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SCIENTIFIC NOTE

OVIPOSITION ACTIVITY OF *MANSONIA* SPECIES IN AREAS ALONG THE MADEIRA RIVER, BRAZILIAN AMAZON RAINFOREST

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ABSTRACT. *Mansonia* are aggressive mosquito species that are abundant in aquatic ecosystems where the macrophyte plants occur. These mosquitoes are commonly found across the Amazon/Solimões River basin. However, little is known about the oviposition behavior of these species. In the present study, we registered observations on the oviposition activity of 3 species: *Mansonia amazonensis*, *Ma. humeralis*, and *Ma*. cf. *titillans*, in 5 species of macrophytes in the vicinities of the Madeira River, Porto Velho, State of Rondônia, Brazil. Overall, 197 egg batches were collected. A greater amount of egg batches was found in *Salvinia molesta* as compared with other macrophytes sampled. In addition, 2 new oviposition habitats were noted in *Ludwigia helmintorrhiza* and *Limnobium spongia*. These findings will be important to understand the reproductive dynamics of these mosquitoes in the Brazilian Amazon basin.

KEY WORDS Amazon River basin, *Mansonia amazonensis*, *Mansonia* cf. *titillans*, *Mansonia humeralis,* oviposition.

Mosquitoes of the genus *Mansonia* are widely found across the Brazilian Amazon River basin (Hutchings et al. 2008, Gama et al. 2012, Palermo et al. 2016, de Araújo et al. 2020, Hutchings et al. 2020). Six species were registered in the region: *Mansonia amazonensis* (Theobald), *Ma. flaveola* (Coquillett), *Ma. humeralis* Dyar and Knab, *Ma. indubitans* Dyar and Shannon, *Ma. pseudotitillans* (Theobald) and *Ma. titillans* (Walker) (Ferreira 1999, Hutchings et al. 2020, Galardo et al. 2022), all of which were recorded in the vicinities of the municipality of Porto Velho, State of Rondônia, Brazil (Galardo et al. 2022).

Aquatic ecosystems with macrophytes can be potential habitats for immatures of *Mansonia* species (Ronderos and Bachman 1963). The female leans on a substrate or water surface and bends the last abdominal segments to reach the abaxial part of the macrophyte leaf to lay numerous egg batches. The eggs are surrounded by a gelatinous substance, forming a cluster, organized in a rosette shape, a vision created due to the funneling of the apical pole of the eggs (Dyar and Knab 1916, Linley et al. 1986, Lounibos and Linley 1987). The main plant species associated with this underwater oviposition activity are the water hyacinth, *Eichhornia crassipes* (Mart.), and water lettuce, *Pistia stratiotes* L. (Dunn 1918, Laurence 1960). The immature forms have siphons (in the larvae) and trumpets (in the pupae) that are adapted to pierce the roots of these macrophytes to obtain oxygen from the aerenchyma of the host plants (Wesenberg-Lund 1918, Guille 1975). Observations in a colony of *Mansonia* (*Mansonioides*) revealed that first larval instars could not breathe for a long time without macrophytes, making this association essential for the survival of these mosquitoes (Samarawickrema 1968).

Although oviposition mechanisms of *Mansonia* are known, it is better documented for the subgenus *Mansonioides*, which has a restricted distribution in Africa and Asia (Laurence 1960). Water hyacinth and water lettuce are the most important macrophytes for oviposition and fixation of larvae and pupae (Lounibos and Linley 1987, Ferreira 1999) for the subgenus *Mansonia* (Nearctic and Neotropical distribution). However, little is known about the role of other macrophytes in the oviposition of these species in the Amazon/Solimões River basin.

Between February 24 and March 17 of 2022, we recorded, for the first time, batches of eggs of *Mansonia humeralis*, *Ma*. cf. *titillans,* and *Ma. amazonensis* on the abaxial surface of the leaves of 5 species of aquatic macrophytes, 3 of which have no previous records of association with the ovipo-

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Table 1. The number of minimum, maximum, and mean (± standard deviation) of eggs of *Mansonia* found in three areas near the Madeira River from February 24 to March 17, 2022.

Area	Macrophytes	*Mansonia* sp.	No. of batches	No. of eggs per batch			
				Min	Max	Mean	SD
Luzitânia	*Ludwigia helminthorrhiza*	*Ma. humeralis*	10	59	123	98	16,3
		Mansonia sp.	3	101	122	111	10,6
	Salvinia molesta	*Ma. humeralis*	102	18	137	103	25,9
		Ma. cf. *titillans*	3	86	125	108	20,0
		Mansonia sp.	20	32	137	112	37,0
	Limnobium spongia	*Ma. humeralis*	4	72	112	91	18,9
		Ma. cf. *titillans*	2	72	137	105	46,0
Point Three	*L. helminthorrhiza*	*Ma. humeralis*	20	33	130	90	32,3
	S. molesta	*Ma. humeralis*	5	76	101	91	9,5
	Eichhornia crassipes	*Ma. humeralis*	9	87	114	100	10,7
		Ma. cf. *titillans*	9	116	213	187	32,2
		Mansonia sp.	7	112	212	168	47,3
Samaúma	*Pistia stratiotes*	*Ma. amazonensis*	2	118	121	120	2,1
		Ma. humeralis	1	24	24	24	0,0
Total			197	18	213	108	35,3

sition of *Mansonia* spp., *Salvinia molesta* Mitch (Salviniales: Salviniaceae), *Ludwigia helmintorrhiza* (Mart.), and *Limnobium spongia* (Bosc) Rich. Ex Steud (Alismatales: Hydrocharitaceae), in 3 breeding grounds near the Madeira River in Porto Velho, State of Rondônia.

The aquatic habitats found with *Mansonia* eggs were characterized for macrophyte species composition, and egg batches were either identified morphologically based on the descriptions of the eggs (Ferreira et al. 2020) or maintained in an insectary until the emergence of adults. Finally, the number of eggs was counted to verify the number of eggs per batch among the different macrophyte species. These values are presented with maximum, minimum, mean, and standard deviation values (Table 1).

Altogether, 13 species of macrophytes were sampled—*Eichhornia azurea* (Sw.), *E. crassipes* , *Limnobium spongia, Limnobium laevigatum* (Humb. and Bonpl. Ex Willd.), *Ludwigia helmothorrhiza* (Mart.), *Ludwigia sedoides* (Humb. and Bonpl.) (Alismatales: Hydrocharitaceae), *Paspalum repens* Bergius (Poales: Poaceae), *Cyperus rotundus* L. (Poales: Cyperaceae), *P. stratiotes*, *Azolla caroliniana* Willd., *Salvinia auriculata* Aublet complex, *Salvinia minima* Baker, and *Salvinia molesta* Mitch. (Salviniales: Salviniaceae), in Luzitânia area (09°03′28.22″S; 64°12′38.52″W), Point Three (09°01′16.46″S; 64°12′21.93″W), and Samauma area (09°11′47.45″S; 64°27′28.02″W). The 3 areas present characteristics of semiperennial lakes, with September being the period with the lowest water level at these points (Fig. 1).

In the Luzitânia area (Fig. 1), 9 species of macrophytes were analyzed: *E. crassipes, L. laevigatum, L. spongia, L. helmothorrhiza, L. sedoides, P. repens, C. rotundus, A. caroliniana*, and *S. molesta*. Egg batches were found in *S. molesta* (*n* = 125–63.5%), *L. helmothorrhiza* (*n* = 13–6.6%), and *L. spongia* (*n* = 6–3.0%). The largest number of eggs batches was found in the Luzitânia (*n* = 144–73.1%). In addition, 2 species of *Mansonia* were identified, *Ma. humeralis* and *Ma.* cf. *titillans*. The eggs that were found hatched were classified as *Mansonia* sp. (Table 1).

In Point Three (Fig. 1), 10 species of macrophytes were identified: *E. azurea, E. crassipes, L. laevigatum, L. helmothorrhiza, P. repens, C. rotundus, A. caroliniana, S. minima, S. auriculata* s.l. and *S. molesta*. The locality had the second highest number of egg batches (*n* = 50–25.4%), found in *E. crassipes* (*n* = 25–12.7%), *L. helmothorrhiza* (*n* = 20–10.2%), and *S. molesta* (*n* = 5–2.5%), which were identified as *Ma. humeralis, Ma.* cf. *titillans,* and *Mansonia* sp. (Table 1).

Finally, in Samauma area (Fig. 1), 5 species of macrophytes were identified: *E. azurea, E. crassipes, L. laevigatum, P. stratiotes,* and *S. molesta*. In addition, egg batches were found in juvenile *P. stratiotes* (*n* = 3–1.5%). The eggs belonged to *Ma. amazonensis* and *Ma. humeralis*, and were found in smaller numbers compared to the other studied locations (Table 1).

Salvinia molesta (*n* = 130–66.0%) presented the highest number of egg batches observed during the study, followed by *L. helmothorrhiza* (*n* = 33–16.8%), *E. crassipes* (*n* = 25–12.7%), *L. spongia* (*n* = 6–3.0%), and *P. stratiotes* (*n* = 3–1.5%). The oviposition distance from the leaf edge, measured with a caliper, ranged from 0.1 to 0.3 mm (*L. helmothorrhiza*), 0.15 to 0.2 mm (*P. stratiotes*), 0 to 0.39 mm (*S. molesta*), 0.1 to 0.3 mm (*E. crassipes*), and 0.1 to 0.2 mm (*L. spongia*). The egg batches of *Ma. amazonensis* were deposited 0.15 mm (SD = 0 mm) from the leaf edges of *P. stratiotes*; in *Ma. humeralis,* the average distance was 0.18 mm (SD = 0.06 mm) (all macrophytes), and in the case of *Ma.* cf. *titillans*, it was 0.10 mm (SD = 0.09 mm) (Table 1).

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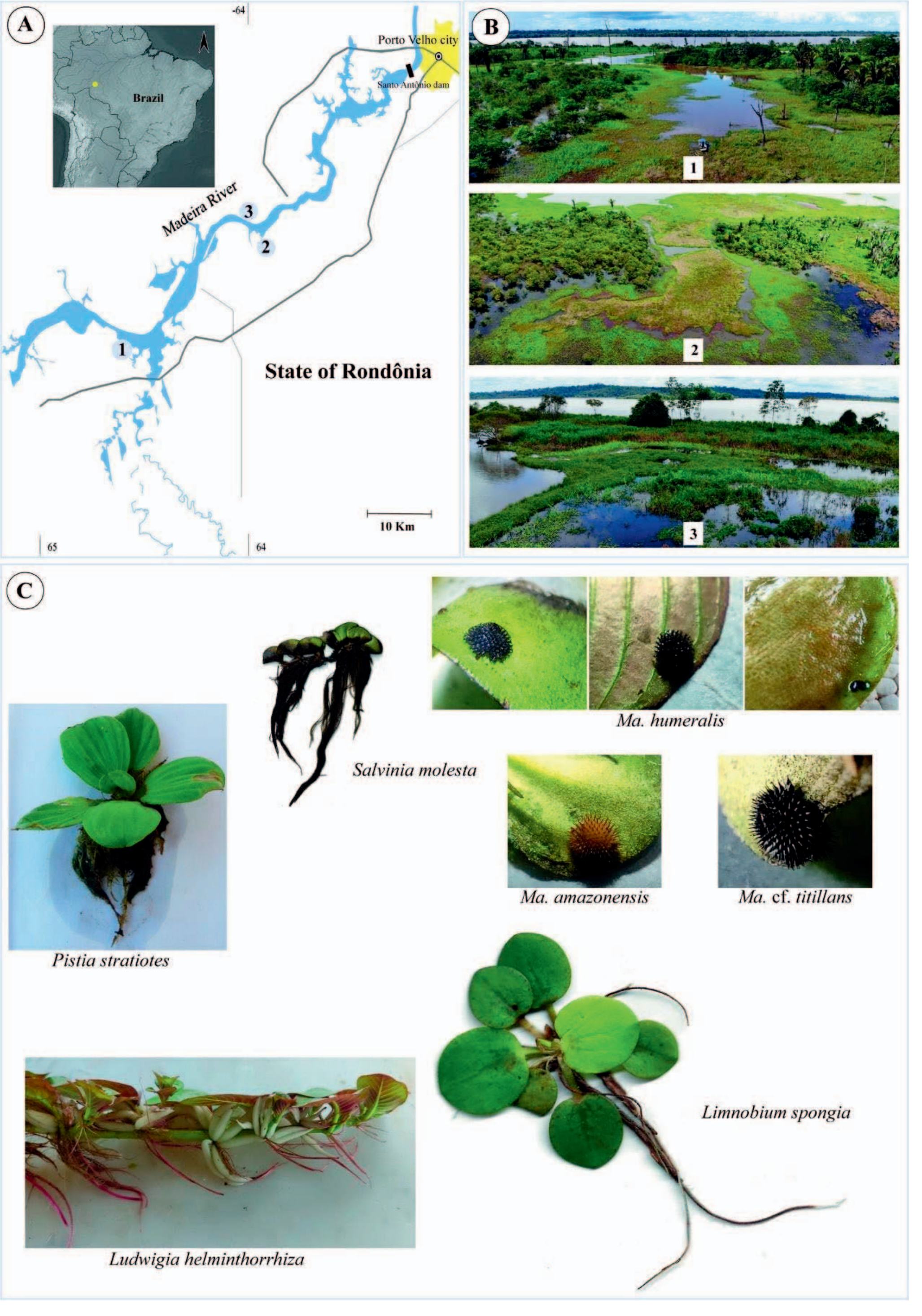

Fig. 1. Oviposition environments of *Mansonia* spp. on the Madeira River. (A) location of sites positive for *Mansonia* spp. eggs, (B) Aerial photographs of *Mansonia* spp. breeding sites, (1) Samauma area, (2) Luzitânia area, and (3) Point Three, (C) Macrophytes associated with oviposition and eggs of *Ma. humeralis*, *Ma. amazonensis,* and *Ma*. cf. *titillans*.

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Results of field collections showed that the highest numbers of egg batches of *Ma. humeralis* and *Ma.* cf. *titillans* were found in *S. molesta*. In addition, the presence of *E. crassipes* and *P. stratiotes* is important for *Mansonia* larvae and pupae in an aquatic ecosystem (Ronderos and Bachman 1963). Our study suggests that the eggs are deposited in shallow breeding sites, densely covered by small macrophytes, where the leaves are in contact with the water surface in flooded areas but with little influence from the main river flow. These more stable breeding sites ensure that the position of the leaves keeps the eggs trapped in the plant tissue and immersed in water until hatching, for their subsequent migration to macrophytes with more extensive roots, such as *E. crassipes* and *P. stratiotes*. In a similar study carried out by Ferreira (1999) on the island of the Marchetaria, the environmental conditions were like that found in this study. However, on Marchetaria Island, egg batches were more frequently collected in *P. stratiotes*, whereas none was found in *Salvinia*. Differences observed may indicate either the behavioral plasticity of these species or differences in *Mansonia* species oviposition preferences.

Laurence and Smith (1958) reported that aquatic grasses such as *Azolla, Salvinia,* and *Lemne* are excellent substrates for the oviposition of species of the subgenus *Mansonioides* in the laboratory. In preliminary studies carried out in our laboratory, engorged field-collected females laid eggs on *S. auriculata* s.l. and thin styrofoam sheets. Still, they did not lay eggs on *E. crassipes* and *P. stratiotes*. This finding can be explained by the position of the young leaves of these macrophytes, which are not in contact with the water surface. However, during field studies, we found *Mansonia* eggs on old or dead leaves of *E. crassipes* and *P. stratiotes*, which were in contact with the water surface; therefore contact with water was an important characteristic of the oviposition sites of the *Mansonia* species studied.

In addition, the association between *Mansonia* larvae and the macrophyte roots to obtain oxygen needs to be considered. Field observations indicated that larvae and pupae are found only in the roots of large macrophytes such as *E. crassipes* and *P. stratiotes* and more recently in the roots of *L. laevigatum* (Amorim et al. 2022), possibly due to the availability of oxygen in the plant aerenchyma. Also, the roots of these macrophytes protect them from predators, making it challenging to find the larvae and pupae throughout the roots (Consoli and Lourenço-de-Oliveira 1994).

Breeding site features, such as macrophytes composition, proper positioning of the leaves on the water surface, larger macrophytes to support the fixation of larvae and pupae, and suitable physicochemical parameters of the water, should also be considered as important criteria for *Mansonia*. Our study not only reinforces the importance of *E. crassipes* and *P. stratiotes* as primary host plants for larvae, but also reveals that oviposition can be carried out in other macrophyte species in the presence of the aforementioned environmental criteria.

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