Short Communication

Sara P. Cobacho*, Nicole A.L.M. Grol, Thomas A. Dros, Dorine T.B.M. Dekkers and Marjolijn J.A. Christianen

First evidence of polyembryony in black mangrove *Avicennia germinans*

https://doi.org/10.1515/bot-2022-0006
Received January 19, 2022; accepted June 7, 2022;
published online July 14, 2022

Abstract: We provide the first documented case of the black mangrove, *Avicennia germinans*, producing multiple seedlings from a single propagule, a phenomenon called polyembryony, on several occasions. There is a lack of knowledge about polyembryony in mangroves, as it is an extremely rare phenomenon previously observed in only three other mangrove species. A higher weight of mature propagules may indicate polyembryony, thus enabling early detection. *A. germinans* may make use of mixed reproductive strategies to ensure the continued survival of the species, or to increase its expansion under favourable environmental conditions. Potential causes and implications of polyembryony in *A. germinans* are discussed.

Keywords: *Avicennia*; mangrove; polyembryony; propagule; seedling.

The black mangrove (*Avicennia germinans* (L.) L., angiosperm, Acanthaceae) is a tropical mangrove species native to the American continent and West Africa (Dodd et al. 2002) that grows into a tree or shrub and reproduces by cryptovivipary (Alleman and Hester 2011). As a common reproductive strategy in mangroves, *A. germinans* produces propagules that, once detached from the parent plant, are aquatically dispersed until they take root in sediment (Alleman and Hester 2011). Once established, propagules develop into saplings, as shown in Figure 1.

Polyembryony in plants is the result of multiple sexually or asexually produced embryos in one seed, resulting in twin (or multiple) seedlings (Batygina and Vinogradova 2007). We collected propagules from a captive mangrove tree from the mangrove ecodisplay at Royal Burgers' Zoo Arnhem, The Netherlands in two consecutive years, 2020 and 2021. Polyembryony was observed in one out of 46 propagules in 2021 (July 28) and 1 out of 15 propagules in 2020 (November 27). Polyembryos were first recognised by the identification of two hypocotyls, one from each seedling, and two independent pairs of cotyledons (Figure 2).

The parent *A. germinans* tree of our polyembryonic propagules was collected in Cuba as a sapling, and grew into an adult tree in captivity. At Royal Burger's Zoo, the parent tree grows alongside another *A. germinans* individual blooming simultaneously, therefore our polyembryonic propagules could have resulted from either cross- or self-pollination. Resembling standard habitat conditions, humidity levels range between 60 and 85%, and water and air temperature are kept constant at 25 °C all year round. Water salinity in the mangrove semi-diurnal tidal basin is 33. Nutrient analyses in the mangrove basin indicate a concentration of 0.018 mg l^{-1} of phosphate and 0.46 mg l^{-1} of nitrate in seawater.

The visual appearance of the 2021 polyembryonic, dry mature propagule before sprouting did not differ from the rest in any morphological parameter (Figure 2A). However, the polyembryonic propagule's initial weight on the day of collection was 2.2 g, 27.5% higher than the average of propagules in a similar development stage (1.7 g, n = 46). This suggests that a higher weight of mature propagules could indicate the presence of multiple embryos, which may enable early detection of polyembryony. Propagules were grown in a greenhouse on waterlogged quartz sand, at 26 °C, salinity of 20, and under a 12-h light regime of 300 μmol photons $m^{-2}\,s^{-1}$. All collected propagules sprouted after 48 h from the time of collection. After five days, hypocotyls were visible and the pericarp had decayed, exposing the cotyledons. In the case of

***Corresponding author: Sara P. Cobacho**, Department of Environmental Sciences, Aquatic Ecology and Water Quality Management, Wageningen University and Research, Droevendaalsesteeg 3a, 6708 PB Wageningen, The Netherlands, E-mail: sara.pinocobacho@wur.nl. https://orcid.org/0000-0001-5262-9474
Nicole A.L.M. Grol and Thomas A. Dros, Royal Burgers' Zoo, Antoon van Hooffplein 1, 6816 SH Arnhem, The Netherlands
Dorine T.B.M. Dekkers and Marjolijn J.A. Christianen, Department of Environmental Sciences, Aquatic Ecology and Water Quality Management, Wageningen University and Research, Droevendaalsesteeg 3a, 6708 PB Wageningen, The Netherlands

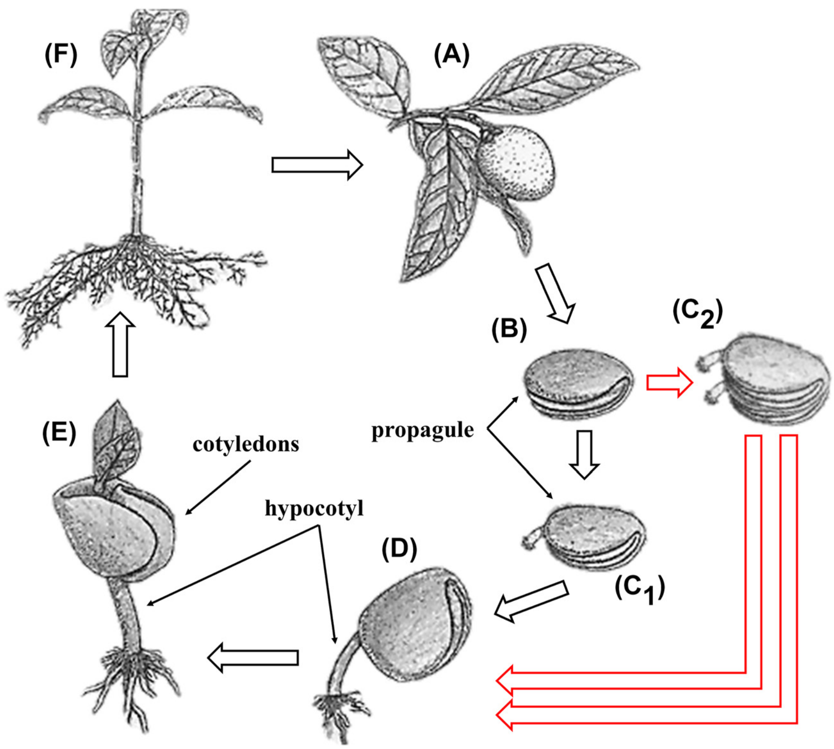

Figure 1: Reproductive cycle of *Avicennia germinans*. Propagules are produced by cryptovivipary thus remaining attached to the parent plant until germination (A and B). Propagules emerge by first developing an hypocotyl (C) and then roots (D). Epicotyl and first leaves emerge from the cotyledons (E), and subsequently the seedling develops into a sapling (F). Red arrows indicate deviations from the standard cycle as a result of polyembryony. Visible differences are the presence of two hypocotyls and a thicker, heavier mature propagule. Adapted from *The mangrove ecosystem–adaptations* (available at: https://themangroveecosystem.weebly.com/adaptations.html (Accessed: 8 September 2021).

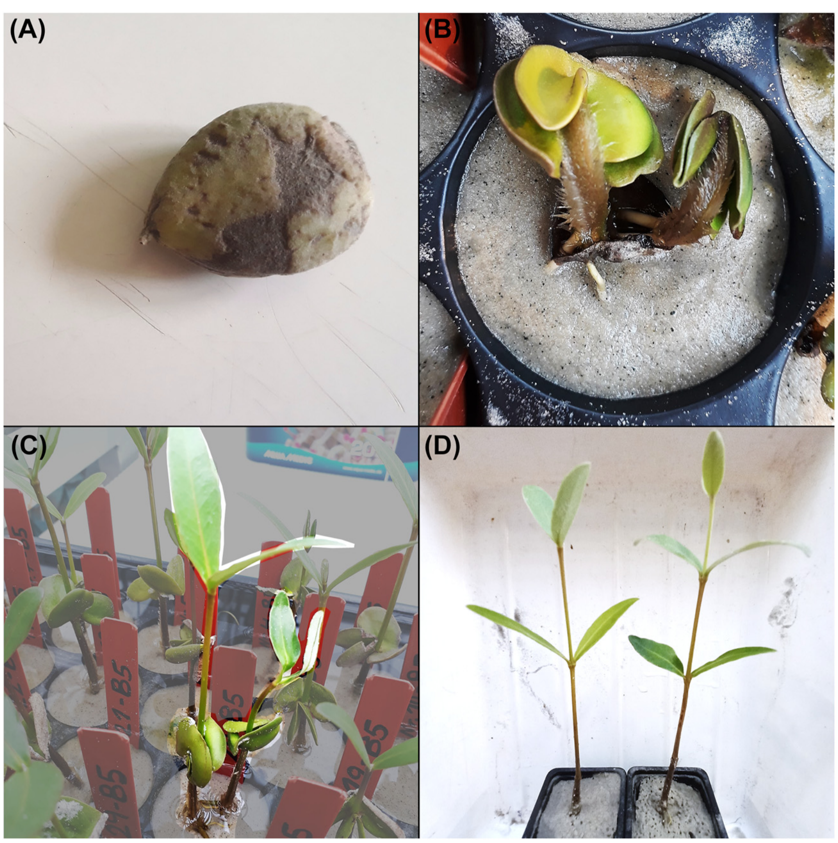

Figure 2: *Avicennia germinans*: polyembryonic, mature propagule (A) and different developmental stages of seedlings at (B) 5 days, (C) 6 weeks, and (D) 19 weeks after germination.

our polyembryonic propagule, each seedling had their own pair of cotyledons and separate, independent hypocotyls from which more roots developed (Figure 2B). These four cotyledons presented an abnormal asymmetrical shape, whereas in normal seedlings it is usually symmetrical. This deviation is due to a limited amount of space under the seed coat that, under normal conditions, only accommodates one pair of cotyledons. This deformity was particularly visible in one of the seedlings (Figure 2C, right). One of the polyembryonic seedlings showed a delayed growth as no epicotyl or leaves had developed from the cotyledons as opposed to its polyembryonic counterpart. Six weeks after germination, an abnormal growth of the epicotyl and deformed leaves became evident in only one of the seedlings (Figure 2C, right). Over time, the abnormally-shaped seedling overgrew its original deformities and developed into a mangrove sapling (Figure 2D).

Polyembryony is an extremely rare phenomenon in mangroves that has only been observed in a few previous occasions and only three other mangrove species. Bowman (1917) reported the observation of polyembryony in *Rhizophora mangle* by other authors in the West Indies and Fiji and mentioned the existence of a heredity factor in certain trees of those regions to develop polyembryony. This phenomenon has also been reported in *Rhizophora mucronata* (Kumar and Joshi 1942) and *Lumnitzera racemosa* (Lakshmanan and Narmatha Bai 1986). To our knowledge, there are no published reports of polyembryony in the mangrove species *A. germinans*.

The genotype of our polyembryonic seedlings of *A. germinans* is unknown. In almond seeds, a slow initial growth and poor development was observed in non-diploid organisms (Martínez-Gómez and Gradziel 2003), however there could be several other causes for the stunting of mangrove seedlings. The fact that our observation corresponds to a propagule produced in captivity raises the question whether the production of polyembryos was due to chance or induced by the more favourable growing conditions provided in the zoo. In citrus fruits, the frequency of polyembryony was found to be increased by environmental factors such as plant nutrition, air temperature, environmental and soil humidity, and wind speed (Andrade-Rodríguez et al. 2005). We cannot completely rule out the possibility that these cases of polyembryony simply resulted from the anomalous development of the original cells. However, our multi-year observations indicate that polyembryony in *A. germinans* could be a genetically regulated character resulting in a deviation from the normal reproduction process. It cannot be determined whether this deviation is an artifact of captivity, but the fact that polyembryony has previously been observed in several natural mangrove communities (Bowman 1917; Kumar and Joshi 1942; Lakshmanan and Narmatha Bai 1986) provides evidence against the artifact hypothesis. In other words, *A. germinans* may make use of mixed reproductive strategies to ensure the continued survival of the species, or to increase its expansion under favourable environmental conditions.

The type of polyembryony determines the genetic diversity of the seeds (Batygina and Vinogradova 2007; Michel et al. 2017). Lower genetic diversity can, in turn, determine the vulnerability of an ecological population to disturbance (Wernberg et al. 2018). Ample environmental tolerance boundaries in highly genetically diverse populations of mangroves could possibly provide an additional source of ecological resilience against the impacts of climate change. Among these impacts, increased evaporation and salinity, water temperature and sea level rise are determining factors for the survival of mangroves (Ward et al. 2016). Therefore, polyembryony could play an important role in the adaptive capacity of *A. germinans* to new, rapidly changing environmental conditions.

Acknowledgements: We thank Prof. Dr. Dolf Weijers for sharing his expert knowledge, and Annie Hunnestad, Rinie Verwoert, Marcel Otten, Rohan van Genderen, and Wilfred Krijnen for the technical support. We also show our appreciation to the Royal Burgers' Zoo for providing the biological material that led to this discovery.
Author contributions: Sara P. Cobacho: conceptualization, investigation, writing- original draft, writing-review & editing, visualization, data curation. Nicole A.L.M. Grol: resources, investigation, writing-review & editing. Thomas A. Dros: resources, investigation, writing-review & editing. Dorine T.B.M. Dekkers: writing-review & editing, access to equipment, availability of crucial research components. Marjolijn J.A. Christianen: writing-review & editing, visualization, supervision, project administration, funding acquisition.
Research funding: This work was carried out under the program of the Netherlands Earth System Science Centre (NESSC). This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement no. 847504 and has been further financially supported by the Dutch Ministry of Education, Culture and Science (OCW).
Conflict of interest statement: The authors declare that they have no conflicts of interest regarding this article.

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Sara P. Cobacho
Department of Environmental Sciences, Aquatic Ecology and Water Quality Management, Wageningen University and Research, Droevendaalsesteeg 3a, 6708 PB Wageningen, The Netherlands
sara.pinocobacho@wur.nl
https://orcid.org/0000-0001-5262-9474

Sara P. Cobacho is a PhD candidate of the Aquatic Ecology and Water Quality Management group at Wageningen University and Research in the Netherlands. Sara's PhD project investigates biogeochemical interactions between mangroves, seagrass and coral reefs under the influence of climate change and anthropogenic disturbances that may play a role in the productivity and resilience of connected tropical seascapes.

Nicole A.L.M. Grol
Royal Burgers' Zoo, Antoon van Hooffplein 1, 6816 SH Arnhem, The Netherlands

Nicole A.L.M. Grol is assistant curator of the Bush, Desert and Mangrove at Royal Burgers' Zoo in Arnhem, The Netherlands. She has an MSc in Animal Health and Welfare from Wageningen University & Research, and works in zoos for 16 years.

Thomas A. Dros
Royal Burgers' Zoo, Antoon van Hooffplein 1, 6816 SH Arnhem, The Netherlands

Thomas A. Dros is a plant and animal keeper at Royal Burgers' Zoo in Arnhem, The Netherlands for 22 years. He is a keeper in the Mangrove ecodisplay since its opening in 2017.

Dorine T.B.M. Dekkers
Department of Environmental Sciences, Aquatic Ecology and Water Quality Management, Wageningen University and Research, Droevendaalsesteeg 3a, 6708 PB Wageningen, The Netherlands

Dorine T.B.M. Dekkers is a Teaching and Research Assistant of the Aquatic Ecology and Water Quality Management group at Wageningen University and Research in the Netherlands. She has an Ir. degree in Biology, and her current research interests are the identification and culturing of marine and freshwater benthos, as well as seagrass and mangroves.

Marjolijn J.A. Christianen
Department of Environmental Sciences, Aquatic Ecology and Water Quality Management, Wageningen University and Research, Droevendaalsesteeg 3a, 6708 PB Wageningen, The Netherlands

Dr. Marjolijn J.A. Christianen is a marine ecologist and an associate professor of the Aquatic Ecology and Water Quality Management Group at Wageningen University and Research in the Netherlands. She is an NWO VENI Scholar and investigates the cascading effects of large consumers, plant invasion and habitat degradation to ecosystem services of coastal ecosystems, both in the tropics and in temperate regions.