

1 **Biology and rearing of the cogongrass gall midge, *Orseolia javanica* Kieffer & Docters**

2 **van Leeuwen-Reijnvaan (Diptera: Cecidomyiidae)**

3  
4 **Purnama Hidayat<sup>1</sup>, Arini<sup>2</sup>, Dwi Guntoro<sup>3</sup>, Keiji Takasu<sup>4</sup>, and William A. Overholt<sup>5</sup>**

5  
6 <sup>1</sup>*Department of Plant Protection, IPB University, Bogor, 16680, Indonesia*

7 <sup>2</sup>*Department of Biology, University of Riau, Riau, 28293, Indonesia*

8 <sup>3</sup>*Department of Agronomy and Horticulture, IPB University, Bogor, 16680, Indonesia*

9 <sup>4</sup>*Faculty of Agriculture, Kyushu University, Fukuoka 812–0395, Japan*

10 <sup>5</sup>*University of Florida, Institute of Food and Agricultural Sciences, Fort Pierce, USA*

11  
12 \*Corresponding author email: [billover@ufl.edu](mailto:billover@ufl.edu)

13  
14 **ABSTRACT**

15  
16 *Imperata cylindrica* (L.) Beauv. (Poaceae) is one of the most harmful weeds in the world  
17 because of its ability to spread and form high density, monospecific stands that exclude other  
18 vegetation. The cogongrass gall midge, *Orseolia javanica* Kieffer & Docters van Leeuwen-  
19 Reijnvaan (Diptera: Cecidomyiidae), is a stem galling insect that is only known to develop in  
20 cogongrass and has only been found on the island of Java in Indonesia. The midge attacks very  
21 young shoots, which stimulates abnormal growth, resulting in the formation of a purplish,  
22 elongate stem gall tapered to a point at the apical end. The aim of the current research was to  
23 describe the biology of the midge and develop a rearing method. *Orseolia javanica* completed  
24 its life cycle in 12-38 days with average egg, larval, and pupal periods of  $4.0 \pm 0.0$ ,  $13.5 \pm 3.8$ ,  
25 and  $8.6 \pm 6.6$  days (mean  $\pm$  SD), respectively. Mated female, unmated female, and male

26 longevities were  $1.7 \pm 0.47$ ,  $1.2 \pm 0.41$ , and  $1.0 \pm 0.00$  days (mean  $\pm$  SD). Galls began to appear  
27 29 days after larval infestation, and stem death coincided with emergence of the adult midge.  
28 The midge may have potential for biological control of cogongrass if future studies confirm a  
29 restricted host range.

30

31 *Additional index words:* Biological control, Indonesia, insect rearing, weed

32

33 Cogongrass, *Imperata cylindrica* (L.) Beauv. (Poaceae), is a serious weed in many areas  
34 of the world including Asia (Brook 1989; Garrity *et al.* 1997), West Africa (Chikoye &  
35 Ekeleme 2001) and the southeastern United States (McDonald 1996). In Asia, the largest  
36 infested area occurs in Indonesia (Garrity *et al.* 1997). The native range of cogongrass is vast  
37 and thought to include Africa, southern Europe, Asia and northern Australia (Hubbard *et al.*  
38 1944), while populations in the southeastern USA are exotic and highly invasive (Burrell *et al.*  
39 2015). There has been some speculation that the center of origin of cogongrass is East Africa  
40 because of the high diversity of plant pathogens (Ivens 1983), lack of weediness and high  
41 genetic diversity (Overholt *et al.* 2016). Cogongrass spreads rapidly by seeds and rhizomes,  
42 often forming monospecific stands that exclude other vegetation, resulting in both ecological  
43 and economic damage (Brook 1989; McDonald 1996). Moreover, cogongrass increases the  
44 frequency and intensity of wildfires (Jose *et al.* 2002).

45 In 2013, the University of Florida initiated exploration for natural enemies of cogongrass  
46 in several countries in Africa and Asia with the objective of identifying insects that may have  
47 value for introduction into the USA as biological control agents (Overholt *et al.* 2016). One of  
48 the insects encountered during surveys in Indonesia was *Orseolia javanica* Kieffer & Docters  
49 van Leeuwen-Reijnvaan (Diptera: Cecidomyiidae). The midge is a gall-forming insect that is  
50 only known to develop in cogongrass (Mangoendihardjo 1980; Soenarjo 1986). Early instar

51 larvae of *O. javanica* colonize young cogongrass shoots which stimulates the formation of  
52 elongate, purplish stem galls, tapered to a point at the apical end (Mangoendihardjo 1980).  
53 Based on field observations, the gall midge can be highly damaging and may have potential for  
54 biological control for cogongrass in the southeastern USA and elsewhere (Overholt *et al.* 2016).

55 The cogongrass gall midge has only been found in West and Central Java in Indonesia  
56 (Mangoendihardjo 1980). The population dynamics of the gall midge and its parasitoids in  
57 Cianjur, West Java were recently reported by Aviansyah (2016), who found that parasitoids  
58 emerged from 40-60% of galled stems. Similarly, Buhl *et al.* (2016) reported that three species  
59 of parasitoids emerged from 64% of galled stems collected in June and July 2015. In addition,  
60 Gumilang (2016) provided information about the distribution of the gall midge in Bogor and  
61 Cianjur Districts in West Java and Magelang and Salatiga Districts in Central Java.

62 The objective of the current study was to provide basic biological information about the  
63 midge, which is essential for developing a laboratory rearing method. The ability to rear the  
64 midge is a critical first step towards the initiation of host ranges studies required to evaluate  
65 the potential safety of the midge as a classical biological control agent.

66

## 67 MATERIALS AND METHODS

68

69 **Plants.** Cogongrass used in the laboratory studies was collected at Leuwikopo, Bogor  
70 Agricultural University. Plants were excised from the soil and planted in 25 plastic trays (40  
71 cm x 27 cm x 15 cm, L, W, H) with each pot receiving 20 stems. Trays were held outdoors  
72 inside screen cages (240 cm x 120 cm x 120 cm, L, W, H) for one month prior to initiation of  
73 studies. The one month period insured that stems were not naturally infested with gall midges.  
74 Studies were conducted at the Laboratory of Insect and Biosystematics, Department of Plant  
75 Protection, Faculty of Agriculture, Bogor Agricultural University.

76 **Insects.** Galled cogongrass stems (45-130) were collected on eight sampling occasions  
77 between August and November 2016 from bunds bordering rice fields near the village of Desa  
78 Cibereum in Cugenang District, Cianjur Regency, West Java. Desa Cibereum is located at -  
79 6.7914 latitude, 107.0687 longitude, at 852 m above sea level. In total, 832 galled stems were  
80 collected on the eight sampling occasions. Galled stems, along with attached rhizome, were  
81 removed from the soil and transported to the laboratory in coolers. Galls were then placed  
82 individually in plastic tubes and held at ambient laboratory conditions ( $27.3 \pm 0.21$  °C,  $68 \pm$   
83  $0.81$  RH) for emergence of adult midges. The size of the tubes varied from 5 cm x 8 cm to 5.5  
84 cm x 30 cm (D, H) depending on the length of the gall. Images of the midge's life stages and  
85 measurements were made using a Leica M205C stereo microscope attached to a Leica DFC  
86 450 camera and processed using Leica Application Suite Version 4.4.0 software. All estimates  
87 of central tendencies are presented as means  $\pm$  standard deviation. Voucher specimens of adults  
88 and immature stages are maintained in the insect collection at the Laboratory of Insect  
89 Biosystematics, Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural  
90 University.

91 **Oviposition, Egg Morphology and Development.** Adult males and females which emerged  
92 in tubes were placed together in the laboratory at ambient conditions in varying numbers in a  
93 plastic box (17 cm x 12 cm x 12 cm, L, W, H) that had a moist tissue paper on the bottom. A  
94 window (13 cm x 7 cm, L, W) was cut in the top of the box and covered with fine mesh cloth  
95 to allow ventilation. Eggs were laid by females on the bottom and side of the plastic box and  
96 collected after adults died. Eggs (20 to 50) were transferred to moist tissue paper in petri dishes  
97 (2 cm x 9 cm, D, H) using a fine brush. The length and width of eggs were measured and then  
98 observed daily until eclosion. Developmental time and the percentage of eggs that hatched were  
99 determined from a sample of 150 eggs.

100 **Larval and Pupal Morphology and Development.** Newly hatched larvae were transferred  
101 using a fine brush to the cogongrass stems which had been planted in trays, with each stem  
102 receiving one larva. Stems were trimmed to 2 cm above the soil surface prior to infestation. A  
103 total of 400 larvae were placed on cogongrass stems. Stems were held outdoors in large screen  
104 cages. Starting five days after infestation, 5-10 cogongrass stems were randomly selected and  
105 dissected every day to access larval colonization and growth of the larvae. In total, 350 stems  
106 were observed over a period of 57 days. Dissections were performed under a stereo microscope  
107 using a scalpel and a micro needle. Larvae and pupae were preserved in 70% ethanol in  
108 Eppendorf tubes (1.5 ml). A subset of 20 larvae was slide mounted to measure their length as  
109 the distance from the sternal spatula to the front of the head, and width at the widest point.  
110 Slides were prepared using a method described in Watson (2007), modified as follows: the  
111 posterior part of the larvae was pierced with a micro needle and then boiled in 95% ethanol for  
112 3 minutes. After 3 minutes, 10% KOH was added until the larvae appeared clear. The body  
113 contents were then removed under a stereo microscope using a micro needle. The larval skins  
114 were washed twice in distilled water and then dehydrated for five minutes each in a series of  
115 ethanol concentrations along with 1-2 drops of acid fuchsin, starting with 50% and proceeding  
116 to 80%, 95%, and finally to 100%. After that, the larvae were immersed in clove oil and slide  
117 mounted in Canada Balsam.

118 **Adult Behavior, Morphology, Fecundity and Longevity.** The behavior of adults was  
119 observed from the time that they emerged until mating. A subset of 38 field collected adults  
120 (19 males, 19 females) were killed in 70% ethanol and measured to determine body length,  
121 wingspan (distal end of left wing to distal end of right wing), and width at the widest point of  
122 the thorax. Sizes of males and females were compared using a two-sample *t*-test. Adult  
123 longevity was determined from a sample of 13 males, 11 mated females, and 30 unmated

124 females. Fecundity was determined by counting the number of eggs laid by 10 mated and 10  
125 unmated females from emergence until death.

126 **Gall Size.** The length and diameter of galls from which males and females (7 each) had  
127 emerged were measured and compared using a two-sample *t*-test. The galls were collected  
128 from the field in Cianjur, West Java.

129

130

## RESULTS

131

132 **Oviposition, Egg Morphology and Development.** From the 832 galls collected, 55 females  
133 and 44 males emerged. Eggs were laid singly or in groups of 2-18, side by side and  
134 longitudinally parallel on the wall of the plastic box or on the moist tissue paper on the bottom  
135 of the box. Eggs eclosed on the fourth days after oviposition. Eggs that failed to hatch became  
136 transparent or moldy.

137 Eggs were oval shaped with a length of  $0.53 \pm 0.004$  mm and a width of  $0.14 \pm 0.001$   
138 mm (Table 1). All freshly laid eggs were yellowish white. On the second day after oviposition,  
139 eggs from mated females became red at both the apical and distal ends. On the third day, the  
140 red color at both ends of the eggs faded while the lateral edges reddened, with the color  
141 becoming increasingly apparent as the eggs matured on the fourth day (Figure 1a-d). Eggs from  
142 unmated females remained transparent and did not undergo any color change.

143 **Larval and Pupal Morphology and Development.** Of the 350 stems that were inoculated  
144 with neonate larvae and dissected, 27% were successfully infested by gall midges and exhibited  
145 signs of deformation, 21% of stems died due to decay at the roots and the remaining 52% of  
146 stems were not colonized. Abnormal stem growth became evident 10 days after inoculation as  
147 an enlarging of the stem at the growing point. During early dissections (< 10 days after  
148 inoculation), some stems were found to be infested with two larvae, but later dissections

149 determined that only one larva survived to pupation. Developmental time and the average  
150 length and width of the body, length of the sternal spatula and width of the head are shown in  
151 Table 1. Newly hatched larvae were  $0.49 \pm 0.03$  mm long and  $0.17 \pm 0.006$  mm wide, reddish  
152 to orange, transparent, and had a black eye spot (Figure 2a). The second instar larva was orange  
153 to white with a black eye spot (Figure 2b). The dorsal side of second instar larvae was convex  
154 whereas the ventral side tended to be flat. Third instar larvae were white to yellowish and their  
155 bodies were firmer than those of second instars. The segments of third instars were more  
156 apparent than earlier instars and the sternal spatula became visible (Figure 2c).

157 Pupae of cogongrass gall midge were first white, and then darkened as they aged,  
158 becoming orange, light brown and dark brown with the eyes, legs and antennae black (Figure  
159 3a-c). The length and width were  $5.89 \pm 0.72$  mm and  $1.61 \pm 0.25$  mm, respectively. The mean  
160 developmental time of pupae was  $8.5 \pm 6.6$  days (Table 1).

161 **Adult Behavior, Morphology, Fecundity and Longevity.** Adult emergence occurred  
162 predominantly in the morning, but some individuals emerged in the afternoon and evening.  
163 After emerging from galls, adults remained on the outside of the gall for several minutes and  
164 then males flew off, while females tended to remain on the gall from which they emerged or  
165 on the soil nearby. Adults were covered with fine hairs on all body surfaces. Males were slender  
166 with black bodies and much smaller than females. Females were much broader than males and  
167 had a brown abdomen (Table 2, Figure 4a-b). Lifetime fecundity of mated females was  $512.1$   
168  $\pm 194.4$  eggs, while unmated female laid  $153.3 \pm 86.3$  eggs (Figure 5a-b). Mated female,  
169 unmated female, and male longevities were  $1.7 \pm 0.47$ ,  $1.2 \pm 0.41$ , and  $1.0 \pm 0.00$  days (mean  
170  $\pm$  SD).

171 **Gall size.** Gall morphology differed depending on whether a male or female emerged. Galls  
172 from which males emerged were longer (male:  $125 \pm 45$  mm, female:  $80 \pm 23$  mm;  $t = 2.4$ , df

173 = 12,  $P = 0.02$ ) and thinner (male:  $2.5 \pm 2$  mm, female:  $3.2 \pm 2$  mm;  $t = 8.9$ ,  $df = 12$ ,  $P <$   
174  $0.00001$ ) than galls from which females emerged.

175

176

## DISCUSSION

177

178 Developmental time of the midge (range = 12-38 days, mean =  $25.4 \pm 7.6$  days) was  
179 surprisingly variable, and much less than that found by Soenarjo (1986) of 35 to 49 days. The  
180 variability in developmental times of larvae and pupae in our study was likely due to  
181 fluctuations in ambient outdoor temperatures where the infested stems were maintained.  
182 Mangoendihardjo (1980) also reported much longer developmental times than those in our  
183 study (33 days for females and 35 days for males) but provided no measure of variability. The  
184 developmental times we found are similar to those reported for other *Orseolia* spp. *Orseolia*  
185 *oryzae* developed in 15 days (Jagadeesha Kumar 2009) or 19-23 days (Rajamani et al. 2004).  
186 *Orseolia oryzivora* was found to complete development in 26 days (Umeh & Joshi 1993).

187 Unfortunately, due to the lack of detail provided in Mangoenhihardio (1980), it is  
188 difficult to compare the success of the two rearing methods. The proportion of stems that were  
189 successfully colonized by the midge larvae in our study (27%) was low. Reasons for the  
190 relatively low success in colonization are unknown but could be due to injury to the delicate  
191 neonate larvae during transfer to stems, or perhaps differences in the susceptibility of stems  
192 due to their age, size or physiological state. Mangoendihardjo (1980) also reported a low  
193 success rate of stem colonization with only 25% of stems exposed to ovipositing females  
194 successfully colonized. Surprisingly, he also found that the majority (98%) of *O. javanica* eggs  
195 were laid on the soil surface, with only 2% laid on the plant. If this is representative of what  
196 occurs in nature, we suspect that there is very high mortality of larvae during their search for  
197 suitable stems due to predation and desiccation.



198 *Orseolia javanica* has three larval instars, as had been found for other *Orseolia* spp.,  
199 including *O. oryzae* (Perera & Fernando 1970) and *O. oryzivora* (Ogah et al. 2010). Similar to  
200 our findings, first and second instars of both *O. oryzae* and *O. oryzivora* had visible eyespots,  
201 and the third instar was characterized by the presence of a sternal spatula (Perera & Fernando,  
202 1970; Ogah et al. 2010).

203 Biological control of weeds utilizes highly host specific insect herbivores to regulate  
204 exotic weeds in their areas of invasion. A narrow host range is required to insure that introduced  
205 biological control agents will have little or no negative impacts to native flora or cultivated  
206 crops after release. Additionally, biological control scientists are increasingly encouraged to  
207 conduct studies on the potential impact of candidate biological control agents to the target plant  
208 in order to avoid the introduction ineffective agents (McClay & Balciunas 2005). In order to  
209 delineate the physiological host range of candidate biological control agents and conduct  
210 impact studies, effective rearing methods are required. Our study provides basic biological  
211 information and describes a rearing method for *O. javanica*, a potential biological control agent  
212 of cogongrass in the southeastern USA (Overholt *et al.* 2016).

213 Limited host range testing of *O. javanica* suggests that it may have the requisite host  
214 specificity for release as a biological control agent in USA. Mangoendihardjo (1980) exposed  
215 cogongrass, three varieties of cultivated rice, two wild rice species of questionable  
216 identification (published as *Oryza fatua*, which is considered a synonym of *O. rufipogon* Griff.  
217 and *O. perennis* which is of doubtful taxonomic status and may also be a synonym of *O.*  
218 *rufipogon* (Terrell *et al.* 2000), sorghum (*Sorghum bicolor* (L.) Moench), maize (*Zea mays* L.)  
219 and two wild grasses (*Paspalum conjugatum* Berguis, *Pennisetum polystachyon* (L.) Schult. )  
220 to the midge and it only completed development in cogongrass. Narrow host ranges of other  
221 *Orseolia* spp. also point towards a possible high specificity of *O. javanica*. The African species,  
222 *O. bonzii* Harris is only known to develop in *Paspalum scobiculatum* (L.), and *O. nwanzei*

223 Harris & Nwilene only in *Eragostris atrovirens* (Desf.) Trin. ex. Steud (Nwilene *et al.* 2006),  
224 while the Africa rice gall midge, *O. oryzivora*, only completes development on cultivated and  
225 wild *Oryza* spp. (Williams *et al.* 1999). The Asian rice gall midge, *O. oryzae* was found only  
226 in cultivated rice during a survey of 10 wild grasses and wild *Oryza* spp. in the state of  
227 Karnataka, India (Kumar *et al.* 2009), while another study in India reported the midge from  
228 two wild grasses and three *Oryza* spp. (Rajamani *et al.* 2004). Twenty-four species of oriental  
229 *Orseolia* are described, and the majority, including *O. javanica*, have only been collected from  
230 one grass host (Gagné & Jaschhof 2017).

231 In summary, our studies provide basic biological information, and describe a rearing  
232 method that can be used to initiate and maintain laboratory colonies of *O. javanica* so that host  
233 range and impact studies can be conducted in order to determine whether the midge may be  
234 considered for release as a biological control agent of cogongrass.

235

## 236 ACKNOWLEDGMENTS

237

238 This research was partly funded by the Thesis and Dissertation Grant from The Indonesian  
239 Endowment Fund for Education (LPDP), Ministry of Finance, Republic of Indonesia and the  
240 Florida Agricultural Experiment Station, Florida Fish and Wildlife Conservation Commission,  
241 the Florida Department of Agriculture and Consumer Services.

242

## 243 LITERATURE CITED

244

245 Aviansyah, I. 2016. Population and parasitoid of the cogongrass gall midge *Orseolia javanica*  
246 Kieffer & Van Leeuwen-Reijnvaan (Diptera: Cecidomyiidae) in Cianjur Regency] [BSc  
247 Thesis]. Institut Pertanian Bogor, Bogor.

- 248 Brook, R.M. 1989. Review of literature on *Imperata cylindrica* (L.) Rauschel with particular  
249 reference to South East Asia. *Tropical Pest Management* 35: 12–25.
- 250 Buhl, P.N. and Hidayat, P. 2016. A new species of *Platygaster* (Hymenoptera: Platygastridae)  
251 reared from *Orseolia javanica* (Diptera: Cecidomyiidae) on cogongrass, *Imperata*  
252 *cylindrica* (Poaceae). *International Journal of Environmental Studies* 73: 25–31.
- 253 Burrell, M., Pepper, A.E., Hodnett, G., Goolsby, J.A., Overholt, W.A., Racelis, A.E., Diaz, R.,  
254 and Klein, P.E. 2015. Exploring origins, invasion history and genetic diversity of  
255 *Imperata cylindrica* (L.) P. Beauv. (Cogongrass) in the United States using genotyping  
256 by sequencing. *Molecular Ecology* 24: 2177–2193.
- 257 Chikoye, D. and Ekeleme, F. 2001 Weed flora and soil seedbanks in fields dominated by  
258 *Imperata cylindrica* in the moist savannah of West Africa. *Weed Research* 41: 475–490.
- 259 Gagné, R.J. and Jaschhof, M. (2017) *A Catalog of the Cecidomyiidae (Diptera) of the World*,  
260 4<sup>th</sup> edn. Systematic Entomology Laboratory, Agricultural Research Service, U.S.  
261 Department of Agriculture, Washington.
- 262 Garrity, D., Soekardiz, M., Van Noordwijk, M., De La Cruz, R., Pathak, P., Gunasenas, H.,  
263 and Van So, N. 1997. The *Imperata* grasslands of tropical Asia: area, distribution, and  
264 typology. *Agroforestry Systems* 36: 3–29.
- 265 Gumilang, B.L. 2016 Distribution and population level of the cogongrass gall midge, *Orseolia*  
266 *javanica* Kieffer & van Leeuwen-Reijnvaan (Diptera: Cecidomyiidae) [BSc Thesis].  
267 Institut Pertanian Bogor, Bogor.
- 268 Hubbard, C.E., Whyte, R.O., Brown, D., and Gray, A.P. 1944. *Imperata cylindrica*: taxonomy,  
269 distribution, economic significance and control. *Imperial Agricultural Bureaux Joint*  
270 *Publication* 7: 1–63.
- 271 Ivens, G.W. 1983. The natural control of *Imperata cylindrica*: Nigeria and northern Thailand.  
272 *Mountain Research and Development* 3: 372–377.

- 273 Jagadeesha Kumar, B.D., Chakravarthy, A.K., Doddabasappa, B., and Basavaraju, B.S. 2009.  
274 Biology of the rice gall midge, *Orseolia oryzae* (Wood-Mason) in southern Karnataka.  
275 *Karnataka Journal of Agricultural Science* 22: 535–537.
- 276 Jose, S., Cox, J., Miller, D.L., and Shilling, D.G. 2002. Alien plant invasions: the story of  
277 cogongrass in southeastern forests. *Journal of Forestry* 100: 41–44.
- 278 Kumar, B.D.J., Charkravarthy, AK, Doddabasappa, B., and Basavaraju, B.S. 2009. Biology of  
279 the rice gall midge, *Orseolia oryzae* (Wood-Mason) in southern Karnataka. *Karnataka J.*  
280 *Agricultural Science* 22: 535-537.
- 281 Mangoendihardjo, S. 1980. Some notes on the natural enemies of alang-alang (*Imperata*  
282 *cylindrica* (L.) Beauv.) in Java, pp. 47-55. In *Proceedings of BIOTROP Workshop on*  
283 *Alang-alang*. Jul 27-29 1976, Bogor, Indonesia (Edited by Soewardi). Seameo Biotrop,  
284 Bogor.
- 285 McClay, A.S. and Balciunas, J.K. 2005. The role of pre-release efficacy assessment in selecting  
286 classical biological control agents for weeds—applying the Anna Karenina principle.  
287 *Biological Control* 35: 197–207.
- 288 McDonald, S.K., Shilling, D.G., Okoli, C.A.N., Bewick, T.A., Gordon, D., Hall, D., and Smith,  
289 R. 1996. Population dynamics of cogongrass, *Imperata cylindrica*, pp. 156. In  
290 *Proceedings of the Southern Weed Science Society*.
- 291 Nwilene, F.E., Nwanze, K.F., and Olahidievlbie, O. 2006. *African Rice Gall Midge, Biology,*  
292 *Ecology and Control. Field Guide and Technical Manual*. African Rice Centre  
293 (WARDA) Cotonou, Benin. 24 pp.
- 294 Ogah, E., Odebiyi, J., Ewete, F., Omoloye, A., and Nwilene, F. 2010. Biology of the African  
295 rice gall midge *Orseolia oryzivora* (Diptera: Cecidomyiidae) and its incidence on wet-  
296 season rice in Nigeria. *International Journal of Tropical Insect Science* 30: 32–39.

- 297 Overholt, W.A., Hidayat, P., Le Ru, B., Takasu, K., Goolsby, J.A., Racelis, A., Burrell, A.M.,  
298 Amalin, D., Agum, W., Njaku, M., Pallangyo, B., Klein, P.E., and Cuda, J.P. 2016.  
299 Potential biological control agents for management of cogongrass (Cyperales: Poaceae)  
300 in the southeastern USA. *Florida Entomologist* 99: 734–739.
- 301 Perera, N. and Fernando, H.E. 1970. Infestation of young rice plants by the rice gall midge,  
302 *Pachytiplosis oryzae* (Wood-Mason) (Diptera: Cecidomyiidae), with special reference  
303 to shoot morphogenesis. *Bulletin of Entomological Research* 59: 605–613.
- 304 Rajamani, S., Pasalu, I.C., Mathur, K.C., and Sain, M. 2004. Biology and ecology of rice gall  
305 midge, *Orseolia oryzae* (Wood-Mason), pp. 7-16. In *New Approaches to Gall Midge*  
306 *Resistance in Rice*. Proceedings of the International Workshop. 22-24 November 1998,  
307 Hyderabad, India (Edited by J Bennett, JS Bentur, IC Pasalu, K Krishnaiah). International  
308 Rice Research Institute, Hyderabad, India.
- 309 Soenarjo, E. 1986. Alang-alang gall midge potential as an alternate host for parasitoids.  
310 *International Rice Research Newsletter* 11: 22–23.
- 311 Terrell, E.E. and Duvall, M.R. 2000. *Oryza*. Catalogue of New World Grasses (Poaceae): I.  
312 Subfamilies Anomochlooideae, Bambusoideae, Ehrhartoideae, and Pharoideae.  
313 *Contributions from the United States National Herbarium* 39: 89–92.
- 314 Umeh, E.D.N. and Joshi, R.C. 1993. Aspects of the biology, ecology and natural biological  
315 control of the African rice gall midge, *Orseolia oryzivora* Harris and Gagné (Diptera:  
316 Cecidomyiidae) in central and southeast Nigeria. *Journal of Applied Entomology* 116:  
317 391–398.
- 318 Watson, G.W. 2007. Identification of whiteflies (Hemiptera: Aleyrodidae), pp. 16-26. In *APEC*  
319 *Re-entry Workshop on Whiteflies and Mealybugs*. 16 – 26 April 2007, Kuala Lumpur,  
320 Malaysia.

321 Williams, C.T., Okhidievbie, O., Harris, K.M., and Ukwungwu, M.N. 1999. The host range,  
322 annual cycle and parasitoids of the African rice gall midge *Orseolia oryzivora* (Diptera:  
323 Cecidomyiidae). *Bulletin of Entomological Research* 89: 589–597.  
324

325 **Table 1.** Sizes and developmental durations (means  $\pm$  SD) of immature stages of cogongrass  
 326 gall midge.

Stages	Length (mm)	Width (mm)	Length of sternal spatula (mm)	Width of head (mm)	Duration (days)	N
Egg	0.53 $\pm$ 0.004	0.14 $\pm$ 0.001	-	-	4.0 $\pm$ 0.0	133
Larva						
First instar	0.59 $\pm$ 0.100	0.19 $\pm$ 0.010	-	0.05 $\pm$ 0.01	7.1 $\pm$ 1.4	10
Second instar	1.18 $\pm$ 0.400	0.43 $\pm$ 0.150	-	0.09 $\pm$ 0.02	2.7 $\pm$ 1.5	12
Third instar	3.30 $\pm$ 1.500	1.00 $\pm$ 0.350	0.09 $\pm$ 0.02	0.08 $\pm$ 0.01	9.8 $\pm$ 4.7	32
Pupa	5.89 $\pm$ 0.700	1.61 $\pm$ 0.250	-	-	8.5 $\pm$ 6.6	30

327

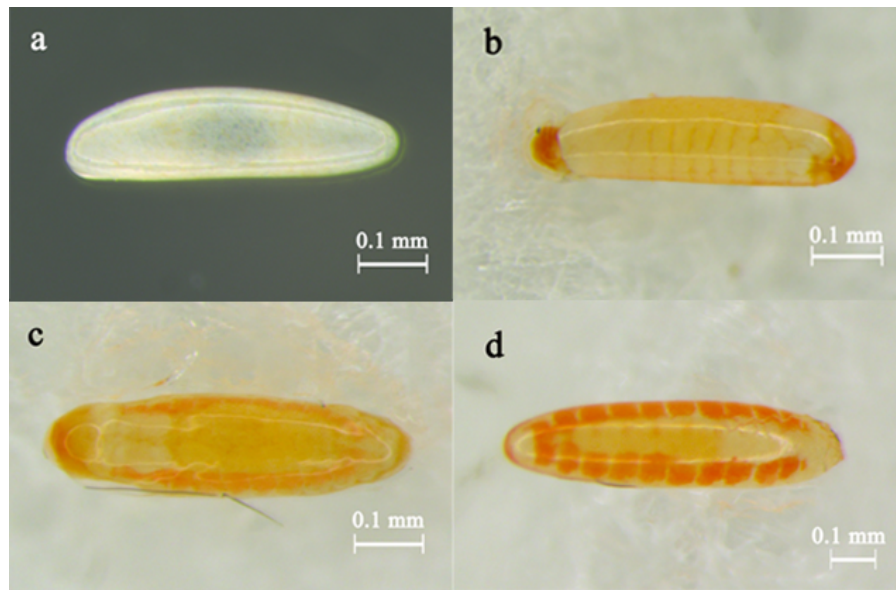
328 **Table 2.** Length, width and wingspan (mean  $\pm$  SD) of adult male and female cogongrass gall  
 329 midge.

Parameter	Male (mm) <sup>a</sup>	Female (mm) <sup>a</sup>	<i>t</i>	df	P
Body length (mm)	2.95 $\pm$ 0.27a	5.65 $\pm$ 1.12b	10.3	36	< 0.00001
Body width (mm)	0.58 $\pm$ 0.06a	1.16 $\pm$ 0.15b	15.8	36	< 0.00001
Wingspan (mm)	6.63 $\pm$ 1.19a	9.76 $\pm$ 0.49b	10.5	36	< 0.00001

330 <sup>a</sup> Means in a column followed by different lowercase letters are significantly different ( $P \leq$   
 331 0.05; ANOVA and LSD test).

332

333



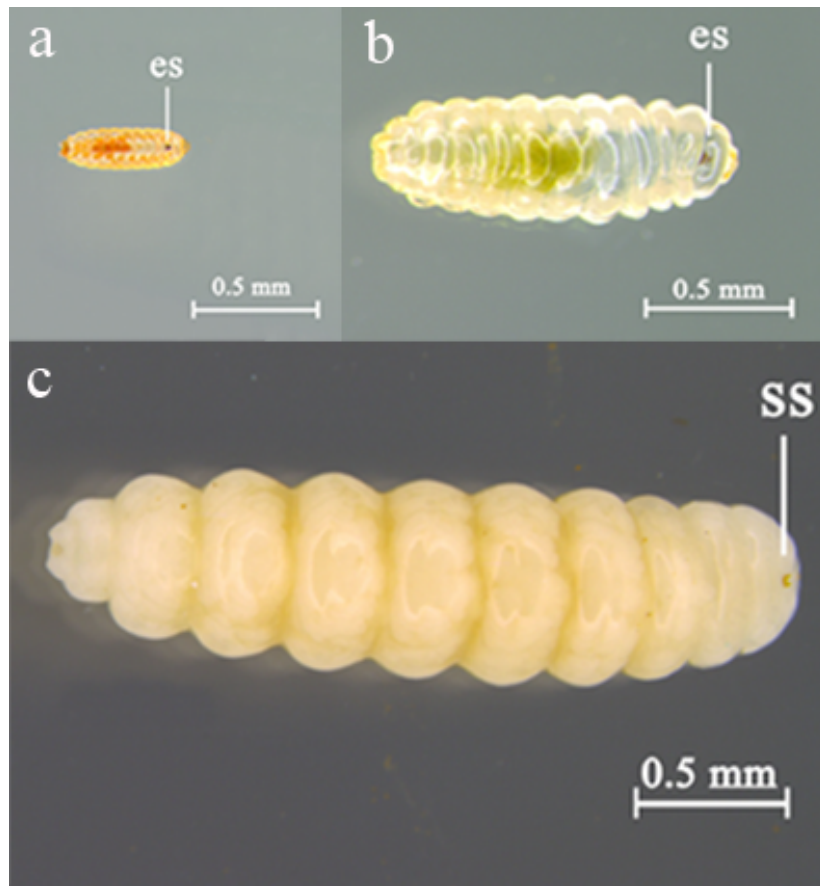
334

335

**Figure 1.** One (a), two (b), three (c) and 4 days (d) old eggs of *O. javanica*.

336





337

338 **Figure 2.** Neonate (a), 11 days old (b), and 21 days old larvae of *O. javanica*, es, eye spot; ss,

339 sternal spatula.

340

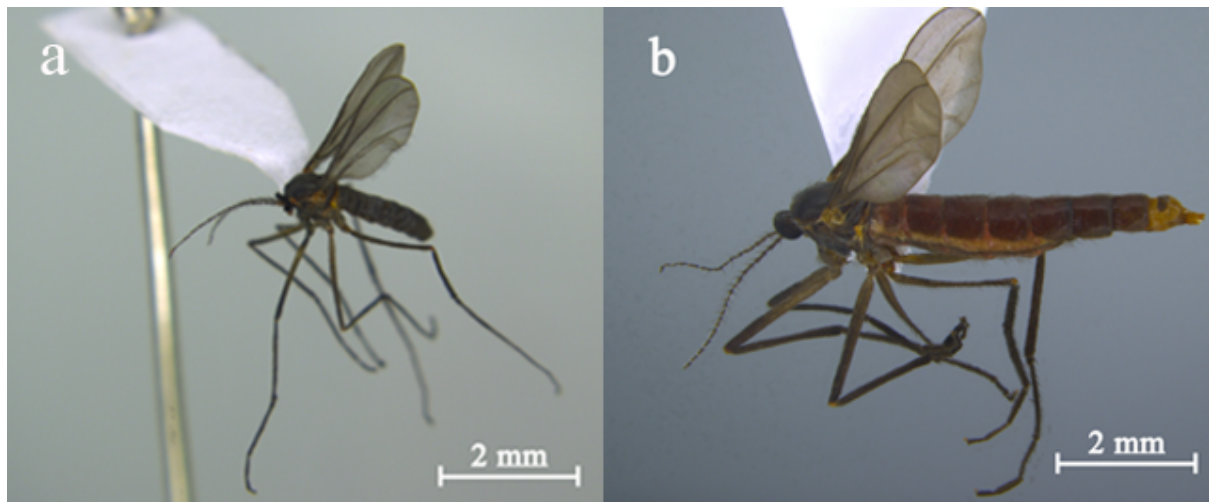


341

342

**Figure 3.** Pupae of *O. javanica* at 7 (a), 15 (b), and 25 days (c) after pupation.

343

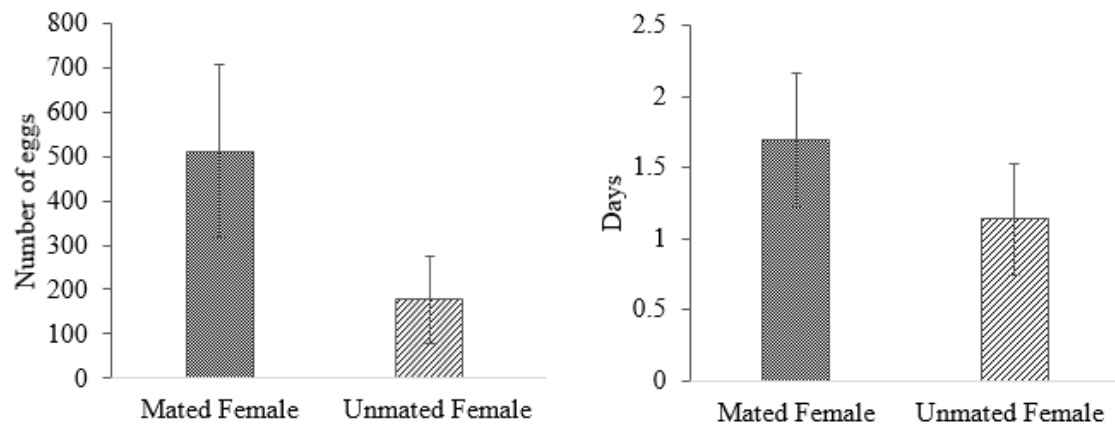


344

345

**Figure 4.** Adult male (a) and female (b) cogongrass gall midges.

346



347

348 **Figure 5.** Fecundity (a) and longevity (b) (mean  $\pm$  SD) of adult female cogongrass gall midges.

349