

**PONTIFICIA UNIVERSIDAD CATÓLICA DEL ECUADOR**

**FACULTAD DE CIENCIAS EXACTAS Y NATURALES**

**CARRERA DE MICROBIOLOGÍA**

**Caracterización de la microbiota en el intestino de *Panstrongylus chinai*, insecto  
transmisor de la enfermedad de Chagas en el Sur de Ecuador**

Disertación previa a la obtención del título de Microbiólogo

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## **CERTIFICACION**

Yo Anita Gabriela Villacís Salazar PhD, certifico que la disertación de Microbiología del estudiante Matías Leonel Cadena Cárdenas ha sido concluida de conformidad con las normas establecidas; por lo tanto, puede ser presentada para la calificación.

A handwritten signature in blue ink that reads "Anita Villacís". The signature is fluid and cursive, with a long horizontal stroke at the bottom.

Anita G. Villacís

**DIRECTOR DE LA DISERTACIÓN**

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## **DEDICATORIA**

A mis padres porque sin ellos no habría llegado hasta este punto, por ser aquellos que me han guiado durante toda mi vida, dándome alientos de seguir adelante, que han velado y han trabajado arduamente para llegar hasta este momento, porque han depositado su confianza en mí, demostrándome lo capaz que soy y haciéndome ver las fortalezas que desconocía de mí mismo, sin ellos y sin su guía no habría llegado hasta este punto. Es por esto y miles de razones más que esta tesis es dedicada a ellos porque me han ayudado a alcanzar mis metas.

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58

59 **Abstract**

60 Chagas disease (CD) is caused by the *Trypanosoma cruzi* parasite and transmitted mainly by  
61 blood-sucking insects of the Triatominae subfamily. This disease is endemic in 21 countries in  
62 America, mainly in rural areas, but spreading to urban areas due to human mobility. Ecuador,  
63 present an incidence of 36 cases per 100,000 inhabitants per year and 16 species of triatomines  
64 were described. *Panstrongylus chinai*, is an secondary vector in Loja provinces, southern  
65 Ecuadorian Region. Insecticides has been used as the main way to control this disease,  
66 however, new strategies need to improve to prevent CD. The intestinal microbiota of insects  
67 can influence in the transmission of *T. cruzi*, for that reason the aim of this study is the  
68 molecular characterization of the gut bacterial microbiota of *Panstrongylus chinai* collected in  
69 three communities of Loja, Ecuador. For this, i) a total of 63 DUs were examined, of those 5  
70 houses were infested (infestation index = 7.9 %) with 12 individuals of *P. chinai*. ii) A total 12  
71 samples collected in the DUs and 50 individuals from laboratory colonies were analyzed for  
72 the bacterial composition. The intestine and DNA were extracted, performing molecular  
73 detection of *T. cruzi* by PCR and sequencing the bacterial 16S region. The results revealed the  
74 presence of *T. cruzi* in a field sample and the presence of *Staphylococcus* genus, with the  
75 species *S. saprophyticus* (75%) and *S. equorum* (25%). This study contributes to the  
76 understanding of the microbiota and open for further research to contribute to the control of  
77 CD.

78 **Keywords:** Chagas disease, Ecuador, microbiota, triatomines.

79

## 80 **Introduction**

81 Chagas disease, also known as American trypanosomiasis, is caused by the protozoan  
82 *Trypanosoma cruzi* (Coura 2013). This disease is primarily transmitted through the feces of  
83 hematophagous insects from the Reduviidae family, Triatominae subfamily (Coura 2007).  
84 However, this disease can also be transmitted through transfusions, congenital, organ  
85 transplants, orally or laboratory accidents (PAHO 2022).

86 Chagas disease is endemic in 21 countries in the Americas, and it is estimated that between  
87 six and seven million people are infected by *T. cruzi* (PAHO 2022). In recent years, the  
88 epidemiological pattern of the disease has shifted from being primarily rural to predominantly  
89 urban, and migratory movements have further spread the disease in the Americas and other  
90 regions of the world (PAHO 2022).

91 Knowledge of the incidence and prevalence of Chagas disease in Ecuador is limited, and  
92 there are no estimated data on the number of cases infected by *T. cruzi* (Grijalva et al. 2015).  
93 The incidence has been estimated at 36 cases per 100,000 inhabitants per year, translating to  
94 4,400 new cases each year (Dumonteil et al. 2015, PAHO 2022). At least 151 species of  
95 triatomines have been reported worldwide (Justi and Galvão 2017). In Ecuador, the presence  
96 of 16 species has been documented, with *Triatoma dimidiata* and *Rhodnius ecuadoriensis*  
97 being the main vectors (Villacis et al. 2020) Additionally, six species of the *Panstrongylus*  
98 genus have been found in Ecuador: *Panstrongylus chinai*, *P. howardi*, *P. geniculatus*, *P.*  
99 *lignarius* / *P. herreri*, and *P. rufotuberculatus*. The significance of these species has  
100 dramatically increased due to their invasion of human structures, acting as secondary vectors  
101 (Villacís et al. 2020). Some of them have a high capacity to transmit Chagas disease due to i)  
102 their longevity, ii) their rapid response to the presence of a host, iii) the large volume of blood  
103 ingested, and iv) frequent defecation during the feeding process (Villacis et al. 2020).

104 *Panstrongylus chinai* is distributed in Ecuador, Peru, and Venezuela (Patterson et al. 2009),  
105 and in Ecuador, this species is found in the provinces of Loja and El Oro, in the southern  
106 Andean region of Ecuador. *P. chinai* can be found in domestic and peridomestic environments,  
107 such as chicken coops, pigeon nests, and rat nests. Inside houses, it is mainly located under  
108 beds or in holes in the walls (Grijalva et al. 2015). Furthermore, *P. chinai* is a competent vector  
109 of *T. cruzi*, since in its different stages of development, defecation occurs during or  
110 immediately after feeding (Mosquera et al. 2016). This fact is important since the development  
111 of this insect takes approximately 12 months (Mosquera et al. 2016), meaning that in all its  
112 stages it can contract and transport the parasite.

113 It is crucial to understand the movement, development and interactions of the parasite, *T.*  
114 *cruzi* enters the insect through blood containing the parasite, migrates to the midgut to survive  
115 and grow, evading the immune system and avoiding harmful interactions with local microbiota  
116 (Garcia and Azambuja1991, Da Motta et al. 2012). Once it reaches the midgut, the parasite  
117 grows. After completing the parasite's life cycle, it is excreted by the insect through feces from  
118 the rectum during feeding, initiating a new infectious cycle (Garcia and Azambuja1991, Da  
119 Motta et al. 2012).

120 Currently, the main method for controlling triatomine population is through the use of  
121 insecticides. However, it has been observed that several triatomine have developed resistance  
122 (Engel et al. 2013), making it necessary to find a new method to control this disease. One of  
123 these alternatives is the use of the indigenous intestinal microbiota of these vectors.  
124 Furthermore, there is increasing evidence that the insect's intestinal microbiota can affect the  
125 ability of pathogens to colonize and persist in the vector or alter the vector's competence to  
126 transmit pathogens to the human host (Waltmann et al. 2019). The microbiota includes a wide  
127 variety of commensal, pathogenic, or mutualistic species that affect the insect host's  
128 reproduction, nutrition, and immune system (Kieran et al. 2019).

129 The triatomine microbiota represents one of the factors that can interfere with the  
130 transmission and virulence of *T. cruzi* in humans. Therefore, it is possible that the transport of  
131 the *T. cruzi* parasite in the insect's intestine may affect the structure of the intestinal microbiota  
132 (Da Mota et al. 2012), making knowledge of its composition important and essential for  
133 initiating new areas of research and disease control. For this purpose, molecular diagnostic  
134 characterization is necessary, as it will provide a diagnostic and a comprehensive view of the  
135 microorganisms present inside the vector insect.

136 Coprophagy is one of the main processes through which intestinal symbiotic microbes are  
137 acquired among insects in the laboratory (Waltmann et al. 2019). In nature, the acquisition of  
138 non-symbiotic/environmental or host-associated microbes can also occur through coprophagy  
139 and during blood meals when in contact with mammalian hosts or other animals (Enge et al.  
140 2013). Therefore, it is important to study and understand the microbiota of insects raised in the  
141 laboratory and those collected in nature to obtain a complete picture of the microorganisms to  
142 be studied.

143 Culture-dependent methods have shown that the spectrum of bacterial species identified in  
144 laboratory cultures does not necessarily reflect the relative frequency of these species in natural  
145 conditions (Vallejo et al. 2009). Previously, research on bacterial diversity in vector intestines  
146 was based on the isolation and identification of cultivable bacteria, a method that inevitably  
147 overlooks many non-cultivable taxa (Oliveira et al. 2018). This is why non-culture-based  
148 studies are needed, in which some characteristics of the intestinal microbiota of triatomines  
149 have been identified. First, their diversity within each host is low, with one or a few dominant  
150 genera. Additionally, some bacterial genera appear to be specific to certain triatomines. This  
151 raises questions about the diversity of intestinal microorganisms in *P. chinai*, as well as whether  
152 it is specific to the insect or not (Díaz et al.2016). So far, there is no documentation on *P.*

153 *chinai's* microbiome, making it interesting to conduct a diagnosis of its microbiota, both in  
154 insects raised in the laboratory and those collected in nature.

155

## 156 **Materials and Methods**

157

### 158 **Study area and collection of *Panstrongylus chinai***

159 The field phase was carried out in the domestic and peridomestic habitats of three rural  
160 communities, Guara, Chaquizhca and Bellamaría in Calvas county Loja province, (Fig. 1).  
161 Chaquizhca (888–1323 meters above sea level [masl], n=23 examined houses, Guara (1064–  
162 1450 masl, n=11 examined houses), and Bellamaria (1000–1384 masl, n=29 examined houses).  
163 We visited the total of the DUs in each community. The exclusion criteria were those  
164 households inhabited, closed or refused.

165 Each domestic unit (DUs), which included domestic and peridomestic habitats were  
166 searched for triatomine using the one-man-hour method as previously described by Grijalva et  
167 al. (2015) and conducted by two-person skilled teams (30 min) from the Ecuadorian Ministry  
168 of Health under the supervision of the personnel from the Center for Research on Health in  
169 Latin America (CISeAL). Collected triatomines were placed in individually labeled plastic  
170 containers under collection permit number MAAE-DBI-CM-2021-0185, and transported to the  
171 insectary of the CISeAL of the Pontificia Universidad Católica del Ecuador (PUCE) in Quito,  
172 Ecuador by the mobilization permits number MAAE-CMARG-2020-0178. Details of place of  
173 capture (domestic and peridomestic habitats, number of insects found dead or alive, and  
174 insects' developmental stages and sex (of adults) were performed using a dichotomous key by  
175 Lent and Wygodzinsky, 1979.

176 The following entomological indexes were calculated: Infestation rate ( $100 \times$  number of  
177 houses infested / number of houses searched), density (number of triatomines captured /  
178 number of houses searched), crowding (number of triatomines captured / number of houses  
179 infested), and colonization index ( $100 \times$  number of houses with nymphs / number of houses  
180 infested) (WHO 2002).

181

### 182 **Panstrongylus chinai colonies**

183 The colonies of *P. chinai* were maintained under controlled conditions of temperature ( $24 \pm$   
184  $6^\circ\text{C}$ ), humidity ( $70 \pm 10\%$ ), and photoperiod (12 [L:D] h) as described Villacís et al. (2008),  
185 simulating the environmental conditions in Calvas County. Among the selected insects we  
186 separate nymphs from 3 to 5 stages and adults of both sexes. The maintenance of the colonies  
187 using mice were approved by the protocol 15-H-034 approved by Ohio University Institutional  
188 Animal Care and Use Committee (IACUC). However, currently the insects were periodically  
189 fed with human blood meal every 15 days with Hemotek membrane feeding system (Hemotek  
190 Ltd., Blackburn, UK) for 45 minutes (Durdén et al. 2023).

191

### 192 **Intestinal extraction of *Panstrongylus chinai***

193 The specimens were dissected following the procedure by Gumiel et al. (2015). This technique  
194 involved the external sterilization of the insect's body, followed by the use of surgical tools to  
195 remove its intestinal tract. This approach was adapted to lower the risk of contamination by  
196 incorporation of a flow chamber.

197 In the cabin, a cut was made in the abdomen and the entire contents of the intestines were  
198 extracted using a micropipette and placed in 200 microliters of sterile PBS. This mixture was  
199 then stored at  $-20^\circ\text{C}$  until further processing for DNA extraction.

200

201 **Microbial Cultures**

202 During the extraction process with the help of a loop, cultures were performed on blood agar  
203 with the sole purpose to observe growth and verify the existence of microorganisms within the  
204 intestinal content. The incubation conditions were as follows: Temperature of 37 °C ±2 and  
205 growth was checked after 24 and 48 hours.

206

207 **DNA extraction**

208 For the DNA extraction process, the procedure described by the DNAzol reagent of the MRC  
209 brand was used. The only deviation from the suggested protocol for the reagent was the method  
210 of storage. After DNA extraction, DNA was stored in Quiagen brand Buffer AE. Subsequently,  
211 the DNA samples were subjected to quantification and purity evaluation using a NANO2000  
212 (Thermo Scientific) drop instrument.

213

214 **Molecular detection of *T. cruzi***

215 To conduct molecular detection, a PCR (polymerase chain reaction) was made using the  
216 following primers: TCZ1 - dCGAGCTCTTGCCCACACGGGTGCT, and TCZ2 –  
217 dCCTCCAAGCAGCGGATAGTTCAGG (Moser et al. 1989). The Promega kit was utilized  
218 for creating the reaction mixture, and the thermocycler settings were: Initial denaturation at  
219 95°C for a duration of 3 minutes; followed by 35 cycles at 95°C for 30 seconds, 52°C for 30  
220 seconds, and 72°C for 30 seconds each, finally, there was a concluding extension step at 72°C  
221 for 10 minutes.

222 Once the PCR procedure was completed, the samples were stored at a temperature of 4°C.  
223 Subsequently, these samples were loaded onto to 2% agarose gels containing 0.5X TAE Buffer  
224 and subjected to electrophoresis at 135V for a duration of 35 minutes. The gel was stained with  
225 SYBR Safe from invitrogen.

226

### 227 **Amplification of the 16s region**

228 For amplification of the 16s region, primers 27F AGAGTTTGATC(A/C)TGGCTCAG and  
229 R1492 TACGG(C/T)TACCTTGTTACGACTT (Heuer et al. 1997) were used. The reaction  
230 mixture, totaling 20 µl, contained 3 µl of the DNA template. The amplification process  
231 involved an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation  
232 for 1 minute at 95°C, annealing for 1 minute at 57°C, and extension for 1 minute at 72°C. A  
233 final extension step of 10 minutes at 72°C was performed. As a quality control measure,  
234 negative controls (lacking DNA) were included in all amplification processes to verify the  
235 absence of contamination or missing DNA. PCR products were subjected to analysis by 2%  
236 agarose gel electrophoresis. Gels were stained with SYBR Safe and electrophoresed at 135 V  
237 for 45 minutes. This step was carried out to ensure the integrity of the extracted DNA samples  
238 (Da Mota et al, 2012).

239

### 240 **Amplicon Sanger sequencing**

241 Once the electrophoresis was performed, the positive bands were identified, so the amplicon  
242 was chosen from the positive samples for this gene and Sanger sequencing was performed. For  
243 this Sanger sequencing process, the samples were sent to the service of Biosequence S.A.S,  
244 located in Quito-Ecuador.

245

246 **Bioinformatic analysis**

247 For the analysis of the sequences provided by Biosequence, the program Geneious Prime  
248 version 2023.2.1 was used to edit all the sequenced chromatograms and eliminate the primer  
249 regions of the sequence. Once this process was completed, we proceeded to use the BLASTn  
250 tool (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Once our sequence was entered, we proceeded  
251 to compare the results with those that showed greater similarity.

252

253 **Results**

254

255 **Entomological indexes**

256 A total of 63 DUs were searched in the three communities (Guara, Chaquizcha and Bellamaria).  
257 Of those, 5 houses were infested with the species of *Panstrongylus chinai* (infestation index =  
258 7.9%). A total of 12 bugs were collected (density = 0.2 bugs / DUs searched, crowding = 2.4  
259 bugs / infested DUs, colonization index = 20% DUs with nymphs) (Table 1).

260

261 **Natural infection with *Trypanosoma cruzi***

262 A total, of 12 individuals of *Panstrongylus chinai* from the three communities were assessed  
263 for the infection of *T. cruzi* by PCR. Of those, only one sample was positive (8.33%).

264

265 **Presence of bacteria in the culture media**

266 In the culture media, the growth of whitish, round, creamy-looking colonies was observed at  
267 24 hours, and at 48 hours only the growth of these same colonies and the appearance of punctate  
268 white colonies was observed (data no show).

269 **Bacterial composition of *Panstrongylus chinai***

270 A total of 12 samples collected in the DUs and 50 individuals from laboratory colonies were  
271 analyzed. A total of 13 samples were selected to amplify the 16s gene on the agarose gel. Of  
272 these amplified samples, 8 samples were from the field and 5 samples were from the laboratory.  
273 These selected samples were performed to Sanger sequencing and bioinformatic analysis.  
274 When the bioinformatic analysis was performed, only four samples showed corresponding  
275 alignments.

276 *Staphylococcus* genus was detected, being the dominant species *S. saprophyticus* with 75%  
277 and *S. equorum* with 25% (Fig. 2). We observed that *S. saprophyticus* predominates in both,  
278 field and laboratory insects, and it predominates in both adults and nymphs (NIV) (Table 2).  
279 The sample that presented *T. cruzi*, no amplification was observed in the 16s region, so it was  
280 not possible to analyze the microbiota.

281

282 **Discussion**

283 We found a low infestation of *Panstrongylus chinai* in DUs located in three communities in  
284 Calvas county. Only adults of this species were found in the domestic habitat, suggesting that  
285 the adults of *Panstrongylus chinai* invaded the DUs attracted to the lights, similar to what was  
286 demonstrated by Villacís et al. (2015) with *P. howardi* in Manabí province and to the reports  
287 of the members of the communities.

288 It has been observed that in insects the variety of microorganisms is not usually very  
289 complex and diverse, so in general the community of symbionts is not usually diverse, and at  
290 the same time this symbiosis is usually beneficial for the insect (Weiss and Aksoy 2011).  
291 Furthermore, it has been observed that in triatomines the predominant bacteria can change  
292 depending on the species (Da Mota et al. 2012). Studies of the intestinal microbiota have been  
293 carried out in other triatomine species; however, this study evaluated the diversity of

294 microorganisms in *P. chinai* as a vector of Chagas disease in the southern Andean region of  
295 Ecuador. Laboratory bred and field insects were analyzed, and it was observed that some  
296 species predominate over others suggesting that these bacterial communities are highly adapted  
297 to their hosts (Da Motta et al. 2012).

298 The blood that triatomines ingested is generally sterile, but the bacteria colonize their  
299 intestinal tract. It is assumed that these bacteria are acquired by contact of the oral apparatus,  
300 before and after blood ingestion, with the skin of the vertebrate host (Guarneri and Schaub  
301 2021), however, the main form of transmission of the microbiota and *T. cruzi* among  
302 triatomines is through cannibalism and coprophagy (Schaub 1988). Perhaps this could be one  
303 of the main ways in which the laboratory insects manage to preserve their microbiota and, also,  
304 this could be one of the ways in which field insects could obtain the blood source and therefore  
305 maintain their microbiota.

306 In this study we observed that *Staphylococcus sp.* is the unique bacterial genus, while in  
307 other studies *Staphylococcus*, *Serratia*, *Enterobacter*, *Burkholderia-Caballeronia*-  
308 *Paraburkholderia* and *Bacteroides* are the five most important genera of intestinal bacteria in  
309 triatomines (Hu et al. 2020). The dominance of this genus is so great that it is considered that  
310 it could have important functions in the insect's physiology especially in survival and  
311 reproduction process (Oliveira et al. 2018). This bacterial genus also was observed in other  
312 species of triatomines such as *Panstrongylous megistus* (Schaub 2020), *Meccus pallidipennis*  
313 (Jiménez et al. 2021) and has been isolated in six individuals of *Triatoma* spp (Mann et al.  
314 2020), considered as a transient microbiota and the impact in the triatomines can cause low  
315 microbiota diversity (Mann et al. 2020).

316 One of the species within the genus *Staphylococcus* that we found was *Staphylococcus*  
317 *equorum*, which was originally isolated from healthy horses (Schleifer et al. 1984), and later  
318 isolated from the milk of a cow with mastitis and from healthy goats (Meugnier et al. 1996). It

319 has also been found in clinical materials (Pinna et al. 1999; Marsou et al. 2001; Alcaráz et al.  
320 2003). Knowing where these microorganisms have been isolated is important since some *P.*  
321 *chinai* were collected in communities where cattle are in the peridomestic habitat (Grijalva et  
322 al. 2015).

323 *Staphylococcus saprophyticus* was found in both, field and laboratory insects, suggesting  
324 that this species could maintain an association with *P. chinai*. In addition, this species of  
325 bacteria was isolated in the intestinal tract and in the feces of the nymphs and adults of *Triatoma*  
326 *dimidiata* (López et al. 2018). As the same of our study, *S. saprophyticus* was found in a NIV  
327 raised in laboratory. However, Lopez et al. (2018) isolated two bacterial species, *S.*  
328 *saprophyticus* and *S. gallinarum*, present in both, field and laboratory insects.

329 Finally, this study presented some limitations, such as: i) lack of information of the food  
330 source of the field triatomines, i.e. most of the *P. chinai* were collected in the domestic habitat,  
331 however, we do not have any information about the host it feeds on. ii) The low number of  
332 individuals of *P. chinai* collected in the field. i.e. in this case we collected 12 individuals. iii)  
333 There is still no information on the insects from other provinces of Ecuador and Peru. iv) The  
334 use of more advanced techniques, such as new generation sequencing (that allows to screen  
335 more samples cost-effectively and detect multiple variants across targeted areas of the genome)  
336 to have a big picture of the different species (bacteria, fungi, virus) of microbiota, because in  
337 our study we used sanger sequencing for the bacterial 16S region.

338 The bacterial diversity within *P. chinai* is low but consistent, with the predominant genus  
339 of *Staphylococcus*, some coincidence was observed with other triatomines. Thus, this genus  
340 can be considered as a symbiont of this species of insects, however, deeper studies need to  
341 carry out about this species of insect, that is considered as a secondary vector of Chagas disease  
342 in the southern Andean region of Ecuador. Assays about the influence of *Staphylococcus*

343 *saprophyticus* and *Staphylococcus equorum* on the development of *P. chinai* to evaluate the  
344 potential use of these bacterial microbiota as possible controls on *T. cruzi* would be crucial.

345 **Acknowledgments**

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347 Ministry of Public Health who participated in the collection of the species of *Panstrongylus*  
348 *chinai*. Also, we want to thanks to Santiago Cadena who is the person in charge in the  
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488 p.

489 **Table 1.** Entomological indices of *P. chinai* in three rural communities (Guara, Chaquizhca and Bellamaria) in the southern region of Ecuador.

490

<b>Communities</b>	<b>Code of the communitites</b>	<b>DUs</b>	<b>Infested DUs</b>	<b>DUs with nymphs</b>	<b>N of bugs collected</b>	<b>Infestation index (%)</b>	<b>Density</b>	<b>Crowding</b>	<b>Colonization index (%)</b>
Guara	GA	11	1	1	8	9.1	0.7	8	100
Chaquizhca	CQ	23	2	0	2	8.7	0.1	1	0
Bellamaria	BM	29	2	0	2	6.9	0.1	1	0
<b>TOTAL</b>		<b>63</b>	<b>5</b>	<b>1</b>	<b>12</b>	<b>7.9</b>	<b>0.2</b>	<b>2.4</b>	<b>20</b>

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499 **Table 2.** BLAST sequences percent identity of the bacterial microbiota species within *Panstrongylus chinai*

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<b>Community</b>	<b>Habitat</b>	<b>Field / Laboratory</b>	<b>Stage</b>	<b>Bacterial microbiota species</b>	<b>BLAST sequence percent identity</b>
Guara	Domestic	Field	Male	<i>Staphylococcus saprophyticus</i>	99
Chaquizcha	Domestic	Field	Male	<i>Staphylococcus saprophyticus</i>	99
Bellamaria	Domestic	Laboratory	Nymph IV	<i>Staphylococcus saprophyticus</i>	99
Bellamaria	Domestic	Field	Male	<i>Staphylococcus equorum</i>	99

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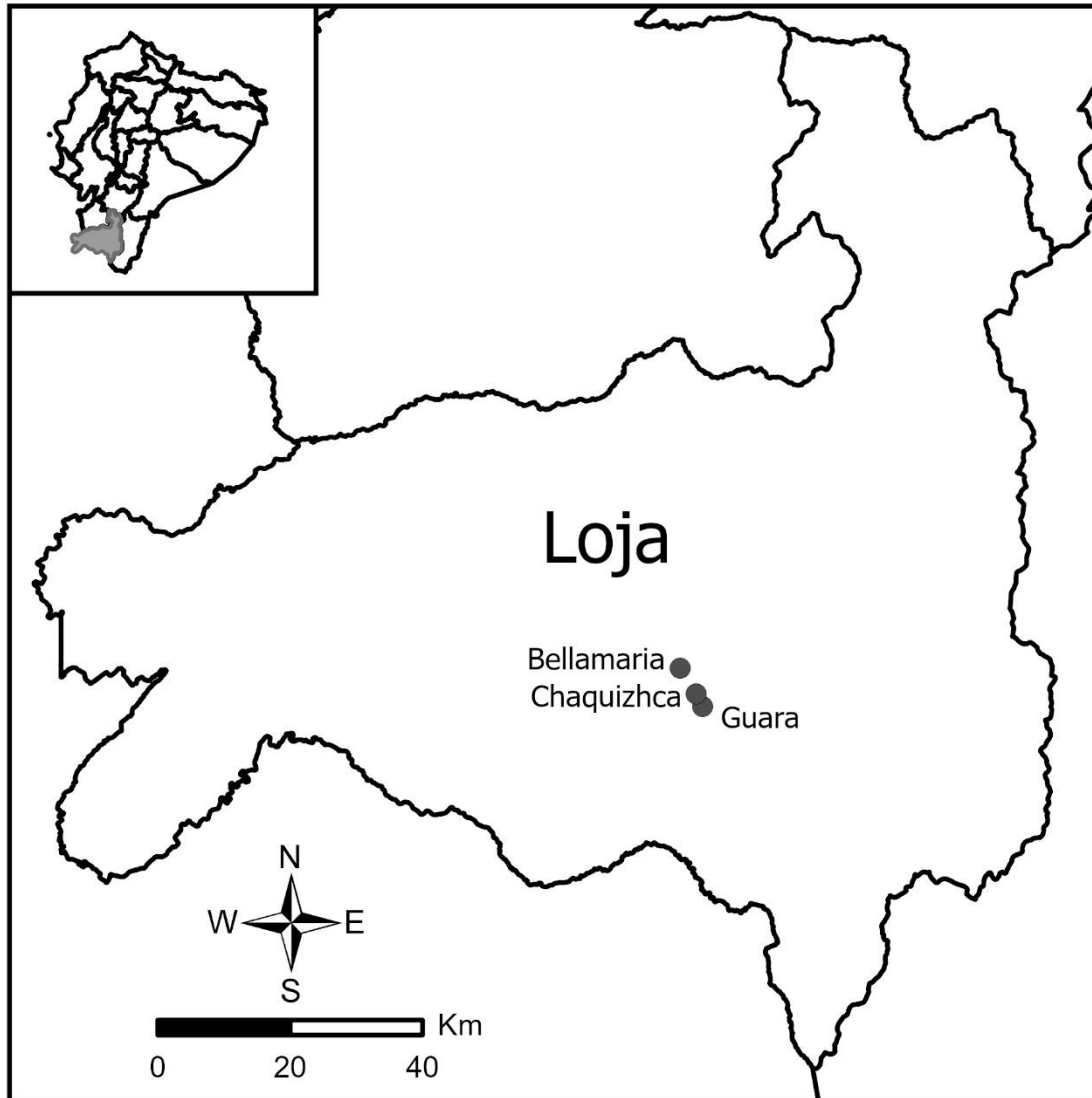
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## Figures legends

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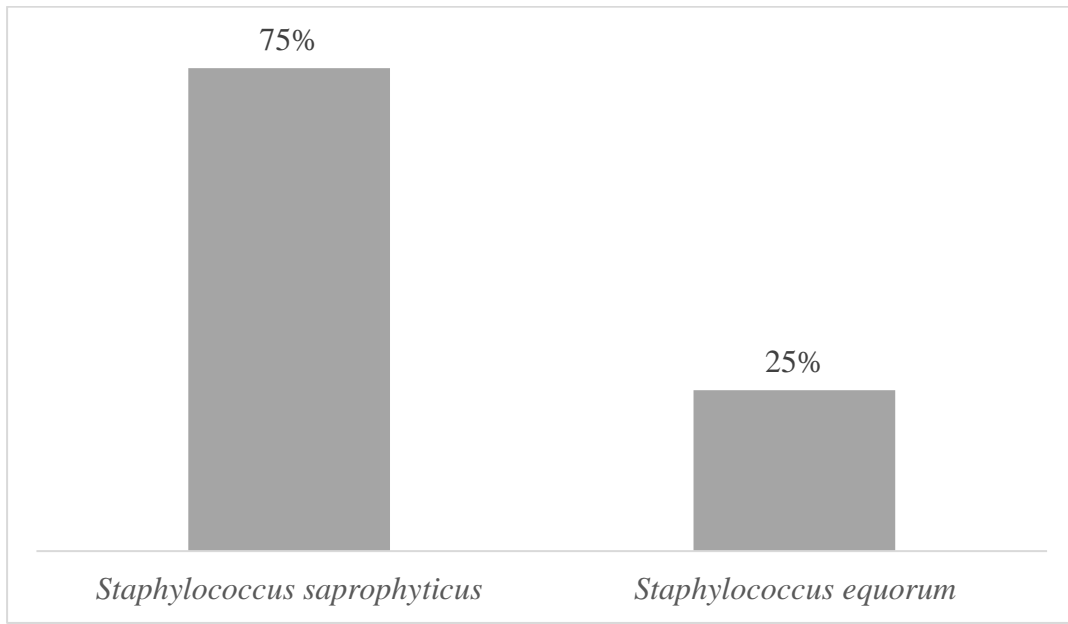
508 **Figure 1.** Map of the three rural communities (Guara, Chaquizcha and Bellamaria).

509 Cariamanga, the county capital. The grey points represent the communities where

510 *Panstrongylus chinai* were collected.

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515 **Figure 2.** Percentage of bacterial predominance in positive samples

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642 the journal’s editorial board. More information on the appeals process can be found on  
643 the [Journal Policies](#) page.

644 Statistics and sample size

645 Statistics should be fully reported (i.e., F-value, both degrees of freedom [treatments and  
646 replicates], and exact P-value [unless it’s less than 0.001]). Furthermore, the paper will be  
647 withdrawn if Duncan’s Multiple Range Test is used for papers that do not deal with plant  
648 resistance. For more information on statistics, please see the Statistics section of these author  
649 instructions.

650 Papers that have insufficient sample sizes (e.g., only a single year of data collected at one  
651 location for either insect surveys, pesticide studies, or other field data) are immediately  
652 withdrawn. The duration and size of trials/sampling must be biologically significant.

653 Plant extracts and essential oils

654 For papers that test the efficacy of plant extracts or other compounds on control or behavior  
655 modification of insects, the concentrations of the chemical constituents must be  
656 listed. Reports on the use of essential oils (EO) and botanically derived compounds as  
657 attractants, repellants, and insecticides must meet the following criteria for publication in  
658 the *Journal of Medical Entomology*.

- 659 1. Manuscripts on essential oils/botanicals should include more information than contact  
660 toxicity or repellency. There should be an element of either physiology, cross-resistance,  
661 environmental persistence/fate, or non-target effects.
- 662 2. All studies should include a positive control so data on multiple compounds can be  
663 directly compared. For repellants, the preferred positive control is DEET (Maia & Moore  
664 2011).
- 665 3. Experiments involving dose responses and estimation of LC50 or EC50s should follow  
666 general guidelines given by (Burgess et al. 2021). The dose range should include  
667 responses between 20 and 80%. Statistics should be reported in accordance with Journal  
668 policies.
- 669 4. Manuscripts should not claim botanical or natural compounds are inherently safer or  
670 more efficacious than synthetic compounds without supporting evidence (Trumble 2002).
- 671 5. Small sample sizes (<20) should not be converted to percentage data for analyses (Jaeger  
672 2008).
- 673 6. Chemical analyses of component compounds must be included in the study.

674

675 Burgess ER, King BH, Geden CJ. 2020. Oral and topical insecticide response bioassays and  
676 associated statistical analyses used commonly in veterinary and medical entomology. *J.*  
677 *Insect Sci.* 20(6):1-9. <https://doi.org/10.1093/jisesa/ieaa041>

678 Jaeger TF. 2008. Categorical data analysis: Away from ANOVAs (transformation or not) and  
679 towards logit mixed models. *J. Mem. Lang.* 59(4):434–446.  
680 <https://doi.org/10.1016/j.jml.2007.11.007>

681 Maia MF, Moore SJ. 2011. Plant-based insect repellents: a review of their efficacy,  
682 development and testing. *Malar. J.* 10(Suppl 1):S1-S11. 10.1186/1475-2875-10-S1-S11

683 Trumble JT. 2002. Caveat emptor: Safety considerations for natural products used in  
684 arthropod control. *Amer. Entomol.* 48(1):7-13. <https://doi.org/10.1093/ae/48.1.7>

685

686 Petri dish tests

687 Petri dish experiments are not accepted unless they contain a field, semi-field, or greenhouse  
688 test.

689

### 690 **Personal communications**

691 Personal communication citations should be accompanied by a letter from the person being  
692 cited giving permission to use him or her as a citation and verifying the claim being cited.  
693 This letter should be uploaded as a supplementary file.

694

### 695 **Abbreviations**

696 Abbreviations should be used sparingly. Standard abbreviations for measurements according  
697 to *Scientific Style and Format*, 8th edition, are acceptable, as well as common abbreviations  
698 that improve the readability of a manuscript (e.g., DNA, PCR). All other abbreviations used  
699 should be defined at the first use.

700

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702 JME is committed to ethical behavior in all aspects of scholarly publishing. Please ensure  
703 your paper meets the following ethical criteria:

- 704 • The author list is complete and correct. Please see the “[Journal Policies](#)” page for  
705 authorship information and policies.

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708 be placed in quotation marks. All manuscripts undergo a plagiarism test before they are  
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711 a paper in another language, may not be published again. If portions of data published  
712 previously are being used, the author must provide explicit written consent from the  
713 publisher of the previous paper to reuse the data.

714

## 715 **Experimental Ethics**

716 Research published in JME must adhere to minimal ethical and compliance requirements for  
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718 include human subjects and/or domestic and wild animals and therefore requires that authors  
719 reference compliance protocols to indicate adherence to federal, state, and local regulations,  
720 permits, and authorizations. International authors should reference similar compliance  
721 documents from their government and/or institution.

722 Listed below is a minimal series of basic requirements requested from USA and international  
723 researchers to be included, as needed, within an ethical section positioned in the Materials  
724 and Methods section of each manuscript:

- 725 1. Biological Use Authorization (BUA). Surveillance, epidemiological, and experimental  
726 infection studies with pathogens require containment for diagnostics and culture  
727 depending upon the virulence of the organism and the risk of vectorborne, contact, and/or  
728 aerosol transmission. Descriptions of appropriate containment for different biosafety  
729 levels can be [downloaded as a pdf](#).  
730 A search engine and database to determine the Risk Group of the organism[s] concerned  
731 can be found at the [ABSA International website](#).  
732 Researchers should report their institution's required compliance review and approved  
733 containment level BUA protocol for the pathogen(s) or arthropods used.
- 734 2. Institutional Review Board (IRB) compliance. Use of human subjects in research must be  
735 approved by IRB committees adhering to [US Department of Health and Human Services](#)  
736 [guidelines](#). Each organization may have different interpretations of guidelines required for  
737 human subject activities which may range from surveys, house entry for arthropod  
738 collection, use of humans to feed arthropods, use of humans as bait for sampling host-  
739 seeking insects, test subjects for candidate repellents, etc.<sup>1</sup>
- 740 3. Institutional Animal Care and Use Committee (IACUC) compliance. Use of animals in  
741 research must adhere to protocols meeting minimal ethical requirements for collection,  
742 maintenance, and experimental procedures. Research done within the USA or funded by  
743 USA agencies must adhere to [requirements described](#) by the US National Institutes of  
744 Health, and these protocols should be appropriately referenced.
- 745 4. Sampling wildlife. Most countries and states/provinces/districts require permits to collect  
746 vertebrate animals and some insects for research purposes. This is especially true for  
747 migratory species, such as birds, where international agreements are in place; for example,  
748 see [permitting requirements](#) for collecting and banding birds in North America.  
749 An example of state permits required for taking or trapping and release of wildlife within  
750 California can be found at [Department of Fish and Wildlife website](#).  
751 Additional permits may be required for sampling on wildlife refuges or nature  
752 conservatory properties. Reference to these permits should be required to ensure sampling  
753 was done in compliance with regional oversight, especially for endangered or threatened  
754 species.

755 5. Transport and release of organisms. With the increasing use of genetically modified  
756 arthropods for population or pathogen control, medical entomologists must adhere to  
757 correct oversight governing production and release. The US Department of Agriculture  
758 has [strict requirements](#) for the transport and/or release of organisms as well as  
759 experimental use permitting for applications of experimental compounds for arthropod  
760 control.  
761 The Biotechnology Quality Management System (BQMS) Program within the USDA  
762 helps organizations, including small businesses and academic researchers, analyze the  
763 critical control points within their management systems to better maintain compliance with  
764 the APHIS regulations (7 CFR part 340) for the import, interstate movement, and field  
765 release of regulated genetically engineered (GE) organisms.

766 <sup>1</sup>Aultman KS, Walker ED, Gifford F, et al. 2000. Managing risks of arthropod vector  
767 research. *Science* 288 (5475): 2321-2322. DOI: 10.1126/science.288.5475.2321

768

## 769 **Conflicts of interest**

770 Potential conflicts of interest include any relationships of a financial or personal nature  
771 between an author or coauthor and individuals or organizations which, in theory, could affect  
772 or bias an author's scientific judgment, or limit an author's freedom to publish, analyze,  
773 discuss, or interpret relevant data.

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775 aspect of a study must be indicated in the Acknowledgments section of the paper. Financial  
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778 financial support, must be revealed to the editor at submission, and disclosed in a statement  
779 immediately following the Acknowledgments.

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783 interpretation of any results of the study, whether or not such veto power was exercised, this  
784 information must be disclosed in a statement immediately following the Acknowledgments.

785

## 786 **Article Types**

### 787 **Research**

788 Research articles report original observations and experiments, the results of the experiment,  
789 and a discussion of the significance of the results. There is no word limit for research  
790 articles.

791

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793 Review articles review and synthesize current information on a topic. Review articles can  
794 also contain historical threads of important ideas (i.e., are not confined to recent citations).  
795 There is no word limit for review articles.

796

### 797 **Forum**

798 Forum articles are authored by acknowledged leaders in the field, review a research area, and  
799 include a stimulating, thought-provoking discussion that focuses on important, and  
800 sometimes controversial, issues. They should provide an innovative approach to current  
801 thought and speculate about future research directions.

802 **Short Communications**

803 Short communications should be similar to a research article, but with briefer Materials and  
804 Methods and Discussion. Total length should be 2,000 words or less.

805

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807 JME will consider submissions in the form of a letter to the editor in which the authors  
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818 our Journal Policies page.

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- 822 1. *Corresponding author*: Include full name, mailing address, telephone number, and  
823 email address.
- 824 2. *Title*: Should be as short as possible. Only include common names that are listed in  
825 the ESA Common Names of Insects & Related Organisms. Do not include authors of  
826 scientific names. Insert “([Order]: [Family])” immediately after the name of the organism.
- 827 3. *Author list*: Include all authors in the order the names should be published.
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829 designate through numbered footnotes.
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  - 831 a. 250 words or less.
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  - 834 d. Refer to results only in the general sense.
  - 835 e. A second abstract in a second language is permitted.
- 836 6. *Keywords*
  - 837 a. Below the abstract, provide three to five keywords, separated by commas.
  - 838 b. Do not use abbreviations, combined keywords, or species names.

839

840 **Body**

841 **Introduction**

842 Clearly state the basis of your study along with background information and a statement of  
843 purpose.

844

845 **Materials and Methods**

846 Include a clear and concise description of the study design, experiment, materials, and  
847 method of statistical analysis.

848

849 **Results**

850 Clearly present the results. Do not include interpretation of results or interpretation of  
851 statistical analysis—simply present the results of the experiment and the results of the  
852 statistical analysis. Data listed in tables should not be listed in the results; instead, refer to the  
853 table.  
854

855 **Discussion**

856 Interpret and discuss results of the study and their implications. Include suggestions for  
857 direction of future studies, if appropriate.  
858

859 **Acknowledgments**

860 Place the acknowledgments after the text. Organize acknowledgments in paragraph form in  
861 the following order: persons, groups, granting institutions, grant numbers, and serial  
862 publication number.

863 Following the Acknowledgments, you may {{include a statement of author contribution  
864 outlining the specific contributions of each author to the article. A statement of author  
865 contribution is welcomed but not required.  
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867 **References and in-text citations**

868 The ESA Journals style is based on the Council of Science Editors 8<sup>th</sup> Edition Name-year  
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873 same author. For materials with two authors use both authors’ surnames and the year of  
874 publication: (Mazan and Hoffman 2001). For 3 or more authors use the first author’s surname  
875 and “et al.” like this example: (Ito et al. 1999). Finally, to cite more than one reference: list  
876 materials chronologically as follows: (Singh 2011, Davidson 2015, Harding 2018).

877 Here are some basic formatting points for the bibliography:

- 878 • Only reference published and formally accepted (in press) articles.
- 879 • List references alphabetically by first author surname. List multiple references from the  
880 same author chronologically.
- 881 • Add an alphabetic designator to the year in the in-text and end reference when there are  
882 references from the same author in the same year, for example: (Johnson 2023a, 2023b)
- 883 • Author names are given surname first, followed by initials—with no punctuation except  
884 for commas between authors and a period at the end.
- 885 • Include all author names unless there are more than 3. If a reference has 4 or more authors,  
886 give only the first 3 names followed by “et al.” and the publication year.
- 887 • Abbreviate journal titles according to the List of Title Word Abbreviations: [Access to the](#)  
888 [LTWA | ISSN](#) (see examples)
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894 publication.
- 895 • When available, include the stable doi URL at the end of the reference or the doi.

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897 *Journal Article*

898 Author surname(s) and initial(s), et al. Publication year. Article title. Abbreviated Journal  
899 Title. Volume number (issue number):start page-end page. [stable doi URL] or DOI:doi  
900 Schmidt LS, Schmidt JO, Rao H, et al. 1995. Feeding preference and survival of young  
901 worker honey bees (hymenoptera: Apidae) fed rape, sesame, and sunflower pollen. J. Econ.  
902 Entomol. 88(6):1591-1595. <https://doi.org/10.1093/jee/88.6.1591>.

903

904 *Book*

905 Author surname(s) and initial(s), et al. Publication year. Editor(s). *Book title*, edition. Place  
906 Published: Publisher. Pages.

907 Gravena S, Sterling W, Dean A. 1985. *Abstracts, references, and key words of publications*  
908 *relating to the cotton worm Alabama argillacea (Huebner), (Lepidoptera: Noctuidae).*  
909 *College Park, MD: Entomological Society of America. 136 p.*

910

911 *Section/Chapter in Book*

912 Author surname(s) and initial(s), et al. Publication year. Title of Chapter in an Edited Book.  
913 In: *Editor(s). Book Title. Edition. Place Published: Publisher. Pages.*

914 ***Corporations or Organizations as authors***

915 Organization. Publication year. Title. Abbreviated journal title. Available from: URL  
916 White House. 2015a. National strategy to promote the health of honey bees and other  
917 pollinators. Pollinator Health Task Force. Available from  
918 [https://obamawhitehouse.archives.gov/sites/default/files/microsites/ostp/Pollinator%20Health](https://obamawhitehouse.archives.gov/sites/default/files/microsites/ostp/Pollinator%20Health%20Strategy%202015.pdf)  
919 [%20Strategy%202015.pdf](https://obamawhitehouse.archives.gov/sites/default/files/microsites/ostp/Pollinator%20Health%20Strategy%202015.pdf)

920 White House. 2015b. Pollinator research action plan. Report of the Pollinator Health Task  
921 Force. Available from

922 [https://obamawhitehouse.archives.gov/sites/default/files/microsites/ostp/Pollinator%20Resear](https://obamawhitehouse.archives.gov/sites/default/files/microsites/ostp/Pollinator%20Research%20Action%20Plan%202015.pdf)  
923 [ch%20Action%20Plan%202015.pdf](https://obamawhitehouse.archives.gov/sites/default/files/microsites/ostp/Pollinator%20Research%20Action%20Plan%202015.pdf)

924

925 *Patents*

926 Inventor(s), Assignee, Assignee name. Year. Title. Country Patent Number.

927 Pound M, Miller A, LeMeilleur A. 1994. Device and method for use as an aid in control of  
928 ticks and other ectoparasites on wildlife. U.S. Patent #5,367,983.

929

930 *Conference Paper*

931 Author surname(s) and initial(s), et al. Publication year. Title. Paper Presented at: Conference  
932 Name. Publisher; Conference Location.

933

934 *Conference Proceedings*

935 Editor(s). Publication year. Title. Conference name; Date year of Conference; Conference  
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938 *Theses/Dissertations*

939 Author surname(s) and initial(s), et al. Publication year. Title [Thesis type]. Place Published:  
940 University. URL if available.

941 Barrufaldi APF. 2015. Temperatures of constant and floating influence on the biological  
942 characteristics of *Euschistus heros* (Fabricius) (Hemiptera: Pentatomidae) in successive  
943 generations [Master's dissertation]. Botucatu (Brazil): Universidade Estadual Paulista Júlio  
944 de Mesquita Filho. <http://hdl.handle.net/11449/135938>.

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963 the article.

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1041 If supplied alongside the manuscript and cited within the article text, published videos will  
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1043 party sites such as YouTube, as the link may expire.  
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1048 mention of the organism. It is the author's responsibility to provide accurate authority,  
1049 order, and family information. Organisms mentioned in passing or whose importance to the  
1050 paper is limited do not need to have full authority, order, and family listed, nor do mentions  
1051 of common names of groups (e.g., mosquitoes, beetles, ticks, etc.).

1052 • If a species name is included in the title of a paper, either the ESA-approved common name  
1053 or the Latin name, the order and family should also be provided. If the order and family of  
1054 an organism is provided in the title of the paper, it does not need to be provided again in the  
1055 abstract and main text.

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1057 be provided at first mention of the organism in both the abstract and the main text.

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1059 time the organism is mentioned in both the abstract and the main text, but not in the title.

1060 For tables that include lists of species, authority should be given for each species if it is the  
1061 first time it is being mentioned in the paper.

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1064 to the paper, are in different genera, but are in the same order and family, order and family  
1065 should be provided at first mention of each organism.

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1067 the text of the paper together, the order and family should be placed after the first species  
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1070 the genus should be spelled out). If two species belong to different genera but the genera  
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1079 Descriptions should include information such as sample sizes and number of replications.

1080 Only t-tests, Chi square, and analyses of variance require no citation. Cite the computer  
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1086 tables in the following order (left to right); n, slope + SE, LD (or LC) (95% CL), and chi-  
1087 square. When a ratio of one LD versus another is given, it should be given with its 95% CI.  
1088 Statistical tests to show what model best fits data intended to estimate the 99.9986% level of  
1089 effectiveness should be presented to justify use of any model, including the probit model.  
1090 Thus, we do not recommend use of the Probit 9 without tests to show that the probit model  
1091 fits the data.

1092

1093 **Analysis of Variance or t-test**

1094 When presenting the results of analysis of variance or a t-test, specify F (or t) values, degrees  
1095 of freedom, and P values. This information should be placed in parentheses in the text.  
1096 Example: (F = 9.26; df = 4, 26; P < 0.001). If readability of the text is affected by the  
1097 presence of repeated parenthetical statistical statements, place them in a table instead.

1098

1099 **Regression**

1100 In regressions, specify the model, define all variables, and provide estimates of variances for  
1101 parameters and the residual mean-square error. Italicize variables in equations and text.

1102

1103 **Variance and sample size**

1104 Include an estimate of the variance (or standard error) and sample size for each mean  
1105 regardless of the method chosen for unplanned multiple comparisons. The use of Duncan's  
1106 Multiple Range Test (DMRT) is not acceptable as a mean separation test as it was designed  
1107 to be a very liberal test intended to find even minor differences in resistance between plant  
1108 lines used for breeding.

1109

1110 Model Analysis, Guidelines, Equations, and Computer Code

1111 **Model Analysis**

1112 At the beginning of the manuscript, authors should state clearly the goals of their model  
1113 construction and analysis. Evaluation by reviewers depends upon these goals and the type of  
1114 model. Authors should attempt to describe the main conclusions, limitations, and sensitivity  
1115 of results to assumptions. For stochastic models, describe the variability in the results.

1116

1117 **Modeling Guidelines**

1118 The following guidelines pertain to any mathematical model calculated for purposes other  
1119 than statistical analysis.

- 1120 • Authors must adequately describe both model structure and model analysis.
- 1121 • Authors must explain and justify original equations and computer programs or justify the  
1122 selection of a published software package used in the computation of models.
- 1123 • Model structure and steps in the analysis must be described in the Materials and Methods  
1124 section.
- 1125 • Without presenting extensive computer code, the text must permit an understanding of the  
1126 model that would allow most mathematically inclined scientists to duplicate the work.
- 1127 • Present all equations that represent the biology of the system being modeled.
- 1128 • Unless their derivation is self-evident, show how the equations were derived and mention  
1129 the underlying assumptions.

- 1130 • Express how the equations are solved over time and space.
- 1131 • Provide references for standard techniques (e.g., matrix manipulation, integration).
- 1132 • Define all variables and parameters in each equation and describe their units (e.g., time,  
1133 space, and mass).
- 1134 • In the Materials and Methods or Results section, present the range of parameter values  
1135 included in the model, and describe the uncertainty in or range of validity of these values.

1136

### 1137 **Equations**

1138 Consult Mathematics into Type for correct formatting of equations and mathematical  
1139 variables. Italicize all mathematical variables.

1140

### 1141 **Validation or Testing of Model Results**

1142 Data used for validation must be independent of data used to build or calibrate the  
1143 model. Authors must state why the model did not require testing (e.g., theoretical study),  
1144 why it cannot be tested (e.g., lack of data), or how it was tested.

1145

### 1146 **Structure of Computer Code**

1147 For models solved or simulated by computers, mention the programming language and  
1148 computer used. Describe the important numerical methods used in calculating the model  
1149 (e.g., integration and random number generation). Mention how the program's logic and  
1150 algorithms were tested and verified. When published software is computed, provide a  
1151 reference and state which procedures were used. Discuss in any section of the manuscript the  
1152 limitations of the published software. Original computer programs should be made available  
1153 at the request of reviewers and readers.

1154

1155 Taxonomic Papers

### 1156 **ICZN compliance**

1157 JIS is compliant with the International Code of Zoological Nomenclature, and the publishing  
1158 processes of the journal ensure that new acts of nomenclature are considered valid under the  
1159 code. JME is permanently archived with CLOCKSS, LOCKSS, and Portico in a manner that  
1160 preserves the content and layout of the work.

1161

### 1162 **Nomenclatural Works and Zoobank Registration**

1163 You will be asked during the submission process whether your article contains a new  
1164 nomenclatural act. If it does, in order to comply with ICZN regulations, the author will need  
1165 to register the article in ZooBank and insert a nomenclatural statement, which includes a Life  
1166 Science Identifier (LSID), into the article. The registration can be made at first revision of the  
1167 article. Instructions for this process can be found [here](#). When published, your article will also  
1168 include the online publication date, and the statement “Version of Record, first published  
1169 online [online publication date], with fixed content and layout in compliance with Art. 8.1.3.2  
1170 ICZN.” Following publication, the editorial office will update your ZooBank entry with the  
1171 DOI, Volume, and Issue information.

1172 When you have registered the paper, please send the article tracking number and LSID to the  
1173 editorial office ([pubs@entsoc.org](mailto:pubs@entsoc.org)) to record in the peer-review system.

1174

1175

1176 **Taxonomic Style**

1177 Follow the *International Code of Zoological Nomenclature* for taxonomic style. Some  
1178 specific areas to note:

- 1179 • Center the heading that indicates the name of the taxon in bold type.
- 1180 • Center figure numbers in parentheses under the main heading; do not use bold type.
- 1181 • Start all synonymies at the left margin with runovers indented.
- 1182 • Include authors and date.
- 1183 • References must appear in References Cited section.
- 1184 • Use telegraphic style throughout descriptions.

1185

1186 **Taxonomy Headings**

1187 Use only acceptable 3rd-level subheadings such as:

- 1188 • Male
- 1189 • Female
- 1190 • Material Examined
- 1191 • Type Material
- 1192 • Distribution
- 1193 • Etymology
- 1194 • Biology
- 1195 • Discussion

1196 Avoid using “Description” as a subheading.

1197

1198 **Dates**

- 1199 • Use Roman numerals I through XII to designate month of collection.
- 1200 • Use Arabic numerals 0000 through 9999 to designate collection years. Do not abbreviate  
1201 other years, including the 21st century, except when explicitly transcribing exactly what  
1202 appears on specimen labels.
- 1203 • Express dates in this format: day-month (use a Roman numeral)-year. Example: 2-V-97.

1204

1205 **Locality Other than Principal Types**

- 1206 • Start with the largest area followed by successively smaller areas separated by colons.
- 1207 • Capitalize countries.
- 1208 • Arrange data for each locality in the following order: count of specimens and sex or stage  
1209 (as applicable), city or vicinity, date, collector, and depository. Example: MEXICO:  
1210 Tamaulipas: 1 male, 1 female, Ciudad Mante, 15-III-97, K. Haack; 5 females, Ciudad  
1211 Victoria, 3-VII-99, C. Hughes, MCZ.
- 1212 • Arrange localities alphabetically.
- 1213 • Use a semicolon to separate data for different localities.
- 1214 • Define depositories in the Materials and Methods.

1215

1216 **Type Material**

1217 Start description with the principal type in capital letters. Follow this immediately with count  
1218 and sex of specimens (use male and female symbols if possible), then place additional data in  
1219 the order of locality, date, additional data, and collector. Separate these items with commas.  
1220 Example: HOLOTYPE: 1 male, Locust Grove, VA, 22-X-98, on *Cercis canadensis*, R. H.  
1221 Foote. PARATYPES: 2 males, same data.

1222

## 1223 **Voucher Specimens**

1224 Voucher specimens of arthropods serve as future reference for published names used in  
1225 scientific publications. Authors are required to deposit voucher specimens in an established,  
1226 permanent collection and to note in the published article that the expected deposition has  
1227 been made, its location, and the collection accession number. Authors should contact the  
1228 curator of a voucher repository before deposition concerning the procedures required for  
1229 curation to ensure that the collection will accept the voucher materials. The designation and  
1230 proper labeling of voucher specimens is the author's responsibility. When available, at least  
1231 three specimens should be deposited. Each specimen should have the following information  
1232 provided at the time of deposition:

- 1233 • Standard label data that are required for the specimens collection (i.e., locality, date of  
1234 collection, collector, host, ecological data, whether the specimen is from a laboratory  
1235 collection, etc.).
- 1236 • An identification label that includes the identifier and date of identification.
- 1237 • A label that designates the specimen as "voucher."

1238

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1240 *JME* publishes articles online ahead of inclusion in an issue via OUP's Advance Access.

1241

1242 In order to comply with the requirements of the International Commission on Zoological  
1243 Nomenclature (ICZN) with regard to nomenclatural works, articles published by ESA's  
1244 journals that include nomenclatural information will be immutable. Additionally, no changes  
1245 will be allowed to any article without the publication of a correction clearly stating the  
1246 changes that have been made. Therefore, it is the responsibility of the authors to carefully  
1247 check their proofs for accuracy, and to notify the publisher of any changes that are necessary  
1248 prior to Advance Access publication.

1249

## 1250 **Gene Sequencing**

- 1251 • Inclusion of a GenBank/EMBL accession number for primary nucleotide and amino acid  
1252 sequence data is required.
- 1253 • Sequences from new species and new genes must indicate the proportion of the gene  
1254 sequenced and should include data from both strands.
- 1255 • The accession number may be included in the original manuscript or the sequence may be  
1256 provided for review and an accession number provided when the manuscript is revised.

1257

## 1258 **Availability of Data and Materials**

1259 Where ethically feasible, *JME* strongly encourages authors to make all data and software  
1260 code on which the conclusions of the paper rely available to readers. We suggest that data be  
1261 presented in the main manuscript or additional supporting files, or deposited in a public  
1262 repository whenever possible. Information on general repositories for all data types, and a list  
1263 of recommended repositories by subject area, is available [here](#).

1264 ESA journals are integrated with Dryad ([www.datadryad.org](http://www.datadryad.org)), an online data repository.  
1265 Upon submission, authors will be sent a link to easily deposit the full dataset(s) behind their  
1266 research in Dryad. Datasets deposited in Dryad are given unique DOIs, linked to the  
1267 corresponding paper, and are available to be reviewed by subject editors of the journal upon  
1268 request. Depositing data in Dryad is optional.

1269 If you would like your paper to link to the dataset, data should be deposited prior to  
1270 acceptance. Authors are required to pay Dryad for depositing data, but this fee is only  
1271 charged if a paper is accepted.

1272

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1275 information on [ORCID and the benefits of using an ORCID iD](#) is available. If you do not  
1276 already have an ORCID iD, you can register for free via the [ORCID website](#).  
1277 Coauthors are encouraged to link their ORCID iDs to their online review system accounts.  
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1279 cannot be completed by the corresponding author or staff.

1280

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1282 Authors retain the right to make an Author's Original Version (preprint) available through  
1283 various channels, and this does not prevent submission to the journal, however, preprint  
1284 servers where papers are formally reviewed and a decision is issued will be considered  
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1292 *JME* publishes articles online ahead of inclusion in an issue via OUP's Advance Access.  
1293 In order to comply with the requirements of the International Commission on Zoological  
1294 Nomenclature (ICZN) with regard to nomenclatural works, ALL articles published by ESA's  
1295 journals, regardless of whether they include nomenclatural information, will be immutable;  
1296 this means that no changes will be allowed to any article without the publication of an  
1297 erratum clearly stating the changes that have been made. Therefore, it is the responsibility of  
1298 the authors to carefully check their proofs for accuracy, and to notify the publisher of any  
1299 changes that are necessary prior to Advance Access publication.

1300

## 1301 **Revised Papers**

1302 For revised papers, we have a few more formatting requirements than we do for new  
1303 submissions, to ensure the paper is ready for publication if it is accepted.

1304 Response to reviews

1305 Provide a document listing how you changed your manuscript in response to each point  
1306 mentioned by the reviewers. If you did not implement a change suggested by a reviewer,  
1307 please explain why.

1308

## 1309 **Style**

1310 For any matters of editorial style not covered in these author guidelines, ESA journals adhere  
1311 to the [Council of Science Editors \(CSE\) Style Guide](#).

1312 Measurements

1313 Use metric units. English units may follow within parentheses if needed.

1314

1315 **Nomenclature**

- 1316 • Give full scientific name and authority at first mention of each organism (including plants  
1317 and non-insect animals) in the abstract and again in the text.
- 1318 • Only use common names listed in the current [ESA Common Names of Insects & Related](#)  
1319 [Organisms](#) online database. Common names should be lowercase, except for proper nouns  
1320 and their derivations.
- 1321 • When possible, please provide as much genetic and/or colony information available is  
1322 useful (for example, “Rockefeller colony of *Aedes aegypti* (L.)”). Including geographic  
1323 origin and generations in culture is also useful, but may not always be known and is not  
1324 required.

1325

1326 **Submissions of Cover Photographs**

1327 Authors are encouraged to submit insect photos for the cover of the journal. Photos must be  
1328 of living (not pinned) insects and must be submitted as high-resolution (300 dpi or higher)  
1329 image files. If you wish to crop your image to the exact dimensions of our cover, the  
1330 dimensions to use are W: 222 mm x H: 203 mm; however, photos that do not match those  
1331 dimensions can still be accepted. The insect species featured on the cover of a given issue  
1332 must be mentioned in the issue in question.

1333 Photographers will be asked to give ESA one-time permission to use the photo on the journal  
1334 cover and will retain their copyright of the photo.

1335 Submit photos to [pubs@entsoc.org](mailto:pubs@entsoc.org).

1336

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1340 or geographic location of the authors. If you have any questions about the cost of publication,  
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1342

1343

1344