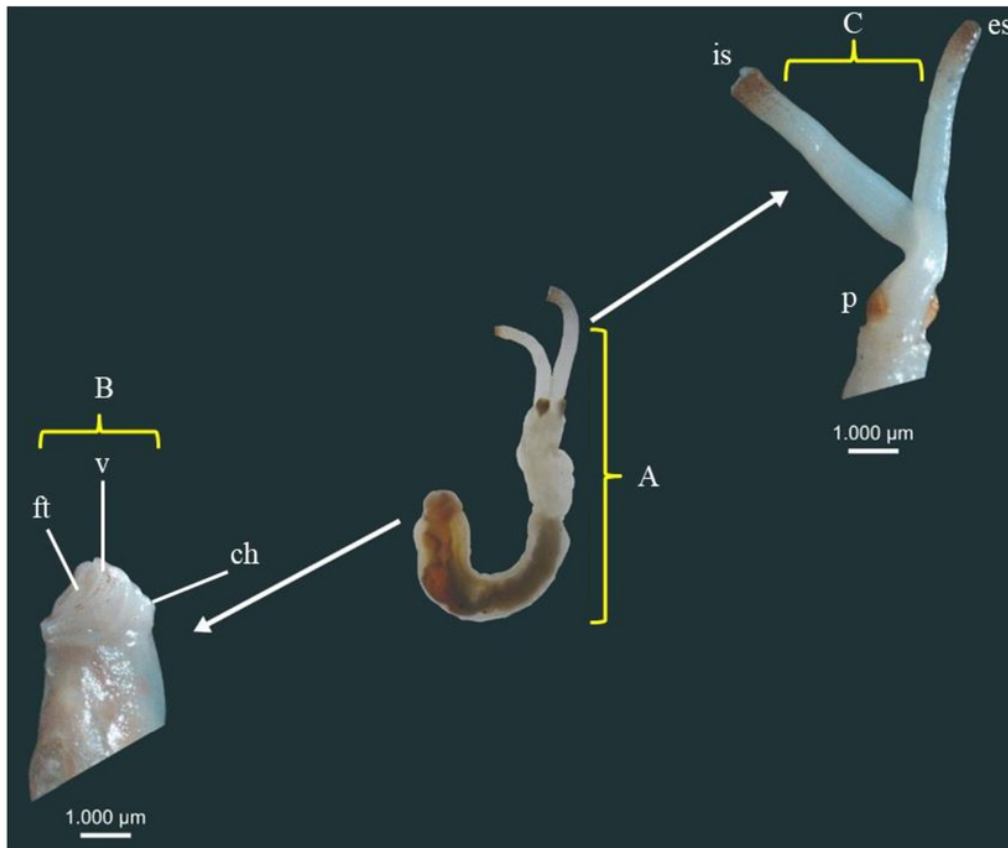


Figure 3. Boring organism found in an *E. acoroides* rhizome in Barrang Caddi, Spermonde Archipelago, South Sulawesi, Indonesia: A. Whole specimen; B. Anterior region (ft: foot; v: valve; ch: cephalic hood); C. Posterior region (p: pallet; is: incurrent siphon; es: excurrent siphon)

Vertical and horizontal sections were made with care, in order to avoid damaging the boring organisms which might be present within the burrows in each rhizome. Although burrows with calcareous linings (calcareous tubes) were present in all 11 *E. acoroides* rhizomes, only three boring organisms were found. These three specimens were preserved in 70% alcohol. The specimens were examined under stereo microscopes (Olympus SZ61 and Zeiss V8). Digital photographs (whole animals and specific body parts) with scale bars were taken through the Zeiss V8 microscope which was connected directly to a computer (e.g. Figure 3B and 3C). Photographs taken through the Olympus SZ61 microscope did not have scale bars (e.g. Figure 3A).

RESULTS AND DISCUSSION

Results

The boring organisms found in *E. acoroides* rhizomes at the study site were identified to the family level as

Teredinids, commonly known as shipworms, following the keys provided by Turner (1966). This identification was based on several morphological features including paired pallets located posteriorly and reduced double-hinged shell valves, with a vermiform body plan (Figures 3 and 4). Only the extreme end of the anterior region was encased in pair of small serrated shells (Figure 3.A and 3.C), while the rest of the body, which was soft in texture and milky white in color, was exposed (Figure 3.A) but protected by a pearly white calcareous tube lining the burrow in which the organisms were found (Figure 2.D). The outer tissues were translucent, so that the internal organs (viscera, and in particular the large gills) could be seen through the body wall (Figure 3.A and 4.D). The shell valves were denticulated as is typical in Teredinids which use the sharp serrations on these vestigial shells to drill into wooden or wood-like materials such as floating wood, mangrove roots, and seagrass rhizomes (Figure 4). A pair of paddle-shaped pallets was present, flanking the base of the siphons (Figure 3.A and 3.C). These pallets are unique to the Family Teredinidae and serve to block the entrance to the

hole when the shipworm is disturbed, and has withdrawn the paired siphons into the burrow (Shipway et al. 2016).

Based on Shipway et al. (2016), the organisms appear to belong to the genus *Zachisia* sp. (Figure 4), the only genus within the Teredinidae which has been reported as a seagrass rhizome borer (Velásquez and Shipway 2018). Specific diagnostic characters included the paired siphons which were predominantly white in color, with a reddish tinge near the posterior end (Figure 3.C). The anterior region of *Zachisia* (Figure 3.A) resembles the description in Shipway et al. (2016): "cephalic hood and foot comparatively enlarged and the shell valves reduced [...]. The foot is bulbous and extends beyond the aperture of the shell valves". Shipway et al. (2016) also describe the translucent cephalic hood, once distended, as being approximately double the size of the shell valves; this feature is not visible in Figure 3.A, as the cephalic hood shrank during preservation but was observed in a live specimen. The pallets of the specimens collected closely resemble those shown in Haga (2006) and Shipway et al. (2016); however they are noticeably different from the pallets of a specimen collected in Malaysia by Yap et al. (2018) (see Figure 4.A, 4.B, 4.H, 4.I, 4.J1, 4.J2). Dwarf males as described by Turner and Yakovlev (1983), Yakovlev and Malakhov (1985) and Shipway et al. (2016) were not seen. Brooded larvae as described by Shipway et al. (2016) were also not seen.

Each of the sampled seagrass rhizome segments collected (average length 10.5cm) contained calcareous tubes (one to four per sample) with a diameter ranging from 2 mm to 5 mm (Figure 2.D). *Zachisia* sp. was predominantly found in conditions where seagrass rhizomes were exposed (e.g. due to erosion of the surrounding sediment), rather than in rhizomes which were covered by (buried under) sediment. Out of the 200 rhizomes sampled in the *Zachisia* burrow detection method validation, burrows lined with calcareous tubes were present in 68% of exposed *E. acoroides* rhizomes (n = 100) but only 4% of non-exposed rhizomes in the natural seagrass bed (n = 100). Applying this method in the *E. acoroides* transplantation area, the symptomatic feel of burrow presence was detected in all exposed rhizomes but was not detected in any of the rhizomes buried in the substrate. Exposed rhizomes were common in the transplant method using metal frames, but not in the other two methods used.

We found that some *E. acoroides* plants infested with *Zachisia* showed signs of stress, both in the natural seagrass meadow and in the transplant area. The seagrass leaves and new shoots were partially or totally tawny brown in color, unlike the healthy green color of the plants without *Zachisia* burrows (Figure 5). Some seagrass rhizomes containing several tubes were visibly decaying and sometimes appeared dead.

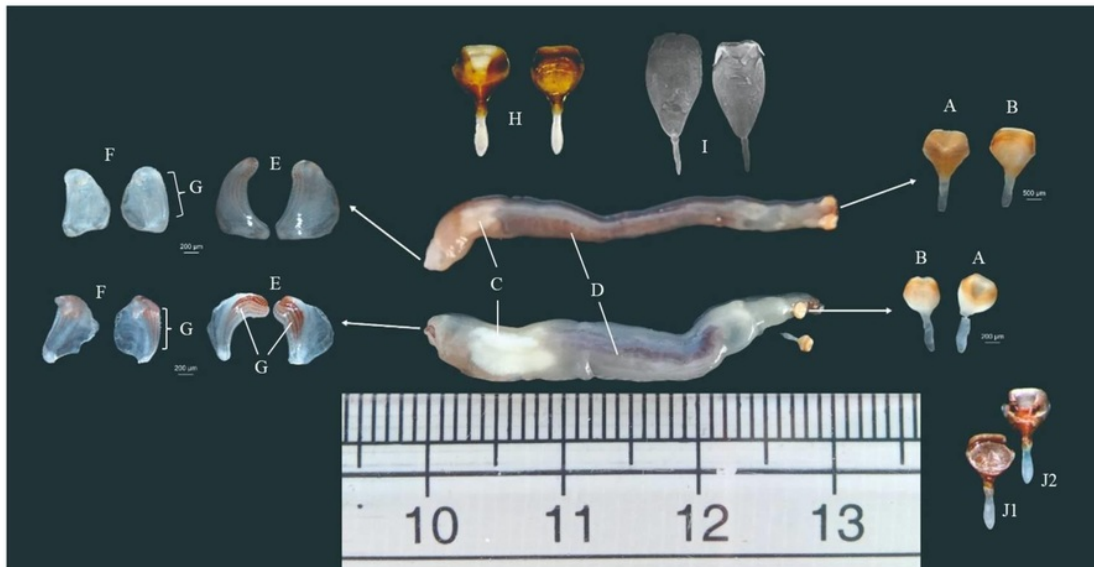


Figure 4. A. Outer margin of the calcareous pallet (this study). B. Inner margin of the calcareous pallet (this study). C. Caecum. D. Internal organs (gills visible in the lower specimen). E. Outer margin of valves. F. Inner margin of valves. G. Denticulation of shell valves. H. Collection of calcareous pallet of *Zachisia zenkewitschi* from Minami-Sanriku town and Ishinomaki city by T. Haga (Shipway et al. 2016, Figure 4.A). I. Calcareous pallet of *Zachisia* sp. From Malaysia (Yap et al. 2018, Figure 4). J1. Collection of calcareous pallet of *Zachisia zenkewitschi* from Shizugawa Bay (Haga 2006, Figure 1B), and J2. Collection of calcareous pallet of *Zachisia zenkewitschi* from Putyatyn Island, Vladivostok by Bulatoff and Rjabtschikoff (Haga 2006, Figure 1.A). Note: scale bars A and B above; 500 um; A and B below; 200 um; E and F: 200um.

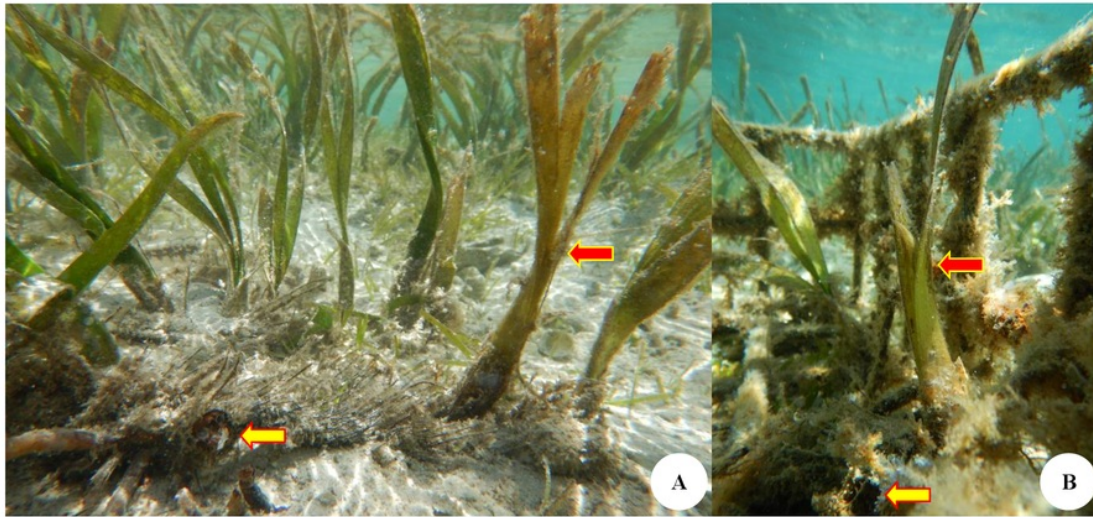


Figure 5. A. Natural seagrass, B. Seagrass transplant in the frame method restoration plot; red arrows indicate tawny brown leaves and yellow arrows indicate calcareous tubes inside exposed rhizomes

Discussion

The boring organism was found in *E. acoroides* rhizomes in both natural seagrass beds and in seagrass transplants within a seagrass restoration plot. This is not, however, the first record of this genus in tropical waters, as *Zachisia* sp. was found in Malaysia, also in rhizomes of the same seagrass species, *E. acoroides* (Yap et al. 2018). Although the two sites (Spermonde Archipelago, Indonesia, and Gaya Island, Malaysia) are relatively close, and both within the Southeast Asian region, it is possible that the organisms found may belong to different species. The morphology of *Zachisia* sp. reported from Malaysia appears to have several differences compared with the Indonesian samples. The pallet of *Zachisia* sp. found in Malaysia appears more elongated with a U-shape and a short stalk, while the pallets of the Indonesian specimens in this study were wider, with a diamond shape and longer stalk (see Figure 4.A, 4.B, 4.I). The pallet shape of the specimens in this study was more similar in shape to those of *Zachisia zenkewitschi* from Japan and Russia (see Figure 4.A in Haga (2006); Shipway et al. (2016); Turner et al. (1983)). Intraspecific variation in the pallets of Teredinidae species has well documented, being principally attributed to their boring lifestyle and environmental conditions (Borges et al. 2012; Velásquez and López 2015). This is one considered one of the principal reasons for erroneous identifications in the past, as demonstrated by a large number of synonym species names (Turner 1966). *Zachisia zenkewitschi* is the only species of the genus *Zachisia* which has been fully described based on morphological and DNA (18S and 28S subunit nuclear rRNA genes) traits. The observed morphological differences are not sufficient as a basis for determining the species of the organisms cofound in the Spermonde Archipelago. Therefore, more detailed

observations and molecular (DNA) research are required to determine the species of the *Zachisia* specimens observed in our study.

Although *Zachisia zenkewitschi* has only been reliably identified to date in temperate seagrasses, it is possible that this species could have a wider distribution, as suggested by Haga (2006) and Yap et al. (2018). Other shipworms have been transported across seas and ocean basins by ships (Shipway et al. 2014) as well as natural events. For example, debris from the Japanese tsunami in 2011 transported living shipworms identified as belonging to eight species to the Northeastern Pacific and Hawaiian shores (Treneman et al. 2018). Furthermore, seagrass rhizomes with live *Zachisia* have been found drifting at sea (Haga 2006). With respect to the possibility of a localized introduction of *Zachisia* through the movement of transplants from one island to another, it is impossible to be certain whether or not any *Zachisia* were originally present in the transplants. However, the ubiquitous presence of *Zachisia* in exposed transplant rhizomes and absence of *Zachisia* in non-exposed transplant rhizomes, coupled with a similar pattern in the natural seagrass, points towards a pre-existing *Zachisia* population in the seagrasses around Barrang Caddi. It seems unlikely that all the *Zachisia* in the natural seagrass around Barrang Caddi were the result of a very recent and small-scale introduction via the transplants. However, *Zachisia* has recently been found around Barrang Lompo (Safruddin 2019, personal communication). The Spermonde Archipelago is situated in Makassar Strait, a seaway traversed by major global shipping lanes (Wajsowicz et al. 2003) as well as the Indonesian Throughflow (Barber et al. 2006), presenting many opportunities for the dispersal of species such as *Zachisia*. It is possible that *Zachisia* may be widespread in the

Spermonde Archipelago, either as a native or introduced (alien) species.

The presence of calcareous tubes inside seagrass rhizomes may affect seagrass health, growth, and survival. Seagrass rhizomes are not only used for vegetative propagation, but also to store energy (Hemminga 1998). The diet of *Zachisia zenkewitschi* in Vostok Bay, Russia, was found to consist almost entirely of seagrass rhizomes (Kiyashko 1986). It can thus be assumed that the boring activity of *Zachisia* will inevitably reduce the volume of rhizome in which food can be stored, as well as making the rhizomes more brittle and likely to break, with negative consequences for survival and spread. Some of the rhizomes of natural and transplanted seagrass shoots that had died were found to have calcareous tubes inside. The possible presence of *Zachisia* was unknown and therefore not recorded in the earlier stages of the transplantation experiment. Therefore these observations are qualitative and we do not have statistical data to estimate how survival of the transplants may have been affected over time by the boring activities of *Zachisia*.

Zachisia has been reported to substantially reduce the growth of *E. acoroides* shoots growing from infested rhizomes (Yap et al. 2018). We hypothesize that the seagrass shoots may not be able to obtain and/or store sufficient nutrients due to the hollowing out and breakage of the rhizomes. Even though the seagrass plants should obtain nutrition from photosynthesis, we observed signs of stress in the leaves of some affected transplants, in particular, a change in color from green to mostly tawny brown (Figure 5). However, if the burrows occupy too large a proportion of the rhizome, they are likely to cause breakage of the rhizome, in which case the borers themselves would become exposed and vulnerable to predation. Thus, there is a level of infestation beyond which both host and parasite may experience high mortality. The opportunistic observations reported here are qualitative, and further research is called for to explore the true effects of these borers on the health and survival of seagrasses.

The presence of *Zachisia* sp. in the study area appears to occur mainly in seagrass rhizomes which have become exposed (visible above the predominantly sandy substrate). Turner et al. (1983) also mentioned repeatedly the finding of *Zachisia* in exposed rhizomes (in this case exposed from a muddy substrate). Seagrass rhizomes can become exposed due to both natural (e.g. wave action) and anthropogenic causes. In the study area, there were indications of sand mining by local people in and around the natural seagrass beds; the hollows made tend to become enlarged due to wave action. Within the seagrass transplantation plots, the exposure of seagrass rhizomes was related to the method of planting. In the frame planting method, where the shoots were attached to a metal frame, the rhizomes were laid on top of substrate without any attempt to bury the rhizomes or to cover them with sand. Signs of *Zachisia* sp. burrows were ubiquitous in this treatment but not found in the two treatments where the rhizomes were buried.

It is worth remembering that *Zachisia zenkewitschi* was found by Haga (2006) in seagrass rhizomes which were drifting, and therefore exposed to attack by organisms in the water column, unlike seagrass rhizomes under a (possibly protective) layer of sediment. In a laboratory experiment observing the dispersal and settlement of *Zachisia* larvae over short distances, it was found that *Zachisia* larvae were more likely to swim close to the substrate, rather than higher in the water column (Turner and Yakovlev 1983; Turner et al. 1983). Thus, any dispersing larvae could be expected to encounter (and therefore be likely to settle on) any exposed rhizomes. Although the initial source of the *Zachisia* sp. which became boring pests in the seagrass transplants during this study cannot be ascertained, it seems likely that these molluscs are widespread in natural seagrass beds. Furthermore, wherever larvae are present in the water column, the prevalence of *Zachisia* could be expected to be higher if disturbance has caused seagrass rhizomes to become exposed. Although further experimental verification is required, our findings indicate that seagrass restoration is more likely to succeed if the transplant rhizomes are buried in the substrate, and if disturbance to nearby natural seagrass beds is minimized (e.g. through cessation of sand mining).

Our findings contribute important information for seagrass conservation, especially seagrass restoration in both temperate and tropical regions, in particular regarding the existence and distribution of seagrass boring molluscs (*Zachisia* sp.). Avenues for further research include species-level identification and a better understanding of their role in and impact on seagrass ecosystems.

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