JULIANA LÍVIA VIEIRA

INSECTICIDE RESISTANCE, GENETIC DIVERSITY, AND ECOLOGICAL NICHE DISTRIBUTION ASSOCIATED WITH THE CO-OCCURRENCE OF RICE STINK BUGS IN NEOTROPICS

Thesis submitted in fulfillment of the requirements for the degree of Doctor (PhD) in Applied Biological Sciences at the Faculty of Bioscience Engeneering of Ghent University, Belgium, with co-tutelage with the Post-Graduate Program of the Federal University of Viçosa, Brazil.

Advisor: Raul Narciso C. Guedes

Co-advisors: Guy Smagghe
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Advisor

A todas as mulheres que me atravessam.

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“Sem ponto, sem virgula, sem meia, descalça
Descascou o medo pra caber coragem
Sem calma, sem nada, sem ar".
(Liniker)

ABSTRACT

VIEIRA, Juliana L., D.Sc., Universidade Federal de Viçosa and Ghent University, December, 2022. **Insecticide resistance, genetic diversity, and ecological niche distribution associated with the co-occurrence of rice stink bugs in Neotropics**. Advisor: Raul Narciso Carvalho Guedes. Co-advisors: Guy Smagghe, Monica Höfte and Kevin Maebe.

Anthropogenic-mediated climate change generally favors the spread and establishment of species better adapted to warmer conditions, while global connectivity increases the likelihood of some species expansion. Additionally, agricultural ecosystems offer ample opportunities for pest species to settle due to the relative impoverishment of local native species and the high availability of resources for better-adapted herbivores. Neotropical rice fields and the rice stink bugs *Oebalus poecilus* and *O. ypsilongriseus* provide a scenario in which apparent area expansion and trade-offs are occurring, favoring the establishment of a species complex, and of which the belated perception of the problem could lay significant economic and management consequences. In light of the main aspects of *O. poecilus* and *O. ypsilongriseus* biology and management, the objective of this thesis is to understand the factors mediating the distribution and overlap of these species. For that, the levels of thiamethoxam insecticide resistance and control failure likelihood were geographically assessed, as were each species' genetic diversity and population structure, attempting to recognize their relative occurrence, potential prevalence, and the likelihood of range expansion. Furthermore, species distribution modeling was used to identify the main predictor variables for the rice stink bug's niche occurrence and overlap. Our findings revealed that resistance to the main insecticide used in their control, thiamethoxam, does not appear to be decisive in species differentiation and prevalence. Nor are genetic divergences found here, considering that both species presented similar levels of diversity and structuring. However, the phenotypic plasticity (or its limitation) expressed in the ecological niche of rice stink bugs appears to indicate that sensitivity to drier periods may be an essential component in the distribution and dominance of these species in Neotropical regions. Thus, by combining control failure likelihood estimates, insecticide resistance, and spatiotemporal dynamics, with population genetics and species distribution modeling, this study allows insights on the relative importance and potential impact of two closely-related species, which in turn could potentially minimize unsuitable pest management practices and rice production losses.

Keywords: Genetic diversity. Insecticide resistance monitoring. Pest management. Species distribution modeling. Species overlap.

RESUMO

VIEIRA, Juliana L., D.Sc., Universidade Federal de Viçosa e Universidade de Gent, dezembro de 2022. **Resistência à inseticida, diversidade genética e distribuição de nicho ecológico associados à co-ocorrência dos percevejos das panículas na região Neotropical**. Orientador: Raul Narciso Carvalho Guedes. Coorientadores: Guy Smagghe, Monica Höfte e Kevin Maebe.

Mudanças climáticas mediadas por fatores antropogênicos geralmente favorecem a disseminação e o estabelecimento de espécies melhor adaptadas às condições mais quentes, enquanto a conectividade global aumenta a probabilidade de expansão de algumas espécies. Adicionalmente, os ecossistemas agrícolas oferecem amplas oportunidades para o estabelecimento de espécies-praga, devido ao relativo empobrecimento local das espécies nativas e a alta disponibilidade de recursos para herbívoros mais bem adaptados. As plantações de arroz neotropicais e os percevejos das panículas, *Oebalus poecilus* e *O. ypsilongriseus*, fornecem um cenário no qual aparente expansão de área plantada e compensações estão ocorrendo, favorecendo o estabelecimento de um complexo de espécies, cuja a percepção tardia do problema pode trazer consequências econômicas e de manejo significativas. A luz dos principais aspectos da biologia e manejo de *O. poecilus* e *O. ypsilongriseus*, o objetivo desta tese é compreender os fatores que mediam a distribuição e a sobreposição destas espécies. Para isto, foram avaliados geograficamente os níveis de resistência e probabilidade de falha de controle ao inseticida tiametoxam, bem como a diversidade genética e estrutura populacional de cada espécie, buscando reconhecer sua ocorrência relativa, a prevalência potencial e a probabilidade de expansão de alcance. Além disso, a modelagem de distribuição de espécies foi utilizada para identificar as principais variáveis preditoras para a ocorrência e sobreposição de nicho dos percevejos das panículas. Nossos resultados revelaram que a resistência ao principal inseticida utilizado no controle dessas, o tiametoxam, não parece determinante na diferenciação das espécies. Tampouco as divergências genéticas encontradas aqui, considerando que ambas as espécies apresentaram níveis semelhantes de diversidade e estruturação. No entanto, a plasticidade (ou limitação) fenotípica expressa no nicho ecológico dos percevejos do arroz parece indicar que a sensibilidade aos períodos mais secos pode ser um componente essencial na distribuição e dominância dessas espécies nas regiões neotropicais. Assim, combinando as estimativas de probabilidade de falha de controle, resistência a inseticidas e dinâmica espaço-temporal com genética de populações e modelagem de distribuição de espécies, este estudo permite reconhecer a ocorrência e importância relativa dessas espécies proximamente relacionadas, potencialmente minimizando práticas inadequadas de manejo e perdas na produção de arroz.

Palavras-chave: Diversidade genética. Monitoramento de resistência à inseticida. Manejo de pragas. Modelagem da distribuição de espécies. Sobreposição de espécies.

SAMENVATTING

VIEIRA, Juliana L., D.Sc., Universidade Federal de Viçosa en Universiteit Gent, December, 2022. **Insecticideresistentie, genetische diversiteit en ecologische niche distributie geassocieerd met het gelijktijdig voorkomen van rijststinkwantsen in de Neotropen**. Promotor: Raul Narciso Carvalho Guedes. Co-promotors: Guy Smagghe, Monica Höfte en Kevin Maebe.

Antropogeen-gemedieerde klimaatverandering bevordert over het algemeen de verspreiding en vestiging van soorten die beter zijn aangepast aan warmere omstandigheden, terwijl wereldwijde connectiviteit de kans op de verspreiding van sommige soorten vergroot. Bovendien bieden landbouwecosystemen voldoende mogelijkheden voor plaagsoorten om zich te vestigen, door de relatieve verarming aan inheemse soorten en de hoge beschikbaarheid van hulpbronnen voor beter aangepaste herbivoren. Neotropische rijstvelden en de rijststinkwantsen, *Oebalus poecilus* en *O. ypsilongriseus,* bieden een context waarbinnen gebiedsuitbreiding en invasies lijken plaats te vinden, wat de vestiging van een soortencomplex bevordert, en waarbij het te laat realiseren van het probleem aanzienlijke economische en beheer gevolgen kan veroorzaken. Het doel van dit proefschrift is om de factoren die de verspreiding en overlap van deze soorten mediëren beter te begrijpen en dit voornamelijk op basis van de belangrijkste aspecten van hun biologie en beheer. Daarvoor werden de resistentie niveaus tegen thiamethoxam-insecticiden en de waarschijnlijkheid van het mislukken van bestrijdingsmaatregelingen geografisch beoordeeld, waarna ook de genetische diversiteit en populatiestructuur van elke soort werd bepaald, om zo hun relatieve voorkomen, potentiële prevalentie en de uitbreidingskans van hun verspreidingsgebied te kunnen achterhalen. Verder werd soortdistributiemodellering gebruikt om de belangrijkste voorspellende variabelen voor de aanwezigheid en overlap van rijststinkwantsen te identificeren. Onze bevindingen toonden aan dat resistentie, tegen het belangrijkste insecticide dat bij de bestrijding van deze soorten wordt gebruikt, thiamethoxam, niet doorslaggevend was voor de differentiatie van de soorten. Evenmin werden genetische verschillen teruggevonden, aangezien beide soorten vergelijkbare niveaus van diversiteit en structurering vertoonden. De aanwezige hoeveelheid fenotypische plasticiteit (of eerder het tekort eraan) binnen de ecologische niche van rijststinkwantsen lijkt er echter op te wijzen dat gevoeligheid voor drogere perioden een essentieel onderdeel kan zijn van de verspreiding en dominantie van deze soorten in neotropische gebieden. Door het inschatten van de kans op falende bestrijding, de resistentie tegen insecticiden en hun tijdruimtelijke dynamiek te gaan combineren met populatiegenetica en modellering van de verspreiding van soorten, biedt deze studie inzichten in het relatieve belang en de potentiële impact van deze twee nauwverwante soorten. Wat op zijn beurt het gebruik van ongeschikte plaagbestrijdingspraktijken en rijstproductie verliezen zou kunnen minimaliseren.

Trefwoorden: Genetische diversiteit. Monitoring van insecticideresistentie. Plaagbestrijding. Modellering van soortendistributie. Soortenoverlap.

SUMMARY

Scope and outline

The current context of climate change and the intensification of agriculture in the savannah-like central Brazil seems to be increasing the importance of the rice stink bugs *Oebalus poecilus* (Dallas) and *O. ypsilongriseus* (De Geer) as the main rice pest species in the country. Although both species can occur in all rice-growing environments, *O. poecilus* has been more frequently reported in (low-land) irrigated rice fields and *O. ypsilongriseus* in upland rice (Ferreira et al., 2001). In addition to the cultivation system employed, the damage intensity of these species depends on the plant phenology and pest population density (Ferreira et al., 2001; Ferreira et al., 2002; Fragoso et al., 2011; Krinski & Foerster, 2017). Thus, determining pest occurrence is a fundamental step to properly estimate their density and distribution, providing support for developing sampling plans, and realistically evaluating yield losses (Couto et al., 2006).

Ecological niche-based models have been successfully used to predict species' potential occurrence and dispersion in response to environmental changes (Kumar et al., 2014; Tonnang et al., 2016; Lantschner et al., 2019). These models are based on the assumption that climatic variables are preponderant in agricultural ecosystems and in the occurrence and distribution of pest species (Jung et al., 2016), which is particularly important in a scenario of climate change and agricultural expansion. Coupled to the description of the potential occurrence and species overlap, spatial mapping of control failure and insecticide resistance allows recognition and determination of the extent of sites that require appropriate control (Guedes, 2017). This is because the emergence of resistance can be understood as a microevolutionary phenomenon (Crow, 1960; Sawicki, 1987), resulting from intensive insecticide use as control practice. The selection of resistant individuals is, therefore, decisive in reducing population diversity by promoting a genetic and demographic bottleneck that can lead to changes in population genetics. Consequently, another component in studying the spatiotemporal dynamics of pest species is identifying population structure and gene flow patterns and assessing the possibility of hybridization between closely related species.

The assessment of the spatial genetic structure of a species may give clues about its evolutionary history and whether biogeographic factors were fundamental in the generation of the population structure. Furthermore, investigating spatial distribution patterns is important to understand species evolution and to design pest management programs (Husch et al., 2018; Porretta et al., 2007). The development of markers capable of capturing the variation in nucleotide sequences in high resolution combined with access to platforms that provide genetic data, allows this type of study. Therefore, understanding the genetic mechanisms of local adaptation is fundamental to predicting species distribution, especially in the case of insect pests responsible for extensive quantitative and qualitative losses in grain production (Ferreira et al., 2002; Ferreira & Barrigossi, 2006; Krinski & Foerster, 2017).

Neotropical rice fields and the rice stink bugs provide a scenario in which apparent area expansion and trade-offs are occurring, favoring the establishment of a species complex with potential economic and management consequences. Changes in the environmental conditions may affect species distribution or favor their establishment in previously inadequate habitats (Early & Sax, 2014), promoting a severe effect on global agricultural production (Lesk et al., 2016). In Brazil, the traditional perception of *O. poecilus* being restricted to irrigated rice fields in the South region (Köppen-Geiger classification Cfa), which has two marked seasons defined by temperature, and the predominance of *O. ypsilongriseus* in the Midwest (Köppen-Geiger classification Aw), where the seasons are based on precipitation (https://pt.climate-data.org/), has being changing (Ferreira et al. 2001; Barrigossi 2008).

Aiming to understand the factors mediating the distribution and overlap of rice stink bugs in the Neotropics, we explored the role of 1) insecticide resistance, 2) genetic diversity and population structuring, and 3) ecological niche, in the *O. poecilus* and *O. ypsilongriseus* context. Due to the lack of internationally published literature (i.e., in English), the first chapter of this thesis summarizes the main aspects of the biology of the species, their current pest status, and some strategies applied in their management in rice fields. Then, the second chapter records the levels of thiamethoxam resistance and control failure likelihood, mapping geographically within rice fields in central Brazil and testing their spatial dependence. The third chapter explores the genetic diversity and population structure of these two rice stink bugs, attempting to recognize their relative occurrence, potential prevalence, and the likelihood of range expansion. Lastly, in the fourth chapter, species distribution modeling was used to identify the main predictor variables for the rice stink bug' niche occurrence and overlap. Integrating the estimates of control failure likelihood, insecticide resistance, and spatiotemporal dynamics (Chapter 2) with population genetics (Chapter 3) and species distribution modeling (Chapter 4), this study provide insights for geographically based decision-making strategies for the management of the two close-related species of rice stink bugs, *O. poecilus* and *O. ypsilongriseus*. Potentially these strategies can minimize the use of unsuitable management practices and rice production losses, opening a door for the proper management of two historically neglected pest species.

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1. Biology and management overview of the rice stink bugs

Considering the lack of internationally published literature (i.e., in English), the objective of this chapter is to summarize the main aspects of the biology of the rice stink bugs, *O. poecilus* and *O. ypsilongriseus*, elucidate their current pest status, and discuss some strategies applied in their management in rice fields.

Genus *Oebalus*

The genus *Oebalus* (Stål, 1862) (Hemiptera: Heteroptera: Pentatomidae) comprises a complex of species, among which *O. ypsilongriseus* (De Geer, 1773), *O. pugnax* (Fabricius, 1775), *O. poecilus* (Dallas, 1851), and *O. insularis* (Stål, 1872) are the most studied due to their economic importance, mainly in rice crops. According to Bhavanam et al. (2021), the first description of *O. pugnax* was made by Fabricius in 1775 under the scientific name *Cimex pugnax*. The genus name was changed to *Oebalus* by Stål in 1862, and revised several times (Bhavanam et al., 2021).

Origin and current occurrence

Native to the Neotropical region (Sailer 1944), the genus *Oebalus* probably originated in the American continent, given its occurrence and distribution. Species occurrence records often underestimate the real situation, as they depend on the official registration with some regulatory agency or institution. Although the rice stink bugs have been neglected species, the records of *O. poecilus* encompass Argentina, Aruba, Bolivia, Brazil, Colombia, Dominican Republic, Ecuador, Guyana, Panama, Suriname, and Venezuela (Vieira et al., 2022). With a broader distribution in the American continent, *O. ypsilongriseus* has occurrence records in Argentina, Bolivia, Brazil, Colombia, Costa Rica, Mexico, Paraguay, and United States (Vieira et al., 2022). The first report of *O. ypsilongriseus* occurring in the U.S. was in Florida in 1983, then some additional records were subsequently reported (Cherry et al., 2018; Bhavanam et al., 2021). Since 1998 it has been indicated as a potentially invasive pest (Cherry et al., 2018).

Bioecology and development

The bioecology of *O. poecilus* and *O. ypsilongriseus* can be illustrated in their activity in the one-year cycle associated to their primary host, rice (*Oryza sativa* L.). Initially, in the driest seasons and with the shortest photoperiod, adults of both species shelter in a variety of substrates, such as straw or wood, tree bark, plant base, and cracks in the soil, during their characteristic diapause period. With the return of the rains, at the beginning of spring, they start their activities by feeding on seeds of alternative hosts existing inside or near the rice fields (Barigossi, 2008). These alternative hosts include plants from Cyperaceae, Fabaceae, Myrtaceae, Malvaceae, Polygonaceae, Solanaceae, and especially Poaceae (Ferreira et al., 2001). The rice stink bugs usually mate in these alternative hosts and perform their first oviposition there (Barigossi, 2008).

As soon as the rice starts to flower, the rice stink bugs move actively and distribute in the fields occupying mainly the panicles (Ferreira et al., 2001). This occupation remains until the milky grain phase, indicating that sampling and decision-making procedures for *Oebalus* control must be carried out during all this settle period (Barrigossi et al., 2005). Concomitant to their occupation and feeding, the rice stink bugs start mating in the rice fields. Although few studies elucidate their chemical ecology, the pheromone produced by males of *O. poecilus* that attract females for reproduction has been described (Oliveira et al., 2013). After chemical attraction, males touch females with their tarsi and antennae in courtship, which may last approximately 5 to 10 minutes, if the female is receptive (Squire, 1934). Following the courtship period, which can last up to 3 days, the individuals copulate, keeping themselves together in the opposite position so they can walk and feed connected. Copulation begins more frequently in the hottest periods of the day, and its maximum duration remains unknown (Ferreira et al., 2001). However, some older references indicate the minimum duration is around five (Amaral, 1949) and eight hours (Squire, 1934).

Oviposition is typically carried out on the rice leaves, but when stink bugs reach high population densities, it can also occur in stalks and panicles (Ferreira et al., 2001). Each female of *O. poeclius* lays batches of about 15 eggs an average of 13 times (Couto, 2005), which are usually laid in double rows (Ferreira et al., 2001). For *O. poecilus* in irrigated rice, there are reports of mass oviposition characterized by thousands of eggs laid in overlapping layers in a few rice plants (Barrigossi, 2008). This phenomenon has not yet been observed in *O. ypsilongriseus* (Barrigossi, 2008).

Under ideal conditions of around 25ºC, the egg phase lasts about 5 to 6 days (Ferreira et al., 2001).

After hatching, first-instar nymphs generally remain grouped in the shells of the eggs, depending solely on the energy previously obtained in the egg (Panizzi, 1991). In the subsequent instars, they show active feeding behavior similar to that of adults. Nymphal instars last an average of 28 days for *O. poecilus* and 24 days for *O. ypsilongriseus* (Ferreira et al., 2001).

Adults of *O. poecilus* and *O. ypsilongriseus* show differences in size, duration of developmental stages, and general body color (Barrigossi, 2008). *Oebalus ypsilongriseus* is usually lighter in color and has a more elongated body, with males measuring 8.5 x 4.1mm in length and width and females 10.0 x 4.2mm. While the males of *O. poecilus* measure an average of 8.1 x 4.1mm and the females 8.9 x 4.2mm. Both have sharp lateral expansions on the prothorax and can be distinguished by the dorsal spots they carry on their thorax. In general, rice stink bug adults are not very active during the hottest hours of the day, remaining immobile and sheltered among leaves and plant stems. When disturbed, they can fly, exhibit thanatosis behavior and release a light yellow, volatile substance with a characteristic unpleasant odor (Ferreira et al., 2001). Additionally, these insects are photosensitive, being attracted to light traps especially on dark and warm nights (Amaral, 1949). In laboratory conditions, the average life span of *O. ypsilongriseus* mated adults is 101 days for males and 88 days for females, while the average of *O. poecilus* is 94 days, with males lasting about two weeks longer than females (Ferreira et al., 2001).

Finally, when the coldest periods of the year approach and the rice stink bugs are exposed to less than thirteen hours of light a day during their first three instars, sexual development is delayed, and body fat accumulates, indicating the onset of diapause (Santos et al., 2003).

Environmental influence on species development

Although the rice stink bugs are found in both cropping systems, *O. poecilus* has been more frequently observed in flooded rice and *O. ypsilongriseus* in upland rice (Ferreira et al., 2001). This observation raises a possible influence of humidity differences or other different microclimatic factors (e.g., temperature) between flooded and upland rice, affecting the development of both species. From the perspective of development *per se*, the duration of the life cycle phases of both species is sensitive to temperature variations, decreasing in the range of 20°C to 30°C (Barrigossi, 2008). As mentioned before in the paragraph “bioecology and development”, the egg phase is also dependent on ideal conditions; at 25°C this phase lasts for 5 to 6 days. Furthermore, the daily activity of nymphs and adults, such as feeding, dispersal, and reproduction, is also determined by environmental conditions. In the hottest period of the day, the insects remain immobile and sheltered between the leaves and stems of the plants, while at night, flights which are normally about 20 to 50m far can reach up to 250m under ideal conditions of temperature and wind (Ferreira & Barrigossi, 2001; 2004). Rainfall is also a critical factor for the survival of *O. poecilus*, determining population increases in the early season when it occurs at high rates (Sutherland & Baharally, 2003). As mentioned before, another interesting fact is the diapause induced in both species with the decrease in photoperiod, a phase that comprises the harvest of the rice crop (Albuquerque, 1993).

Damage

Oebalus poecilus and *O. ypsilongriseus* have a rostrum-shaped piercing-sucking mouth apparatus consisting of mandibles and maxillae modified into two pairs of tubular stylets, the inner pair distinctly forming the salivary and food canals (Grazia et al., 2012). Upon introducing the stylets into the panicle tissues, these bugs secrete two types of saliva, a coagulant that is responsible for forming a sheath where the stylets are introduced, and another more aqueous, containing enzymes that liquefy the solid and semi-solid portions of the cells allowing ingestion (Ferreira et al., 2001). This way of feeding mainly causes a lot of damage to the panicles, where *O. poecilus*, for example, concentrates around 30% of its bites in the rachis ramifications and 70% in the spikelets (Barrigossi, 2008).

Quantitative damage caused by both stink bugs involves reducing the number and mass of grains. This takes place mainly when the attack occurs soon after the fertilization of the flowers, resulting in the formation of empty spikelets (Barrigossi, 2008). The damage intensity of those insects depends on the cropping system used, the rice variety or genotype, the stage of development of the spikelets, and the observed infestation density (Ferreira et al., 2001; Ferreira et al., 2002; Fragoso et al., 2002; Fragoso et al., 2011; Krinski & Foerster, 2017). According to a Ferreira et al.

(2001), *O. poecilus* caused an average total loss of 52.7% in ten genotypes of flooded rice and 25.2% in upland rice. Comparatively, the average total losses in upland rice genotypes were 36.4% for *O. ypsilongriseus*.

Once the spikelets have a liquid (milky) or mass (pasty) endosperm, the injury caused by the rice stink bug feeding favors the establishment of microorganisms which in turn contribute to increased staining and reduced seed germination (Ferreira et al., 2001). In the subsequent stages, the attack results in the formation of dark areas on the seed coat and white or dark areas on the endosperm, reducing the grain's commercial value. Furthermore, the injury structurally weakens the grains in the damaged regions, leading to irregular shapes, and causing them to break during processing (Barrigossi, 2008; Krinski & Foerster, 2017).

In addition to the damage caused in the rice grains, *O. poecilus* and *O. ypsilongriseus* are oligophagous species capable of feeding on panicles of barley, corn, oats, rye, sorghum, and wheat, causing quantitative and qualitative losses in these grains production (Ferreira et al. 2001; Bhavanam et al., 2021).

Integrated management

For the management of *O. poecilus* and *O. ypsilongriseus* in rice fields, the first monitoring should be started when 50-75% of the panicles are formed (Rashid et al., 2006). After this first sampling, one to two weekly samplings should be carried out in the early morning or late afternoon, avoiding the hottest period of the day. These samplings should be performed at arbitrary chosen points, separated by approximately 100 meters in fields of up to 15 ha, and using a standard entomological sweep net at an angle of 180º. Ten sweeps should be performed at each sampling point, advancing one to two paces in each sweep. According to the sampling plan developed for the integrated rice stink bugs management, control measures should be taken when, on average, five or more insects are collected in the first two weeks after flowering and when ten or more are contained in the following weeks (Barrigossi, 2008).

Strategies to avoid rice crop losses include using varieties more tolerant to phytophagous attacks and cultural practices, which can impact the rice stink bugs' demography. Some relevant cultural practices are the prevention of the rice staggered planting in nearby areas; removal of straw and other plant material that could harbor the insects in the off-season; control of weeds andpotential secondary hosts such as

Digitaria spp. and *Echinochloa* spp.; and assessment of the potential consequences of early and late plantings. The former can be used as a trap crop, and the latter can receive insects from harvested fields. Additionally, when the rice stink bug population reaches the economic threshold in the crop, biological (i.e., natural and induced) and chemical control are the strategies adopted to drastically reduce the pest density (Martins et al., 2009).

Biological control

The rational use of insecticides, respecting the maintenance of natural biological control, allows predators of nymphs and adults and various parasitoids to remain at densities capable of contributing to the maintenace of the stink bug population below the economic threshold (Barrigossi, 2008). Another alternative is the applied biological control through the mass release of predators and/or parasitoids. In this sense, it can be mentioned for both species of rice stink bugs the predator of nymphs and adults *Apiomerus flavipennis* Herr. Schaff (Hemiptera: Reduviidae), the adult and nymph parasitoid *Beskia cornuta* (Diptera: Tachinidae); and the egg parasitoids *Microphanurus mormidae* Lima, 1935 and *Telenomus mormidea* Lima, 1935 (Hymenoptera: Platygastridae), the latter considered the most important (Ferreira et al., 2001).

Stink bugs are generally resistant to fungal infection, as the aldehydes constituting their defense secretions act as antimycotic agents for a wide range of entomopathogenic fungi (Sosa-Gómez et al., 1997). Therefore, only a few specific studies report the use of microbial agents for the biological control of *Oebalus* spp. For instance, Martins et al. (1987) verified the effect of *Metarhizium anisopliae* (Metsch.) Sorok on *O. poecilus* under field conditions and reported that the CP172 isolate at a concentration of $5x10^{13}$ spores.ha^{-1} caused mortality of 76.2% in the individuals analyzed. Furthermore, based on the previous tests in other pentatomids (Borges et al., 1993; Moraes, 2008), Mascarin et al. (2013) investigated the action of *Metarhizium anisopliae* strain CG168 isolated and combined with the insecticide EngeoPleno® (thiamethoxam + lambda cyhalothrin) under sub-lethal concentration. However, the residual contact effect of the treatments (i.e., isolated or combined with the insecticide) was ineffective in controlling adults of *O. poecilus* in flooded rice under field conditions.

Santos et al. (2002) published a scientific note after identifying individuals of *O. poecilus* in diapause sites dead and infested by *Beauveria bassiana* (Bals.) Vuill. The isolate obtained from the collection of these insects proved to be pathogenic at all concentrations used, with the highest percentage of mortality (84.4%) recorded at the concentration of $1.25x10^9$ conidia.mL^{-1}. Additionally, Pinto et al. (2013) tested the efficiency of different bacterial suspensions of *Bacillus thuringiensis* strains on rice stink bugs, identifying higher mortality (87%) for *O. poecilus* with the dendrolimus HD-37 strain.

Chemical control

Chemical control only should be applied if the economic threshold defined in the integrated management program is reached. The choice of the method used must be based on the loss estimate, method efficiency, control cost, and environmental impact (Barrigossi et al., 2005). Chemical control should never be performed preventively, as the insecticides used do not have a prolonged residual effect, and can affect populations of natural enemies, in addition to increasing production costs (Barrigossi, 2008). The inadequate use of insecticides could cause unnecessary population losses of pollinators, predators, and parasitoids, compromising the balance of intricate ecological relationships (Guedes et al., 2016), and reducing the efficiency of rice stink bug control. Thus, only products registered and regulated should be used to control *Oebalus* spp. in rice, and the recommendations of the manufacturer of each product must be followed.

In Brazil, the pyrethroids lambda-cyhalothrin, bifenthrin, and fenpropathrin, together with etofenprox, all sodium channel modulating, are currently recommended for the control of *O. poecilus* and *O. ypsilongriseus* (Agrofit, 2022). The agonists of nicotinic acetylcholine receptors in the nervous system, including the neonicotinoids thiamethoxam and acetamiprid, and the derived sulfoxaflor, are also recommended (Agrofit, 2022). In general, few products are registered to control rice stink bugs in Brazil and the United States (Agrofit, 2022; Bhavanam et al., 2021). There are published studies in which other insecticides were used to control these bugs, such as endosulfan, banned in Brazil in 2010, malathion, fipronil, and commercial formulations of neem oil (Silva et al., 2013; Pinto et al., 2013; Pinheiro & Quintela, 2010). In the United States, according to Cherry et al. (2018), carbaryl, lambda-cyhalothrin, malathion, zeta-cypermethrin, and gamma-cyhalothrin were registered for the control of *Oebalus* spp. in Florida in 2017. Mascarin et al. (2013) combined thiamethoxam and lambda-cyhalothrin with the fungus *Metarhizium anisopliae*, and Pinto et al. (2013) used thiamethoxam, lambda-cyhalothrin, malathion, and fipronil with the bacterium *Bacillus thuringiensis*. Each of these studies showed different efficiencies for controlling rice stink bugs.

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2. Area-wide survey of thiamethoxam resistance and control failure likelihood in the rice stink bugs *Oebalus poecilus* and *O. ypsilongriseus*

VIEIRA, J.L.; CAMPOS, S.O.; SMAGGHE, G.; FRAGOSO, D.B.; BARRIGOSSI, J.A.F.; GUEDES, R.N.C. Area-wide survey of thiamethoxam resistance and control failure likelihood in the rice stink bugs *Oebalus poecilus* and *O. ypsilongriseus*. **Journal of Pest Science**, v.95, p.1151–1161, 2022. https://doi.org/10.1007/s10340-021-01445-5

Area-wide survey of thiamethoxam resistance and control failure likelihood in the rice stink bugs *Oebalus poecilus* and *O. ypsilongriseus*

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Abstract

Insecticide resistance survey is an *in-situ* biomonitoring method to assess potential impact of pesticides that exhibit direct economic consequences when leading to control failure of insect pest species. Nonetheless, the latter phenomenon is frequently neglected. Their spatial-dependence and mapping are also seldom considered and when complexes of related pest species are involved, such as the rice stink bugs from the Neotropics, *Oebalus poecilus* and *O. ypsilongriseus*, the scenario is even worst. Insecticide use is common against both species, particularly with the neonicotinoid thiamethoxam, and despite suspicion of a shift in *O. poecilus* historical dominance and complains of control failure, the role of this insecticide in this context was never tested. Thus, we screened populations from both species for thiamethoxam resistance within rice fields from central Brazil. The levels of thiamethoxam resistance and control failure likelihood were recorded and their spatial dependence was tested and geographically mapped. The thiamethoxam potency was similar between species, which also exhibited overlapping levels of resistance. Thus, this insecticide does not seem involved in eventual shifts in species dominance and the occurrence of *O. ypsilongriseus* is frequent. Thiamethoxam resistance was detected in both species, nearly half of the populations of *O. poecilus* and about a third of *O. ypsilongriseus*, but at low levels (< 10-fold). As a consequence, the risk of control failure with thiamethoxam was also low. Spatial dependence was significant for both species and phenomena (i.e., thiamethoxam resistance and control failure), prevailing in about the same area and likely reflecting the local pattern of insecticide use.

Keywords: insecticide use, pest management, insecticide resistance monitoring, neonicotinoids, Neotropical rice, spatial survey

Key message

- Insecticide resistance and control failure are particularly important in related co-occurring species
- Spatial prevalence of the rice stink bugs *Oebalus poecilus* and *O. ypsilongriseus* were studied
- Thiamethoxam resistance and risk of control failure in both rice stink bugs were also assessed
- No spatial prevalence exists and levels of thiamethoxam resistance were similar in both species
- The levels of resistance were low, although frequent, and the risk of control failure was also low

Author contribution statement

JLV, JAFB and RNCG conceived and designed the study. DBF, JAFB, GS and RNCG provided the materials and tools. JLV and SOC performed the experiments and analyzed the data. JLV, GS and RNCG structured and wrote the manuscript. All authors revised the material.

Introduction

Out of sight, out of mind is a common enough notion since its alleged 15th century conception by the German-Dutch canon Thomas à Kempis. Regardless, it is suitable enough for two contexts of environmental and economic significance. The first is the concept of insecticide control failure, which is a core consequence of insecticide resistance, but usually neglected or confounded with the latter. The second is that of neglected pest species complexes where one of the species overshadows the other in its perceived importance without a clear rational. Such is the case of rice stink bugs in the US and Neotropical America, a species complex that, in itself, is frequently and unduly relegated.

Insecticide resistance is a phenomenon resulting from local selection by insecticide application usually aimed at protection against pest species (Crow and Kimura 1960, Sawicki 1987), as a microevolutionary response to withstand this ubiquitous anthropogenic disturbance. The surveying of the phenomenon is an important strategy for *in situ* biomonitoring of environmental impact allowing the tracking of changes in the genetic makeup of the exposed (insect) population (Hopkins 1998; Walker et al. 2001). However, insecticide resistance also exhibits practical importance as a common economic and management concern since it frequently leads to increase insecticide use, higher control costs, and eventual insecticide control failure. The latter possibility is likely a reason why both phenomena – insecticide resistance and control failure, are frequently mixed with assessments focusing on the first, and leading to the neglect of the second (Guedes 2017).

Insecticide control failure is the significant reduction of insecticide efficacy, preventing it from reaching the expected level of control when used following the label rate (Guedes 2017). Thus, insecticide resistance is one of the potential causes of control failure, but such relationship requires proper testing as even existing resistance may not necessarily compromise control if in relatively lower levels (e.g., lower than 10-fold in most cases) and lower frequency of incidence (e.g., < 10%) (Roush and Miller 1986; Scott 1990, Guedes 2017). Furthermore, factors other than insecticide resistance may also lead to control failure (Guedes 2017). Therefore, assessing both insecticide resistance and control failure with suitable methods adds value to the determinations, what is meriting growing of attention (e.g., Roditakis et al. 2018; Silva et al. 2019; Leite et al. 2020; Li et al. 2020).

Control failure is not a sole target of neglect, and such inattention is even more worrisome when species complexes are considered; if also neglected (pest) species, all the worst. Such is the case of rice stink bugs in the Americas. These pest species are all of the genus *Oebalus* and are primarily Neotropical in their distribution although three species do occur in the US – *Oebalus pugnax* (Fabricius), *O. insularis* (Stål), and *O. ypsilongriseus* (De Geer) (McPherson and Bundy 2018). The latter two are of recent introduction in the US with potential to become invasive (Cherry et al. 1998; Awuni et al. 2015; Vanweelden et al. 2020). A fourth species, *O. poecilus* (Dallas), is the historically prevalent late-season rice pest in flooded irrigated rice in Brazil, while *O. ypsilongriseus* is a more recent concern initially directed to upland rice (Ferreira et al. 2001). Nonetheless, both species are recently observed in all rice-growing regions of the country in both upland and flooded irrigated fields (Ferreira et al. 2001, Weber et al. 2020). Their damage level is allegedly associated with the cultivation system, pest population density and rice spikelet developmental stage (Ferreira et al. 2002; Fragoso et al. 2011; Krinski and Foerster 2017).

Records of the establishment of *O. ypsilongriseus* in Brazil and particularly in regions of *O. poecilus* prevalence are not available, and their relative importance was not yet determined. The prevailing notion is that *O. ypsilongriseus* is invasive in flooded rice, but *O. poecilus* seems also to be expanding its range to upland rice and even infesting rice fields edging the Amazon rainforest (Ferreira et al. 2001, Krinski and Foerster 2017). However, both species co-occur and their hybridization is possible, although not yet assessed. This possibility adds further concerns to the scenario, as the determinants of each species dominance and the potential impact of their hybrids remain unknown. Differences in damage potential and insecticide susceptibility, as these compounds are the frequent management tools used against rice stink bugs (Barrigossi 2008; Blackman et al. 2015; Cherry et al. 2018), are two problems potentially associated with related species within a complex and their potential hybridization (Corrêa et al. 2019). Both have economic, environmental and pest management consequences (Barrigossi et al. 2005; Barrigossi 2008), which motivated the present study.

Insecticide resistance, control failure likelihood and occurrence of both species in rice fields are all spatial-dependent phenomena although such a fact is rarely considered. The spatial component should not be ignored as it allows the mapping and recognition of trends and patterns useful for environmental and pest management minimizing insecticide distribution. Therefore, the present study aimed at circumventing these shortcomings assessing the spatial occurrence (and dependence) of both species, and their resistance and control failure likelihood to the neonicotinoid thiamethoxam, one of the main compounds used against these rice stink bugs in Brazil (MAPA 2021).

The study was carried out sampling insects from four rice cultivation areas in central Brazil and the most densely sampled area encompassing two neighboring counties was subjected to spatial-dependence assessment. We hypothesized that: (i) spatial prevalence of both species is distinct, based on the historical perception of distinct preference to upland and flooded rice; (ii) thiamethoxam susceptibility differ between species and may play a role in their spatial distribution; and (iii) thiamethoxam resistance occurs in populations of both species and is translated into control failure likelihood, what may contribute to their recent overlap and spread.

Material and methods

Insects and insecticide

The populations of the rice stink bugs *Oebalus poecilus* and *O. ypsilongriseus* were collected between 2019/2020 from irrigated rice fields from central Brazil in the states of Goiás (three sampling sites) and Tocantins (25 sampling sites) (Table 1; Fig. 1). The samplings were all carried out during the rice reproductive phase after 75% of the panicle emergence (Rashid et al. 2006). All of the sampled fields, one in the county of Goianira (Goiás) and the remaining in the neighboring counties of Formoso do Araguaia and Lagoa da Confusão (Tocantins) (Fig. 1), were subjected to the common agriculture practices of the region including irrigation, fertilization, and weed control, but without insecticide use. The sampling was carried out using sweep net (38 cm diameter) in sequential sweeps directed at the upper third of the plants and at an angle of 180°. The collected stink bugs were stored in polypropylene bags (60 x 80 cm) containing a cluster of rice panicles and transported to the laboratory, where the time-mortality bioassays were carried out under controlled conditions. Each sampling point was georeferenced using cellular phone equipped with an applicative of global positioning system (UTM Geo Map. App., Bandung, Indonesia).

The neonicotinoid thiamethoxam, the main insecticide presently used against rice stink bugs in Brazil, was used in the time-mortality bioassays with a commercial formulation (water dispersible granules, 250 g a.i./Kg; Syngenta, São Paulo). The insecticide was used at the recommended label rate (150 g formulation per hectare in 200 L water, or 0.19 mg a.i./mL), always following the manufacturer recommendations (MAPA 2021).

Time-mortality bioassays

The time-mortality toxicity bioassays were carried out at fixed concentration (i.e., the label rate) by immersing the rice panicles in the insecticide solution for 10 s, and subsequently letting them dry at the environmental temperature. After a 4 h acclimation period in laboratory conditions, groups between 10 to 20 adults collected from each field population were transferred to 500 mL transparent plastic containers containing two standardized rice panicles previously treated with thiamethoxam (except for the untreated control treatment where only water was used). Insect mortality was recorded every 15 min during the first hour, then every hour for eight hours, at every two hours subsequently until 12 hours, and finally every four hours until reaching 24-h exposure. The insects were considered dead if unable to move when prodded with a fine hair brush (Miller et al. 2010). Each bioassay was replicated three times for each insect population obtained from each sampling site.

Control failure likelihood

Final mortality after 24 hours exposure was recorded using the same methods described for the time-mortality bioassays, but with this single exposure time and the same insecticide concentration (i.e., the label rate). Again, untreated controls were used for each population to correct for natural mortality (Abbott 1987). Insect mortality was recorded and used to calculate the control failure likelihood (CFL) under thiamethoxam use, using the formula CFL = 100 - [(observed and corrected mortality (%) x 100)/expected mortality (%)] (Guedes 2017). The expected mortality was 80% following the guidelines of the Brazilian Ministry of Agriculture of a minimum threshold of efficacy for conventional insecticides (MAPA 1995). CFL values below zero were considered as negligible risk (or control failure likelihood), and equaled to zero as minimum value.

Statistical analyses

The differences in sampling site occurrence and co-occurrence of both species of rice stink bugs was tested using qui-square contingency table (2 x 2; $P < 0.05$) (FREQ procedure, SAS; SAS Institute, Cary, NC, USA). In addition, eventual prevalence of a given species or their simultaneous occurrence was tested by Kruskal-Wallis ($P < 0.05$) (NPAR1WAY procedure; SAS, SAS Institute, Cary, NC, USA).

The time-mortality data was subjected to probit analysis to assess thiamethoxam toxicity for each population sampled and tested using the modified protocol to account for repeated measures in time using Mathematica (Wolfram Mathematica 9, Champaign, IL, USA; Throne et al. 1995). Resistance ratios were estimated based on the 2nd most susceptible population and using the Relpot protocol (from Mathematica 9); this was done because one population from each species exhibited unusually high susceptibility to thiamethoxan not allowing proper estimation of their respective LT_{50}s and derived resistance ratios. The resistance ratios at median lethal time (LT_{50}) were considered significant when the 95% confidence interval estimated for the ratios did not include the value 1 (Robertson et al. 2017). The correlation between resistance ratios of both populations from rice stink bug species in their co-occurring sites was tested to recognize if such traits are associated (CORR procedure; SAS). The significant control failure likelihood was recognized by contrasting the observed mortality (after corrected for natural mortality) with 80%, the minimum accepted threshold of efficacy (MAPA 1995) using the one-sided Z-test at 95% confidence level with correction for continuity (Roush and Miller 1986).

Geospatial analyses were carried out to estimate the spatial dependence between neighboring sampling sites and their differences in response to thiamethoxam (both level of resistance and control failure likelihood). The use of regular kriging methods was not possible because of the relatively reduced number of insect samples available on the main stretch of rice fields extending from Formoso do Araguaia to Lagoa da Confusão in Tocantins. Therefore, ordinary cokriging was used for *O. poecilus* and inverse distance was used for *O. ypsilongriseus*, where the number of samples was smaller.

The semivariogram functions that relate spatial distance between sampling sites of *O. poecilus* and their respective difference in response to thiamethoxam were selected through cokriging and subsequently used for spatial mapping (Isaaks and Srivastava 1989). The parameters range (h), model contribution or partial sill (C), nugget (C_o) and sill ($C+C_o$) were used for the semivariogram model selection. Range and partial sill refer to the distance and the semivariance in thiamethoxam response in which a plateau is reached, respectively (i.e., maximum distance and response where spatial dependence exist). The nugget refers to the value in which the model intercepts the y axis and represents measurement error or resolution achieved. Sill refers to the semivariogram value in which the range is reached and provides the maximum variability in the data.

Two further parameters were obtained from these four initial ones, and used for further model characterizations: proportion ($C/C+C_o$) and randomness (C_o/C). Spatial dependence was considered strong when proportion is higher than 0.75 (i.e., > 0.75), moderate when between 0.25 and 0.75, and weak if small or equal do 0.25 (≤ 0.25).

Randomness is the ratio between nugget and partial sill of a particular point and allows testing if the potential association between two data points is random or not. Isotropy or anisotropy in the semivariance (i.e., variability variation with spatial direction or not, respectively) was calculated for four horizontal directions: 0°, 45°, 90° and 135°. The generated semivariogram models were subjected to cross validation to recognize the best data fit (Isaaks and Srivastava 1989), and that with the mean error closer to zero, lowest mean square error and mean standard error, and standardized mean square error close to one was selected for spatial mapping.

Inverse distance was the technique used for spatial interpolation for *O. ypsilongriseus*, as previously mentioned, and the model was selected by cross validation. The criteria used for such selection were the regression slope closer to one, intercept and mean error closer to zero and lowest possible mean square error. Again, the selected model was used for spatial interpolation, as with *O. poecilus*, for mapping the thiamethoxam resistance levels and control failure likelihood. All the spatial analyses were carried out with ArcGIS 10.5 (ESRI, Redlands, CA).

Results

Species occurrence of rice stink bugs

Rice stink bugs were collected in 28 sampling sites stretching into two counties in the State of Tocantins, and one county in the State of Goiás. The overall frequency of site occurrence of both species, and lack thereof, did not vary significantly ($\chi^2 = 3.12$, $P = 0.08$) with rice stink bugs collected in about half of the sampling sites (51%). The Kruskal Wallis test to recognize eventual prevalence of a given species, or their co-occurrence, also did not indicate significant differences between occurrence of each individual stink bug species and their co-occurrence ($H = 2.00$, $P = 0.37$). These results indicate lack of prevalence of either species and their distribution seem independent from one another with *O. poecilus* occurring alone in 43% of the samples, *O. ypsilongriseus* occurring alone in 25% and they both co-occurring in 32% of the samples.

Thiamethoxam species susceptibility

The time-mortality probit model used with correction for successive observations through time was suitable based on the goodness-of-fit values obtained ($\chi^2 < 8.28$, *P*

> 0.05), allowing the estimate of the desired toxicological endpoint – the median lethal times (LT_{50}) and the subsequent resistance ratios and their respective 95% confidence intervals. The insecticide potency and thus efficacy was similar for both species when the pooled data of every population sampled was considered with ratio of 1.24 between them and 95% confidence interval encompassing the value of one (0.13-2.85) (Table 2).

Thiamethoxam resistance within species

The insect populations of each species did vary in their susceptibility to thiamethoxam, but they either were susceptible or exhibit low levels of resistance (i.e., < 10-fold) (Table 2). The frequency of thiamethoxam resistance among populations of *O. poecilus* was 52.38%, while for *O. ypsilongriseus* the frequency of resistant populations was 37.50%. The highest level of resistance was detected for an *O. poecilus* population from Formoso do Araguaia reaching 10.75-fold (Table 2). The levels of thiamethoxam resistance from co-occurring populations of both species was not significant indicating their likely independence (n = 9, r = 0.23, *P* – 0.55).

Thiamethoxam efficacy and control failure likelihood

Thiamethoxam exhibited high levels of overall efficacy against both species of rice stink bugs with an average mortality (± SE) of 97.69 ± 1.42% for *O. poecilus* and 93.01 ± 2.61% for *O. ypsilongriseus* (Table 3). However, a few pointed populations did exhibit significantly lower efficacy incurring in significant risk of control failure with a single population from *O. poecilus* (P02 from Formoso do Araguaia) and two from *O. ypsilongriseus* (P25 and P28, both also from Formoso do Araguaia) in the range of 10.40 to 13.80% (Table 3). The low incidence of risk of control failure with thiamethoxam prevented the testing of the association between thiamethoxam resistance and control failure likelihood.

Spatial analyses and mapping of resistance and risk of control failure

There was significant directionality (i.e., isotropy) in the spatial dependence of thiamethoxam resistance and control failure likelihood among populations of *O. poecilus* and the best semiovariogram models selected for distance interpolation using cokriging are presented in Table 4. The analyses indicated local variability and distance-dependence for both phenomena with prevalence of problems at southeast (Figs. 3 and 4). Cokriging was not possible for *O. ypsilongriseus* and inverse distance was used to test spatial dependence among sampling sites of this species population. The model selected through cross validation indicated mean errors of 0.03 and 0.22 and root means square values of 1.69 and 4.81 from levels of resistance and control failure respectively. Local variability for both traits does also exist for *O. ypsilongriseus* with higher incidence of thiamethoxam resistance and risk of control failure in the south (Figs. 3 and 4).

Discussion

Agriculture is one of the Anthropocene´s heralds, but the chemical signature of pesticides is a more recent scar in the globe (Davis 2017; Hayes and Hansen 2017; Arcuri and Hendlin 2019), whose onset took place with the extensive use of organo-synthetic compounds by the 1940´s (Davis 2017; Hayes and Hansen 2017). Nonetheless, pesticide usage is only one among myriad of agriculture practices and impacts taking place for the last century. Climate change, global traveling, new agricultural frontiers and biological invasions go hand-in-hand shaping the new epoch and interfering with species distribution, dominance and their consequences. Rice fields in Neotropical America have been affected by this scenario and the two existing cultivation systems, flooded and upland rice, are facing a further stink bug species, *Oebalus ypsilongriseus*, in addition to its previous scourge, *O. poecilus.* Again, pesticides and insecticides more particularly are suspected culprits in balancing this relationship in addition to climate change and other environmental changing conditions.

Our study aimed at testing three hypothesis. First, we tested the spatial prevalence of both stink bug species in flooded rice fields to assess if the historical dominance of *O. poecilus* still exists. Second, we assessed if the neonicotinoid thiamethoxam largely used in the area against rice insect species plays any role in mediating eventual rice stink bug shift in dominance, as reported for other species (Cordeiro et al. 2014; Guedes et al. 2016, 2017; Mohammed et al. 2019). Last, we assessed if thiamethoxam resistance and control failure likelihood as spatially-dependent phenomena exist in populations of both stink bug species that may contribute to their co-existence and spread. The two first hypotheses were rejected based on our results, unlike the third.

The co-occurrence of both *O. poecilus* and *O. ypsilongriseus* was frequent in flooded rice fields and similar to the frequency of their isolated occurrence. This finding is a counterpoint to the established perception of *O. poecilus* prevalence in flooded rice, as earlier recognized (Ferreira et al. 2001). Some twenty years of changes in cultivation system and environmental conditions, including climate change, are reason enough for eventual range expansions of pest species without shortage of examples (e.g., Campos et al. 2017; Pantoja-Gomez et al. 2019). Even among rice stink bugs, the range expansion of *O. ypsilongriseus* and *O. insularis* to the Florida rice fields in the US and the shift in dominance away from *O. pugnax* (Cherry et al. 1998; Awuni et al. 2015; Vanweelden et al. 2020), the previously key rice pest species, are revealing in suggesting that this may also be taking place in Brazil. The reporting of both *O. poecilus* and *O. ypsilongriseus* occurring and co-occurring in flooded and upland rice in Brazil lays further credence for this perceived shift in range (Ferreira et al. 2001, Weber et al. 2020), and growing importance of *O. ypsilongriseus* in flooded rice.

Insecticides are able to mediate or contribute with such changes in range and dominance among insect species (Cordeiro et al. 2014; Mohammed et al. 2019), not to mention populations within species (Oliveira et al. 2007). If the susceptibility of both rice stink bugs species significantly differed, such a role would be possible. Thus, the time-mortality toxicity bioassays with pooled population data of both species. Nonetheless, their susceptibilities to thiamethoxam were similar largely overlapping with one another and leading to the rejection of their potential role in determining relative dominance and/or prevalence. This finding is consistent with the co-occurrence of both species on the sampled sites in the region, which were already an indication of this fact subsequently reinforced by the similar susceptibility to thiamethoxam. These results contrasts with those of stored grain beetles and aphids (Cordeiro et al. 2014; Mohammed et al. 2019), although these were carried out comparing more distantly related species than both stink bug species studied here and in *Aedes* mosquitos (Alto et al. 2013).

Resistance to thiamethoxam varied among populations of *O. poecilus* and *O. ypsilongriseus*, but within a similar range and restricted at mild levels (≤ 10-fold) although affecting nearly half of the populations. The phenomenon seems independent in each species with little coincidence on sampling incidence where thiamethoxam resistance was detected for both species at the same sampling sites. Again, this finding reinforces the perception that this neonicotinoid insecticide is not mediating the interaction, or lack thereof, of both species. Nonetheless, thiamethoxam resistance was frequent and significant, albeit low, in both species signaling emerging concerns regarding the future use of this insecticide against rice stink bugs in central Brazil. Estimates of control failure likelihood under thiamethoxam use were therefore obtained indicating that such a risk is negligible at present although potentially requiring future attention.

Beyond occurring frequency and level of thiamethoxam resistance in rice stink bugs, their spatial relationship and that of potential risk of control failure are also worthy of attention as these phenomena are not of general occurrence. Indeed spatial-dependence does exist for both species and even the limited nature of our spatial sampling allows the recognition of hot spots of incidence of thiamethoxam resistance and likelihood of control failure, as also reported for other insect pest species (Gontijo et al. 2013; Tuelher et al. 2017; Leite et al. 2020). These were higher at central and southeast for *O. poecilus* and at south for *O. ypsilongriseus*, which partially overlap despite the distinct sampled points for each species likely reflecting the local pattern of insecticide use (and selection). This spatial pattern again reinforces the apparent independent evolution of thiamethoxam resistance in populations of both species. Nonetheless, the question remains if both species are able to interbreed and if any is favored by the current prevailing conditions of rice fields in the study region and cultivation system, what deserves future attention.

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Compliance with ethical standards

Conflict of interest: The authors declare that they have no known conflict of interest.

Ethical approval: This article does not contain any studies with human participants or animals unduly performed by the authors.

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Table 1. Identification and geographical coordinates of the sampling sites for populations of the rice stink bugs *Oebalus poecilus* and *Oebalus ypsilongriseus*.

State	County	Sampling site	Latitude	Longitude	Species
Goiás	Goianira	P34	- 16º 26' 11.42"	- 49º 23' 45.51"	Op
		P35	- 16º 26' 11.41"	- 49º 23' 45.52"	Op
		P36	- 16º 26' 3.019"	- 49º 23' 44.69"	Op
Tocantins	Formoso do Araguaia	P01	- 11º 47' 58.90"	- 49º 37' 18.83"	Oy
		P02	- 12º 00' 10.01"	- 49º 40' 51.26"	Op
		P03	- 11º 47' 31.88"	- 49º 41' 11.67"	Oy
		P04	- 11º 54' 53.29"	- 49º 40' 59.03"	Op, Oy
		P05	- 11º 57' 11.64"	- 49º 40' 59.12"	Op, Oy
		P06	- 11º 58' 47.75"	- 49º 40' 59.34"	Op, Oy
		P07	- 11º 53' 50.28"	- 49º 40' 59.11"	Op, Oy
		P08	- 11º 29' 10.93"	- 49º 58' 57.98"	Op
		P09	- 11º 29' 40.37"	- 49º 58' 53.67"	Op
		P15	- 11º 58' 52.94"	- 49º 41' 04.74"	Op
		P16	- 11º 58' 17.06"	- 49º 41' 40.77"	Op,Oy
		P22	- 11º 58' 18.06"	- 49º 41' 08.11"	Op
		P24	- 11º 57' 42.25"	- 49º 40' 59.18"	Op, Oy
		P25	- 11º 57' 09.25"	- 49º 41' 45.93"	Oy
		P26	- 11º 56' 35.06"	- 49º 41' 30.65"	Op, Oy
		P27	- 11º 50' 23.83"	- 49º 39' 01.01"	Oy
		P28	- 11º 51' 19.24"	- 49º 41' 11.85"	Oy
		P29	- 11º 46' 06.07"	- 49º 44' 21.13"	Oy
		P39	- 11º 47' 15.86"	- 49º 41' 37.12"	Op
		P42	- 11º 46' 05.95"	- 49º 43' 15.69"	Op
		P50	- 11º 27' 46.81"	- 49º 58' 40.04"	Op
		P51	- 11º 28' 43.05"	- 49º 59' 32.91"	Op
	Lagoa da Confusão	P31	- 10º 55' 17.03"	- 49º 51' 42.35"	Oy
		P32	- 10º 54' 12.67"	- 49º 52' 27.79"	Op, Oy
		P33	- 10º 48' 21.37"	- 49º 45' 07.33"	Op, Oy

Table 2. Relative toxicity of thiamethoxam to Brazilian populations of the rice stink bugs *Oebalus poecilus* and *Oebalus ypsilongriseus*. The asterisk in the resistance ratio indicates significant difference from the standard susceptible population when the confidence interval does not include the value 1, following Robertson et al. 2007.

Species	Sampling site	No.	LT_{50}(95% CI) Hours	Slope ± SE	χ 2	Df	P	Resistance ratio at LT_{50} [RR_{50}] (95% CI)
O. poecilus	P34	120	3.00 (2.26-3.73)	0.38 ± 0.05	5.73	4	0.16	9.38 (4.04-14.71)*
	P35	120	1.05 (0.69-1.41)	0.80 ± 0.12	4.28	2	0.12	3.28 (1.28-5.29)*
	P36	84	2.60 (1.94-3.25)	0.42 ± 0.05	8.28	4	0.07	8.13 (3.47-12.78)*
	P02	120	3.44 (2.81-4.20)	0.44 ± 0.06	7.09	5	0.14	10.75 (4.82-16.68)*
	P04	120	0.73 (0.39-1.04)	0.90 ± 0.12	0.79	2	0.67	2.31 (0.77-3.86)
	P05	120	0.36 (0.12-0.56)	1.44 ± 0.26	5.52	2	0.07	1.13 (0.25-2.00)
	P06	60	0.64 (0.17-1.11)	0.73 ± 0.19	0.16	2	0.92	2.00 (0.37-3.63)
	P07	80	1.26 (1.04-1.48)	1.29 ± 0.15	1.20	3	0.48	3.94 (1.79-6.08)*
	P08	100	0.37 (0.26-0.46)	3.03 ± 0.41	0.27	2	0.80	1.16 (0.49-1.83)
	P09	80	1.09 (0.87-1.31)	1.32 ± 0.16	2.45	2	0.29	3.41 (1.52-5.29)*
	P15	120	0.04 (0.00-0.32)	1.10 ± 0.30	0.90	2	0.54	0.13 (0.00-1.21)
	P16	120	0.32 (0.12-0.47)	1.82 ± 0.30	1.59	2	0.45	1.00
	P22	54	0.98 (0.57-1.37)	1.05 ± 0.20	5.22	2	0.07	3.06 (1.10-5.02)*
	P24	90	0.95 (0.65-1.22)	1.02 ± 0.15	5.54	2	0.06	2.97 (1.21-4.73)*
	P26	44	0.51 (0.18-0.79)	1.69 ± 0.43	1.68	2	0.43	1.59 (0.43-2.76)
	P39	87	1.85 (0.87-2.73)	0.31 ± 0.04	2.39	4	0.36	5.78 (1.68-9.88)*
	P42	50	0.79 (0.49-1.16)	1.76 ± 0.48	3.16	2	0.21	2.47 (0.91-4.02)
	P50	53	2.08 (0.82-3.33)	0.41 ± 0.10	8.03	4	0.07	6.50 (1.65-11.35)*
	P51	120	1.85 (0.41-3.12)	0.22 ± 0.04	2.87	3	0.32	5.78 (1.08-10.48)*
	P32	50	0.81 (0.24-1.31)	0.82 ± 0.18	4.95	3	0.15	2.53 (0.53-4.53)
	P33	60	0.61 (0.05-1.06)	1.12 ± 0.27	0.13	3	0.27	1.91 (0.21-3.60)
O. ypsilongriseus	P01	120	< 0.01 (0.00-0.00)	0.07 ± 0.02	6.67	5	0.16	0.00 (--)
	P03	120	2.89 (1.23-4.23)	0.21 ± 0.04	4.58	3	0.17	5.07 (1.89-8.25)*
	P04	80	1.25 (0.53-1.87)	0.44 ± 0.07	6.99	4	0.11	2.19 (0.78-3.60)
	P05	120	1.22 (0.03-2.24)	0.28 ± 0.05	1.34	2	0.51	2.14 (0.14-4.14)
	P06	100	1.04 (0.08-1.82)	0.36 ± 0.06	2.32	2	0.31	1.82 (0.23-3.42)
	P07	120	1.42 (1.20-1.66)	1.24 ± 0.15	2.78	3	0.33	2.47 (1.43-3.51)*
	P16	80	1.02 (0.57-1.41)	0.70 ± 0.09	3.25	4	0.32	1.79 (0.80-2.78)
	P24	37	1.47 (0.87-2.10)	0.88 ± 0.18	1.89	2	0.39	2.58 (1.17-3.39)*
	P25	90	3.18 (2.52-3.94)	0.42 ± 0.06	5.68	4	0.17	5.57 (3.10-8.05)*
	P26	49	0.95 (0.42-2.10)	1.04 ± 0.39	4.48	2	0.11	1.67 (0.54-2.79)
	P27	114	0.57 (0.33-0.80)	1.27 ± 0.24	4.36	2	0.11	1.00
	P28	120	1.95 (1.24-2.72)	0.40 ± 0.07	5.28	4	0.19	3.44 (1.63-5.25)*

	P29	62	1.10 (0.74-1.84)	1.17 ± 0.35	4.13	2	0.13	1.93 (0.88-2.97)
	P31	33	1.00 (0.39-1.61)	0.91 ± 0.21	5.63	3	0.11	1.75 (0.59-2.92)
	P32	31	0.58 (0.02-1.06)	1.84 ± 0.67	3.07	2	0.22	1.02 (0.27-1.76)
	P33	120	1.76 (1.34-2.19)	0.66 ± 0.09	6.69	3	0.07	3.09 (1.69-4.49)*
O. poecilus	*Total*	*1852*	*0.86 (0.23-1.37)*	*0.61 ± 0.13*	*0.44*	*2*	*0.80*	*1.00*
O. ypsilongriseus	*Total*	*1396*	*1.07 (0.70-1.70)*	*0.87 ± 0.23*	*1.31*	*2*	*0.52*	*1.24 (0.13-2.85)*

Table 3. Estimated thiamethoxam mortality (%) and control failure likelihood (%) of populations of the rice stink bugs *Oebalus poecilus* and *Oebalus ypsilongriseus*, using Brazilian recommended label rates. Mortalities were not significantly lower (one-sided Z-test at 95% confidence level with correction for continuity; n = 80) than 80%, which is the minimum efficacy threshold of efficacy required by the Brazilian legislation for registering an insecticide for pest control (MAPA 1995).

Species	Sampling site	No.	Mortality [control failure likelihood] (%)
O. poecilus	P34	60	100.0 [0.0]
	P35	60	100.0 [0.0]
	P36	60	95.0 [0.0]
	P02	60	70.2 [12.3]
	P04	60	100.0 [0.0]
	P05	60	100.0 [0.0]
	P06	40	97.4 [0.0]
	P07	60	100.0 [0.0]
	P08	60	100.0 [0.0]
	P09	60	100.0 [0.0]
	P15	60	96.7 [0.0]
	P16	60	100.0 [0.0]
	P22	30	100.0 [0.0]
	P24	60	100.0 [0.0]
	P26	24	95.6 [0.0]
	P39	60	100.0 [0.0]
	P42	30	100.0 [0.0]
	P50	33	100.0 [0.0]
	P51	60	98.3 [0.0]
	P32	30	96.3 [0.0]
	P33	30	100.0 [0.0]
O. ypsilongriseus	P01	60	100.0 [0.0]
	P03	60	100.0 [0.0]
	P04	60	100.0 [0.0]
	P05	60	100.0 [0.0]
	P06	60	100.0 [0.0]
	P07	60	98.1 [0.0]
	P16	60	100.0 [0.0]
	P24	20	100.0 [0.0]
	P25	60	68.9 [13.8]
	P26	30	82.4 [0.0]
	P27	60	89.2 [0.0]
	P28	60	71.7 [10.4]
	P29	45	86.7 [0.0]
	P31	20	100.0 [0.0]
	P32	20	94.5 [0.0]
	P33	60	96.7 [0.0]
O. poecilus	*Total*	*1057*	*97.6 [0.0]*
O. ypsilongriseus	*Total*	*795*	*93.0 [0.0]*

Table 4. Semivariogram models and parameters of thiamethoxam resistance and control failure likelihood in populations of the rice stink bug (*Oebalus poecilus*).

Response	Cokriging	Model	Nugget (C_0)	Partial sill (C)	Sill ($C+C_0$)	Proportion ($C/C+C_0$)	Range (h_r, m)	Random-ness (C_0/C)	Mean errors	Root mean square error (RMSE)	Mean square error (MSE)	Root Mean square scaled error (RMSSE)	Average square error (ASE)
Resistan-ce ratio	Ordinary	Spherical	0.5824	12.4803	13.0627	0.9554	4296.7610	0.0466	-0.2141	1.4442	-0.1319	1.5884	2.6579
Control failure likelihood	Ordinary	Gaussian	0.0207	20.7171	20.7378	0.9990	5744.4540	0.0010	-0.5986	2.7464	-0.1508	0.9919	3.0241

Figure captions

Fig. 1. Distribution of the sampling sites of populations of the rice stink bugs *Oebalus poecilus* and *Oebalus ypsilongriseus* used in the spatial survey of thiamethoxam resistance in the states of Tocantins and Goiás (A), then in the counties of Formoso do Araguaia and Lagoa da Cunfusão (B), and in the county of Goianira (C). Identification for each sampling site and its coordinates are found in Table 1. The axes correspond to the geographical coordinates of each map, and also the geographical location of the sites depicted in Figs. 2 and 3.

Fig. 2. Contour maps of the levels of thiamethoxam resistance in populations of the rice stinkbugs *O. poecilus* and *O. ypsilongriseus*. The maps were generated using spatial interpolation and the color legend indicates the range of resistance ratios observed for the rice stink bugs. The axes correspond to the geographical coordinates of each map, which are also represented within the broader geographical region depicted in Fig. 1.

Fig. 3. Contour maps of the levels of thiamethoxam control failure likelihood in populations of the rice stinkbugs *Oebalus poecilus* and *O. ypsilongriseus*. The maps were generated using spatial interpolation and the color legend indicates the range of resistance ratios observed for the rice stink bugs. The axes correspond to the geographical coordinates of each map, which are also represented within the broader geographical region depicted in Fig. 1.

Fig. 1.

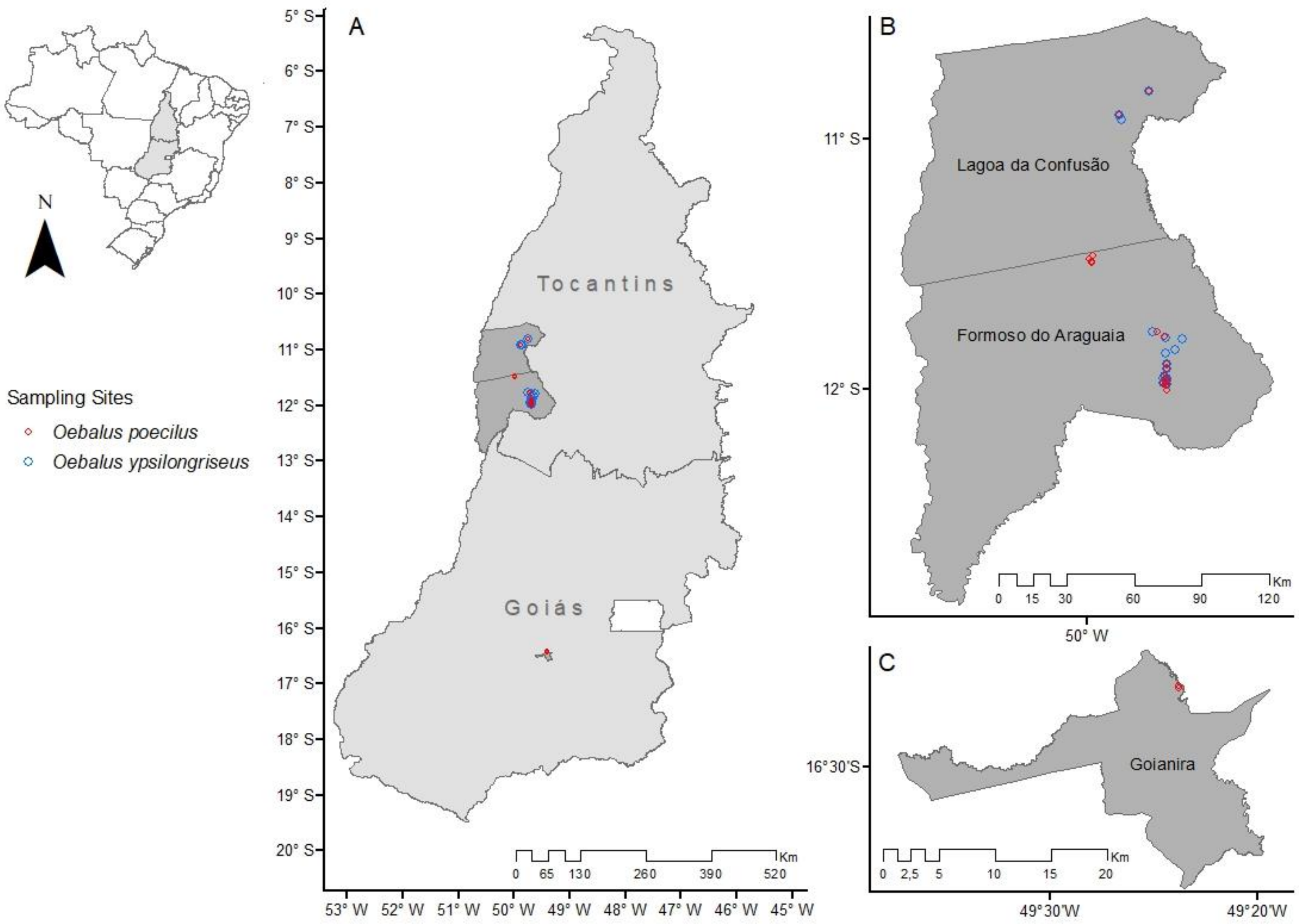

N
Sampling Sites
Oebalus poecilus
Oebalus ypsilongriseus
A
5° S
6° S
7° S
8° S
9° S
10° S
11° S
12° S
13° S
14° S
15° S
16° S
17° S
18° S
19° S
20° S
Tocantins
Goiás
0 65 130 260 390 520 Km
53° W 52° W 51° W 50° W 49° W 48° W 47° W 46° W 45° W
B
11° S
12° S
Lagoa da Confusão
Formoso do Araguaia
0 15 30 60 90 120 Km
50° W
C
16°30'S
Goianira
0 2,5 5 10 15 20 Km
49°30'W
49°20'W

Fig. 2

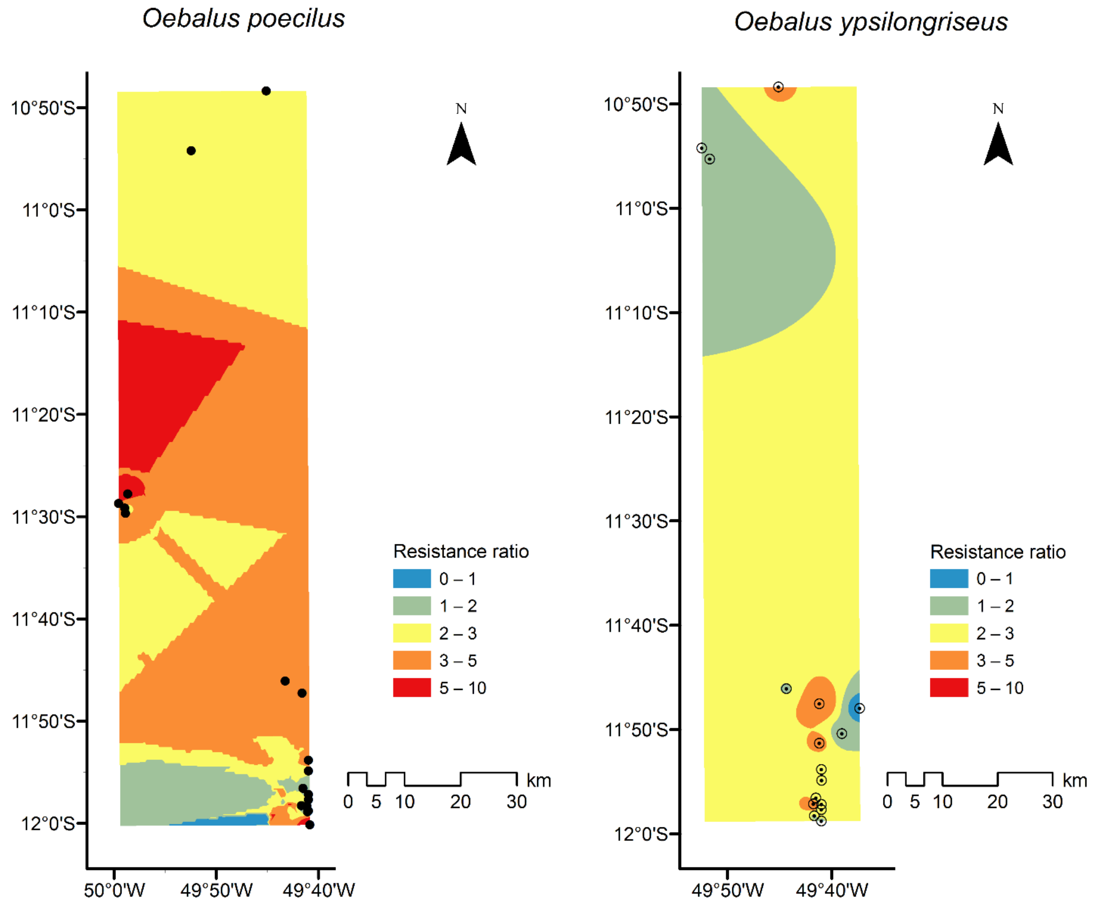
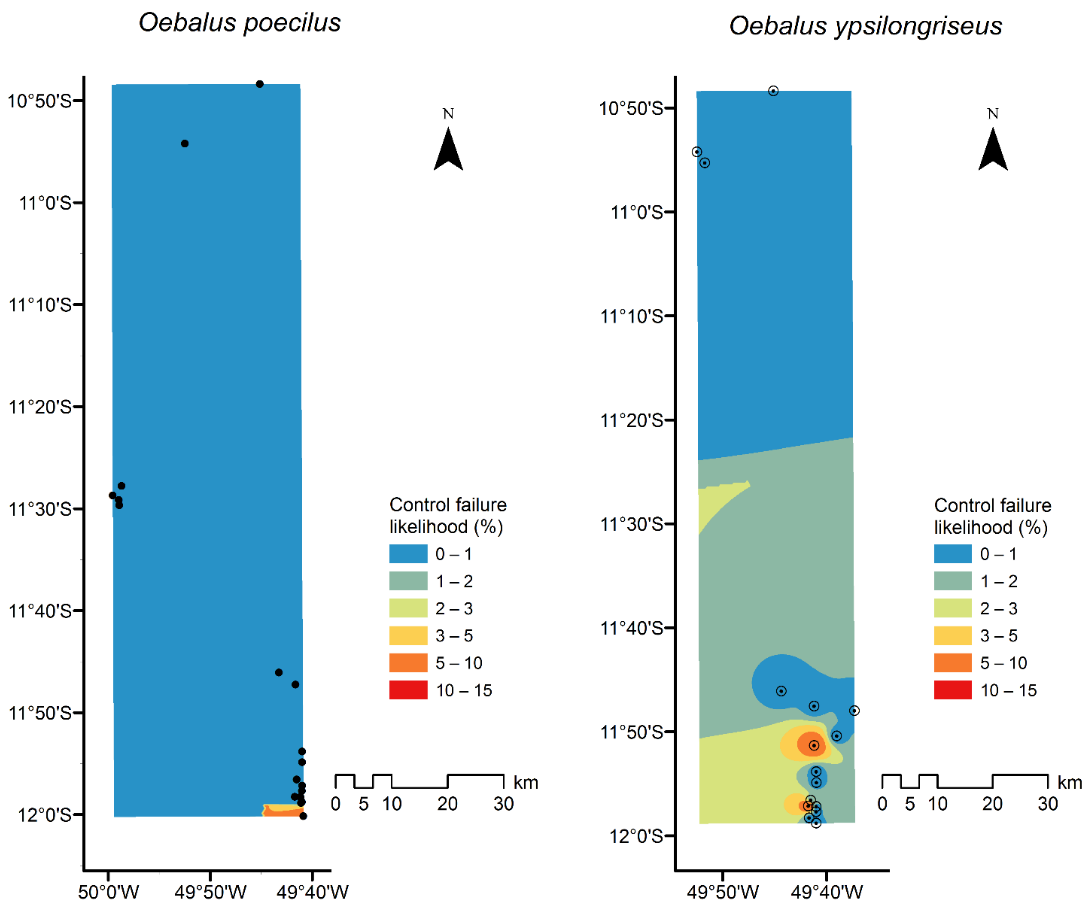

Oebalus poecilus
10°50'S
11°0'S
11°10'S
11°20'S
11°30'S
11°40'S
11°50'S
12°0'S
50°0'W
49°50'W
49°40'W
N
Resistance ratio
0 – 1
1 – 2
2 – 3
3 – 5
5 – 10
0 5 10 20 30 km
Oebalus ypsilongriseus
10°50'S
11°0'S
11°10'S
11°20'S
11°30'S
11°40'S
11°50'S
12°0'S
49°50'W
49°40'W
N
Resistance ratio
0 – 1
1 – 2
2 – 3
3 – 5
5 – 10
0 5 10 20 30 km

Fig.3


Oebalus poecilus
Oebalus ypsilongriseus
10°50'S
11°0'S
11°10'S
11°20'S
11°30'S
11°40'S
11°50'S
12°0'S
50°0'W
49°50'W
49°40'W
N
Control failure likelihood (%)
0 – 1
1 – 2
2 – 3
3 – 5
5 – 10
10 – 15
0
5
10
20
30
km

3. Disentangling a Neotropical pest species complex: Genetic diversity and population structure of the native rice stink bug *Oebalus poecilus* and the invasive *O. ypsilongriseus*

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Disentangling a Neotropical pest species complex: Genetic diversity and population structure of the native rice stink bug *Oebalus poecilus* and the invasive *O. ypsilongriseus*

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ABSTRACT

BACKGROUND: A first step in any pest management initiative is recognizing the existing problem – identifying the pest species and its abundance and dispersal capacities. This is not simple and even more challenging when insidious (invasive) species are involved constituting a pest complex. Understanding a species' population diversity and structure can provide a better understanding of its adaptation and relative pest potential. Such is the need for the native rice stink bug *Oebalus poecilus* and the invasive *O. ypsilongriseus* in low and high flatlands of South America.

RESULTS: The genetic structure differed between both rice stink bug species (F_{ST} = 0.157, P = 0.001), where 84% of the overall genetic variability takes place within species and three genetic groups were recognized through Bayesian approach (K = 3). *Oebalus poecilus* exhibited slightly higher genetic diversity (H_E = 0.253) and structuring (F_{ST} = 0.050, P = 0.001) than the invasive *O. ypsilongriseus* (H_E = 0.211; F_{ST} = 0.038, P = 0.013). Nonetheless, only the former exhibited significant correlation between genetic and geographic distances (r = 0.48, P = 0.013).

CONCLUSION: Despite the pointed peculiarities, the obtained results indicate overlap in both species' occurrence and similar genetic structure allowing for a compound problem to be dealt with as the complex requires managing without, as yet, a prevailing species or a niche specialization.

Keywords: Species overlap, invasive pest species, rice, Neotropical America, population diversity

1 INTRODUCTION

The Confucian notion that silence is a true friend that never betrays holds serious implications when applied to insidious pest species, an ever growing problem in the Anthropocene where anthropogenic stressors are changing the globe, [1,2] but sudden changes draw the bulk of attention while subtle ones remain neglected until too late.[3–5] Anthropogenic-mediated climate change for instance usually favors the spread and establishment of species better adapted to warmer conditions,[6,7] while other current megatrends like increased technology and human demographic shifts enhance world connectivity,[8] and thus the likelihood of pest species invasion. However, the concern is not only the arrival and immediate impact of invader species, but their establishment and dispersal, which are also important conceptual stages of species invasion.[9,10]

More importance is usually given to the arrival and short-term impacts of invasive species, [11–13] while their establishment and subsequent dispersal come as an afterthought. The latter two stages are subtler and long-lasting, and insidious pest species are usually detected too late for action in the early stages of arrival and immediate impact, making them particularly troubling. If such invader is a sister species of a native one, the derived complex may afford chances of hybridization, niche partitioning and/or range expansion that are rather difficult to predict.[14–16] Agriculture ecosystems offer ample opportunities for invaders to settle due to the relative impoverishment of local native species and by being rich in resources for well-adapted herbivores that facilitate the establishment and spread of non-native species and populations.[17] Neotropical rice fields and rice stink bugs provide a context in which apparent range expansion and invasions are occurring with the establishment of a species complex, and with the late perception of the problem and large management and economic consequences. This makes the group an appealing, albeit challenging, study model.

Gene flow between populations of pest species can alter genetic diversity levels and their dynamics in agricultural ecosystems, where the introduction of new genetic variation may assist in the subsequent selection of better adaptive traits. This is often studied among populations usually neglecting the relationship between related species in the species complex,[15] as that of rice stink bugs that seems to be taking place in the US and also in Brazil. The native *Oebalus poecilus* (Dallas) and the invasive *O. ypsilongriseus* (De Geer) are the actors of the latter case, both reported in flooded and upland rice fields stretching from South to Center-North Brazil[18]. The former species, *O. poecilus*, is a common pest in rice fields of Neotropical America, particularly in Brazil, while the latter, *O. ypsilongriseus* is a more recent concern also extending its range into the US through recent introduction and recognition of its invasive potential.[18–20]

The invasive scenario in rice fields in the Americas, and particularly in the US and Brazil where the issue has attracted attention, remains largely neglected. The dominance and occurrence of these rice stink bugs are barely studied despite their rather considerable economic impact on yield and grain quality,[21–23] not to mention the impact of current management strategies used against them, where insecticide use prevails.[24] An early concern was about the role of the neonicotinoid thiamethoxan potentially mediating or contributing with the distribution and dominance of the rice stink bug species,[24] which proved unfounded but raised concerns regarding these species' genetic relationship and population structure. However, such studies are lacking for rice stink bugs.

A plethora of molecular markers can be used to assess genetic diversity.[25,26] Inter simple sequence repeats (ISSR) markers allow the detection of polymorphisms in inter-microsatellite loci regions among individuals of the same species, as well as from different species by using specific primers designed from di-, tri-, or tetra-nucleotide sequence repeats. In addition, ISSR markers combine the benefits of random amplified polymorphic DNA (RAPD) with reproducibility and specificity without requiring prior target sequence knowledge.[26] These traits led to the use of ISSR markers in several studies during the last decade attempting to characterize the genetic structure of species from different insect taxa, from Hemiptera,[27–29] to Lepidoptera,[30–32] Coleoptera,[33,34] Diptera,[35,36] and Hymenoptera.[37] Thus, suggesting its potential usefulness for rice stink bugs.

The goal of the present study was to understand the genetic diversity and population structure of the two rice stink bug species occurring in Brazil, attempting to recognize their relative origin of occurrence, potential prevalence and likelihood of range expansion. Higher genetic diversity and population structure were expected in *O. poecilus*, as the native existing population, while an overlap in occurrence, genetic diversity levels, and structure among populations of *O. poecilus* and the invasive *O. ypsilongriseus* would be suggestive of a lack of a dominant species, and could lead to future range expansion, in turn leading to added concerns for the management of rice stink bugs in the region.

2 MATERIALS AND METHODS

2.1 Insect field populations and sampling

The rice stink bugs *Oebalus poecilus* (Op) and *O. ypsilongriseus* (Oy) were collected in rice-producing regions in Brazil (Table 1). The collected insects were stored in 90% ethanol for transport to the Federal University of Viçosa (UFV), where they were kept frozen at -20°C until the DNA extraction. Each field population was circumscribed within a 5.7km diameter collecting site, based on the recognition of the maximum distance at which the spatial dependence is detected.[24] Overlapping collecting sites were considered as a single population. The sampling sites were georeferenced during the collection, and in the case of overlapping sites, the average of decimal coordinates of each location was used (Table 1). Seven insects were the minimum sample size for each field population.

2.2 DNA extraction

The DNA extraction of rice stink bugs was performed with Wizard Genomic DNA Purification Kit (Promega; São Paulo, SP, Brazil), following the manufacturer's protocol with adjustments. Before adding lysis solution, each sample was subject to the bead-beating method for mechanical cellular disruption. For better separation of the organic phase, an extra step was added consisting of 300 µl of mixed alkyltrimethyl ammonium bromide (MATAB, 2%) and 300 µl of chloroform-isoamyl alcohol (24:1), vortexing for 20 s and centrifuging for 10 min at 14000 rpm. The precipitation with 70% ethanol (cold; -20°C) was repeated three times. To avoid alcohol contamination, the pellet was resuspended in 50 µl of Tris-EDTA (ethylenediaminetetraacetic acid) buffer after air-dried overnight. RNase solution was only added at the end of the extraction, followed by incubation at 37°C for six hours. The integrity, size, and quality of the DNA samples obtained were checked by NanoDrop spectrophotometry and electrophoresis on 1% agarose gel using 1X TBE buffer (Tris/Acid Boric/EDTA) at 100 V.

2.3 ISSR amplification

From ISSR primers previously used in a study of genetic diversity of predatory pentatomids,[29] fifteen primers with similar structures were selected to be tested for both species (S1).[38] In addition to these primers, seven other primers previously used for the soybean looper *Chrysodeixis includens*,[32] were also checked (S1). Altogether, ten suitable polymorphic ISSR primers (Table 2) were obtained to perform the molecular analysis of both rice stink bug species.

ISSR polymerase chain reactions (PCR) were carried out for a total number of 348 individuals of *O. poecilus* and 251 of *O. ypsilongriseus*. All the amplifications were carried out in a final volume of 10 µl containing 1 µl of genomic DNA, 0.5 µM of primer, 200 µM of dNTP, 1 µl of PCR buffer (Qiagen 10x), and 1U HotStar Taq DNA polymerase. In reactions including CI primers, samples were initially denatured at 94°C for 15 min, followed by 37 cycles of 30 s at 94°C, annealing at their respective temperature (Table 2) for 45 s, extension at 72°C for 90 s, and a final extension step at 72°C for 20 min. The protocol for the UBC primers differed by having 35 cycles of 45 s at 94°C, annealing for 90 s, and the final extension step lasted 10 min. Negative control PCR tubes were run to ensure there was no contamination.

The PCR products were electrophoresed in 1.5% agarose gels at 100V for 40 min in 0.5X TAE (Tris/Acetate/EDTA) buffer and stained with ethidium bromide buffer. The amplification fragments were visualized and photographed in ultraviolet (UV) light. A mass Ruler DNA Ladder Mix (Thermo Scientific, Pittsburg PA, USA) was used in all gels as DNA molecular weight standard. Some samples were randomly chosen to be repeated to confirm the primers' capability of producing a reproducible banding pattern.

2.4 Data analysis

Amplification products were image-analyzed using the software Gel Analyzer 19.1 and scored for presence (1) or absence (0) of bands per primer, which allowed obtaining binary matrices (1/0) for both species with up to 139 multiple bands. Only fragments showing a good resolution pattern were considered. The binary matrices obtained for each species were subjected separately and then analyzed together in the GenAlEx 6.5 software to estimate the genetic diversity through the percentage of polymorphic loci (P), the mean effective number of alleles (Ne), the mean expected heterozygosity (H_E), and mean Shannon's Information Index (I) of each population and for both species.[39,40] Analysis of molecular variance (AMOVA) was carried out to calculate variance components among and within populations and species.[41] The genetic differentiation between populations and species was estimated as F_{ST} after 999 permutations. From the determination of F_{ST}, the gene flow (Nm) was calculated as Nm = $(1- F_{ST})/4\ F_{ST}$.[42]

Three approaches were used to set the population structure, a Mantel test, a Principal Coordinate Analysis (PCoA), and a Bayesian method. The Mantel test was executed with 9,999 permutations assuming a linear correlation between geographic distance and genetic distance, while the PCoA was run with the pairwise genetic distance matrix. Both tests were also performed in the software GenAlEx 6.5.[39,40] Based on a model of populations admixture, the Bayesian approach started from a single random-mating cluster to project the number of populations (K) more suitable for the data interpretation. For populations and then comparing species, the number of clusters (K) was estimated in the STRUCTURE v. 2.3.4 from 1 to 10 for each value with 200,000 burn-in iterations and 500,000 data collecting steps, repeated five times.[43] The most likely number of clusters was determined using the ΔK approach,[44] as implemented in STRUCTURE HARVESTER v. 0.6.94.[45] The optimal alignment of five replicate analyses of the 'best' K was determined through CLUMPAK 1.1,[46] and graphically visualized using DISTRUCT 1.1.[47]

PCoA and the Bayesian analyses were also used to compare species clustering. After the genetic clustering, aiming to validate the species' identity, a nucleotide BLAST analysis was also carried out with individuals from each theoretical genetic group (K) used for COI gene amplification. The components of each 20 µl reaction were 2 µL DNA, 10 µM of each primer (F and R)[48], 10 mM dNTP, 2 µl of PCR buffer (Qiagen 10x), and 1U HotStar Taq DNA polymerase. The following sets of primers were used: LCO1490 5′-GGTCAACAAATCATAAAGATATTGG-3′ (forward; F) and HCO2198 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ (reverse; R) for the COI gene[48]. Samples were initially denatured at 94°C for 15 min, followed by 35 cycles of 1 min at 95°C, annealing with the forward and reverse primers at 40°C for 1 min, extension at 72°C for 90 s, and a final extension step at 72°C for 10 min. The PCR product was purified using EXOFastAP (Fermentas, Germany) and sequenced by LGC Genomics, Germany (www.biosearchtech.com), using the same primers as the COI amplification.

3 RESULTS

3.1 Genetic diversity

Ten ISSR primers tested provided consistent and reproducible band patterns suitable to the intended study objectives. A total of 139 bands, varying from 180 to 4800 bp, were identified for the 348 individuals of *O. poecilus* and 251 of *O. ypsilongriseus*. All loci were polymorphic. The average number of bands produced was 13.9, varying from 12 to 16 bands, produced by the primers CI05 and UBC808 respectively (Table 2).

The overall *O. poecilus* populations showed higher mean percentage of polymorphism (P) and a mean effective number of alleles (Ne), 85.47% and 1.419, respectively, compared to *O. ypsilongriseus* (P = 78.78% and Ne = 1.335) (Table 3).

Moreover, the mean expected heterozygosity (H_E) and Shannon's information index (I), both genetic diversity parameters, were higher but not significant ($F \leq 1.13$, $p \leq 0.05$) in *O. poecilus* populations (Table 3). Among *O. poecilus* populations, FO 03 had the highest percentage of polymorphism (99.28%), while population GO 01 showed the highest genetic diversity ($H_E = 0.289$ and $I = 0.440$). Population IT 01 had the lowest percentage of polymorphism (66.91%) and the lowest genetic diversity ($H_E = 0.188$ and $I = 0.293$). Among the *O. ypsilongriseus* populations, the highest percentage of polymorphism was found in FO 04 (98.56%), while the lowest occurred in LA 02 (62.59%) (Table 3). The population FO 04 showed also the highest genetic diversity ($H_E = 0.273$ and $I = 0.425$), while FO 3.2 showed the lowest ($H_E = 0,169$ and $I = 0.271$). The mean percentage of polymorphism was 99.28%, the mean expected heterozygosity was 0.257, and Shannon's index was equal to 0.406 across both stink bug species (Table 3).

3.2 Population structure and species clustering

The analysis of the molecular variance (AMOVA) of the *O. poecilus* populations indicated a low but significant genetic differentiation among populations ($F_{ST} = 0.050$; $p = 0.001$), with 95% of the overall genetic variation occurring within and 5% occurring among populations. Similarly, *O. ypsilongriseus* populations had $F_{ST} = 0.038$ ($p = 0.001$), exhibiting 96% of the overall genetic variation within populations and 4% among them. When comparing the species, the genetic structure indicated significant differentiation ($F_{ST} = 0.157$; $p = 0.001$), with 84% of the overall genetic variation occurring within species (Table 4). Moreover, the Mantel test detected structuring with a significant correlation between genetic and geographic distances in *O. poecilus* populations ($r = 0.484$; $p = 0.013$), but no significant correlation between genetic and geographic distances was detected for *O. ypsilongriseus* ($r = -0.18$; $p = 0.18$). The estimated amount of gene flow, indirectly calculated from F_{ST}, was moderate between *O. poecilus* populations (Nm = 4.728), and between *O. ypsilongriseus* populations (Nm = 6.281). Theoretically, the calculated number of migrants between the species was 1.340.

The PCoAs (i.e., isolated species, and pooled species) revealed the existence of two groups. However, no clear geographical pattern was observed in the analysis. Among *O. poecilus* populations, the first Principal Component (PC1, Component X) explained 14.46% of the total variance, while the second Principal Component (PC2, component Y) explained 5.48% of the total variance. *O. ypsilongriseus* populations exhibited similar results, with PC1 explaining 13.96% of the variance, and PC2 4.52%. Pooling both species led to PC1 explaining 13.10% of the variance, and PC2 8.68% (Fig. 1).

The Bayesian cluster analyses indicated two different genetic groups for each species. Indeed, the best value of K (or the number of populations) that fitted the data, identified by the Evanno method, was K = 2 for both *O. poecilus* and *O. ypsilongriseus* (Fig. 2A and 2B, respectively). Each specimen was assigned to one of these genetic groups (Fig. 2D). The suggestion of two groups in each species was similar to the division obtained by the PCoA, indicating structuring by location just in *O. poecilus* (Fig. 3). Contrasting with the *a priori* expectation, the clustering among species suggested three genetic groups (Fig. 2C). To validate the species identity, the nucleotide BLAST analysis run indicated >99% of genetic correspondence between *O. poecilus* and the NCBI database (S2). Unfortunately, there were not enough samples deposited in NCBI to validate the identity of *O. ypsilongriseus*. Analysis of the three Bayesian models (*O. poecilus*, *O. ypsilongriseus*, and both species pooled together) indicate species overlap, and with the overlapping individuals of both species recognized as a third cluster (Fig. 2C).

4 DISCUSSION

The design of suitable pest management programs requires the proper recognition of the pest problem taking place, which includes the species identification, dispersal and density of occurrence. When the case is of a pest complex including an invasive species, the issue is even more concerning and challenging requiring the disentangling of the species relationships to properly assess the potential risk of establishment and spread of each species, and the expected loss associated with their respective occurrence. The current occurrence and range expansion of rice stink bugs in flooded and highland rice crops is an example of such a scenario and urgently needs more attention. Thus, in this study we targeted the population diversity and structure of both species, the native *O. poecilus* and the invasive *O. ypsilongriseus*. Higher population diversity and structure were expected for the native species, as the introduction and range of the insidious *O. ypsilongriseus* is likely recent, dating from the 1950s, and in expansion[18]. However, no such difference was observed with both species exhibiting similar levels of population and structuring.

Analyzing both species separately, the populations had K = 2 as the best number of genetic clusters. The binary matrices obtained indicate the samples arrangement in more and less polymorphic clusters independently of any previous classification (i.e., populations), which corroborates with the higher variation detected within populations rather than among them. Moreover, low levels of population structuring (e.g., low F_{ST} index) and moderate gene flow (Nm) were detected for both species, which resemble one another. *Oebalus ypsilongriseus* populations presented higher gene flow and lower genetic divergence among populations, but no samples from the South were analyzed, unlike for *O. poecilus*. The sampling of populations in more distant regions will bias the divergence between them in their overall assessment. Besides that, *O. ypsilongriseus* is described more recently in Brazil,[49,50] and with potential to become invasive, as reported after its recent introduction in the US.[19,20] The colonization of new areas determines demographic shifts, which can shape genetic diversity or homogeneity, mainly when facing population bottleneck events.[9,34–36] Curiously, such shifts are not yet clearly characterized for *O. poecilus* and *O. ypsilongriseus*.

Populations of the native species *O. poecilus* exhibited mildly higher levels of structuring than those of the invasive *O. ypsilongriseus*, and with a significant correlation between genetic and geographic distances. This analysis included populations of *O. poecilus* from both the Brazilian central savannah-like region and southern Brazil, unlike *O. ypsilongriseus* where only populations from the former region were collected. However, it is important to notice that *O. poecilus* exhibits a prevailing genetic cluster in the South, the more traditional rice cultivating region of the country. The populations of *O. poecilus* are broadly well-established in this region,[21,24,49,50] which likely serves as a source of individuals to other regions exhibiting a better determined gene pool. In addition, thiamethoxam, the main insecticide used against these stink bug species in Brazilian rice fields, did not play a relevant role in their distribution nor in their prevalence.[24] In this context, the predominance of one genetic cluster in the southern *O. poecilus* populations requires further investigation expanding the area surveyed and increasing the sampling sites and number of insects sampled.

The mildly higher genetic diversity observed in *O. poecilus* is a potential consequence of the higher number of individuals analyzed in some of the collected populations and maybe due to a higher intrinsic capability in annealing with the nonspecific ISSR primers.[25,26] However, the genetic diversity reflects adaptive processes, which in the case of agricultural insect pest species may result from their evolutive association with the host species. For instance, *O. poecilus* exhibits diapause behavior when the photoperiod is shortened during the late fall, which coincides with the rice harvest. During this period, the species migrates to native hosts, remaining there until the next rice crop blooms.[49,51,52] Periodic migration from native forest fragments to disturbed agricultural environments and *vice-versa* can promote the maintenance of the species' global gene pool in a region, preserving existing polymorphism. This is important mainly because *O. poecilus* is considered a native species in Brazil occurring in several natural hosts and widely distributed in rice crops from North to South,[21,49,50] what potentially favors the maintenance of its levels of gene polymorphism.

Another interesting result is the occurrence of genetic traits of both species in a few individuals from the central savannah-like region, as observed with the Bayesian analysis. This result added to the observed PCoA pattern raises the possibility of overlaping between *O. poecilus* and *O. ypsilongriseus* in this region. Although the species co-occur in flooded and upland rice, both in the traditional South region and in the recently expanded Central Savannah-like region, *O. ypislongriseus* is not a native species in Brazil. Biological invasions and the expansion of agricultural frontiers, the latter which is taking place for the last 30 years in the central savannah-like region, are the main drivers of a potential species gene exchange, particularly considering the ongoing scenario of climate change.[15,53]

The hybridization between related species or even the gene flow among populations can alter the population dynamics of key pest species with overlapping occurrence leading to shifts in host preference, as observed in the Neotropical brown stink bug *Euschistus heros.*[54] In the USA, *O. pugnax*, another species of the rice stink bug complex, is already reported occurring in secondary host crops such as wheat, corn, cotton, soybeans and sorghum,[55–57] although shifts in host preference were not investigated yet. In addition to these potential economic implications, gene exchange directly interferes with the effectiveness of pest management practices, especially insecticide use, by promoting resistance selection and dictating changes in demographic dynamics,[15] which is also potentially affected by patterns of insecticide use.[58–60] Thus, studies of genetic diversity and population structuring in pest species, and pest species complexes like the rice stink bug studied here, is fundamental to understand shifts in insect pest dominance and to adopt suitable pest management practices to prevent the establishment and/or contain the spread of invasive species, besides of containing the associated economic and environmental losses.[15,53]

In summary, despite pointed peculiarities, populations of both stink bug species exhibited similar levels of diversity and structuring, which was not expected as *O. poecilus* is regarded as a native species and *O. ypsilongriseus* is an invasive one. How many introduction events of *O. ypsilongriseus* and of which origin are questions that comes to mind and deserve answers by future research. Both species seem still in range expansion and climate change is a likely contributor for that, and respective niche prevalence of each species remains undetermined keeping their management as a complex of co-occurring species. Furthermore, the existence of an intermediary genetic group overlapping both species of rice stink bugs is suggestive for added adaptation and range expansion, which also deserves attention.

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AUTHORS´ CONTRIBUTION

JLV, KM, JAFB, GS and RNCG conceived and designed the study. GS and RNCG provided the materials and tools. JLV, and KM performed the experiments and gathered the data, and JLV, KM, and LOO analyzed the data. JLV and RNCG structured and wrote the manuscript draft, which was read, corrected, and approved by all authors. The manuscript was written through the contributions of all the authors.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Table 1. Identification and geographical coordinates of the sampling sites of the collected populations of the rice stink bugs *Oebalus poecilus* and *O. ypsilongriseus* (coded as Op and Oy, respectively).

Region	County (State)	Code	Latitude	Longitude	Collection date	Sample size Op	Sample size Oy
North Center	Lagoa da Confusão (TO)	LA 01	-10° 48' 21.37"	-49° 45' 7.33"	Fev/2020	09	09
	Lagoa da Confusão (TO)	LA 02	-10° 50' 53.15"	-49° 41' 2.07"	Mar/2020	-	08
	Lagoa da Confusão (TO)	LA 03	-10° 53' 52.26"	-49° 51' 52.24"	Fev/2020	28	23
	Dueré (TO)	DU 01	-11° 12' 10.26"	-49° 23' 18.95"	Jan/2020	-	14
	Dueré (TO)	DU 02	-11° 23' 54.26"	-49° 34' 43.08"	Jan/2020	10	-
	Formoso do Araguaia (TO)	FO 01	-11° 27' 55.83"	-49° 57' 54.99"	Jan-Mar/2020	54	15
	Formoso do Araguaia (TO)	FO 02	-11° 41' 12.39"	-49° 51' 43.70"	Jan/2020	10	9
	Formoso do Araguaia (TO)	FO 03	-11° 49' 42.57"	-49° 40' 59.32"	Jan-Mar/2020	100†	-
	Formoso do Araguaia (TO)	FO 03.1	-11° 46' 40.77"	-49° 44' 55.42"	Jan/2020	-	11
	Formoso do Araguaia (TO)	FO 03.2	-11° 47' 23.87"	-49° 41' 24.40"	Jan-Mar/2020	-	12
	Formoso do Araguaia (TO)	FO 03.3	-11° 47' 58.89"	-49° 37' 18.83"	Jan/2020	-	09
	Formoso do Araguaia (TO)	FO 03.4	-11° 51' 1.27"	-49° 40' 31.88"	Jan/2020	-	22
	Formoso do Araguaia (TO)	FO 03.5	-11° 54' 21.78"	-49° 40' 59.07"	Jan/2020	-	11
	Formoso do Araguaia (TO)	FO 04	-11° 58' 15.21"	-49° 41' 13.07"	Jan/2020	78†	71†
	Goianira (GO)	GO 01	-16° 26' 8.61"	-49° 23' 45.24"	Jan/2020	29	17
	Santo Antônio de Goiás (GO)	GO 02	-16° 29' 57.60"	-49° 17' 41.32"	May/2020	-	20
South	Itajaí (SC)	IT 01	-26° 57' 3.42"	-48° 45' 42.91"	Mar/2020	10	-
	Pelotas (RS)	PE 01	-31° 48' 54.36"	-52° 27' 52.17"	Mar/2020	20	-

†Overlapped populations sum.

Table 2. List of ISSR primers used for characterization of *Oebalus poecilus* and *O. ypsilongriseus.*

Code	Sequence (5’-3’)	Annealing temp (°C)	Size range (pb)	Number of multiple bands
CI01	$(CA)_{10}A$	56	260-4800	13
CI02	$(CA)_{10}T$	56	280-4200	13
CI03	$(CA)_{10}G$	57	210-3700	13
CI04	$(CA)_{10}C$	54	180-4000	15
CI05	$(GACA)_4$	54	230-2700	12
CI07	$(CT)_4(CA)_5$	55	260-4200	15
UBC808	$(AG)_8C$	50	300-2500	16
UBC811	$(GA)_8C$	53	300-3000	13
UBC827	$(AC)_8G$	53	430-2500	14
UBC828	$(TG)_8A$	50	500-3500	15
Total				139

Table 3. Genetic diversity of the sampled populations of *Oebalus poecilus* and *O. ypsilongriseus*.

Region	Code	*Oebalus poecilus*				*Oebalus ypsilongriseus*			
		P (%)	*N*e	*I*	H_E	*P* (%)	*N*e	*I*	H_E
North Center	LA 01	80.58	1.432	0.396	0.260	75.54	1.337	0.336	0.214
	LA 02	-	-	-	-	62.59	1.305	0.291	0.188
	LA 03	88.49	1.366	0.355	0.225	91.37	1.340	0.354	0.220
	DU 01	-	-	-	-	75.54	1.263	0.278	0.171
	DU 02	80.58	1.461	0.408	0.271	-	-	-	-
	FO 01	95.68	1.451	0.427	0.276	83.45	1.445	0.408	0.268
	FO 02	80.58	1.497	0.429	0.288	72.66	1.339	0.330	0.212
	FO 03	99.28	1.415	0.406	0.259	-	-	-	-
	FO 03.1	-	-	-	-	73.38	1.300	0.304	0,192
	FO 03.2	-	-	-	-	68.35	1.260	0.271	0,169
	FO 03.3	-	-	-	-	74.82	1.328	0.323	0,205
	FO 03.4	-	-	-	-	83.45	1.324	0.334	0,208
	FO 03.5	-	-	-	-	76.98	1.347	0.343	0,219
	FO 04	96.40	1.471	0.437	0.285	98.56	1.440	0.425	0.273
	GO 01	92.81	1.488	0.440	0.289	82.73	1.318	0.328	0.205
	GO 02	-	-	-	-	83.45	1.341	0.341	0.215
South	IT 01	66.91	1.306	0.293	0.188	-	-	-	-
	PE 01	73.38	1.302	0.303	0.192	-	-	-	-
Populations Mean ± SE		85.47 ± 3.40	1.419 ± 0.009	0.390 ± 0.006	0.253 ± 0.005	78.78 ± 2.47	1.335 ± 0.007	0.333 ± 0.005	0.211 ± 0.004
		P (%)	*N*e	*I*	H_E	*P* (%)	*N*e	*I*	H_E
Species Mean ± SE		100	1.449 ± 0.027	0.429 ± 0.017	0.276 ± 0.013	98.56	1.369 ± 0.025	0.382 ± 0.017	0.239 ± 0.013
Comparison between species Mean ± SE						99.28 ± 0.72	1.409 ± 0.018	0.406 ± 0.012	0.257 ± 0.009

†*P*, percentage of polymorphic loci; *N*e, mean effective number of alleles; *I*, mean Shannon's information index; H_E, mean expected heterozygosity.

Table 4. Summary of the molecular variance analysis (AMOVA) for the collected populations of *Oebalus poecilus* and *O. ypsilongriseus*.

Species	Source of variation	D.F.	Sum of squares (SS)	Variance components	% of variation
O. poecilus	Among populations	9	546.602	1.197	5
	Within populations	338	7650.703	22.635	95
	Total	347	8197.305	23.832	100
O. ypsilongriseus	Among populations	13	489.512	0.896	4
	Within populations	237	5335.392	22.512	96
	Total	250	5824.904	23.408	100
	Among species	1	1301.270	4.381	16
	Within species	597	14022.209	23.488	84
	Total	598	15323.479	27.869	100

Figure Captions

Figure 1. Principal coordinate analysis (PCoA) of populations of (A) *Oebalus poecilus*, (B) *O. ypsilongriseus*, and (C) pooled from both species.

Figure 2. Summary of ADMIXTURE analysis. ΔK was calculated by the Evanno's method with populations of (A) *Oebalus poecilus*, (B) *O. ypsilongriseus*, and (C) the pooled populations of both species, from K = 1 to K = 10. (D) Cluster analysis using the STRUCTURE software. Different colors represent different genetic groups. Each column represents an individual. K = 2 for populations of *Oebalus poecilus* and *O. ypsilongriseus*; K = 3 when the species were analyzed together.

Figure 3. Distribution map of the genetic groups identified with Structure for populations of (A) *Oebalus poecilus* and (B) *O. ypsilongriseus*, in central savannah-like region, and (C) *Oebalus poecilus* in the South region. Pie charts are arranged on the geographical position of each sampled population. Each pie chart indicates the proportion of each group in the populations. Codes correspond to the populations described in Table 1.

Figure 1.

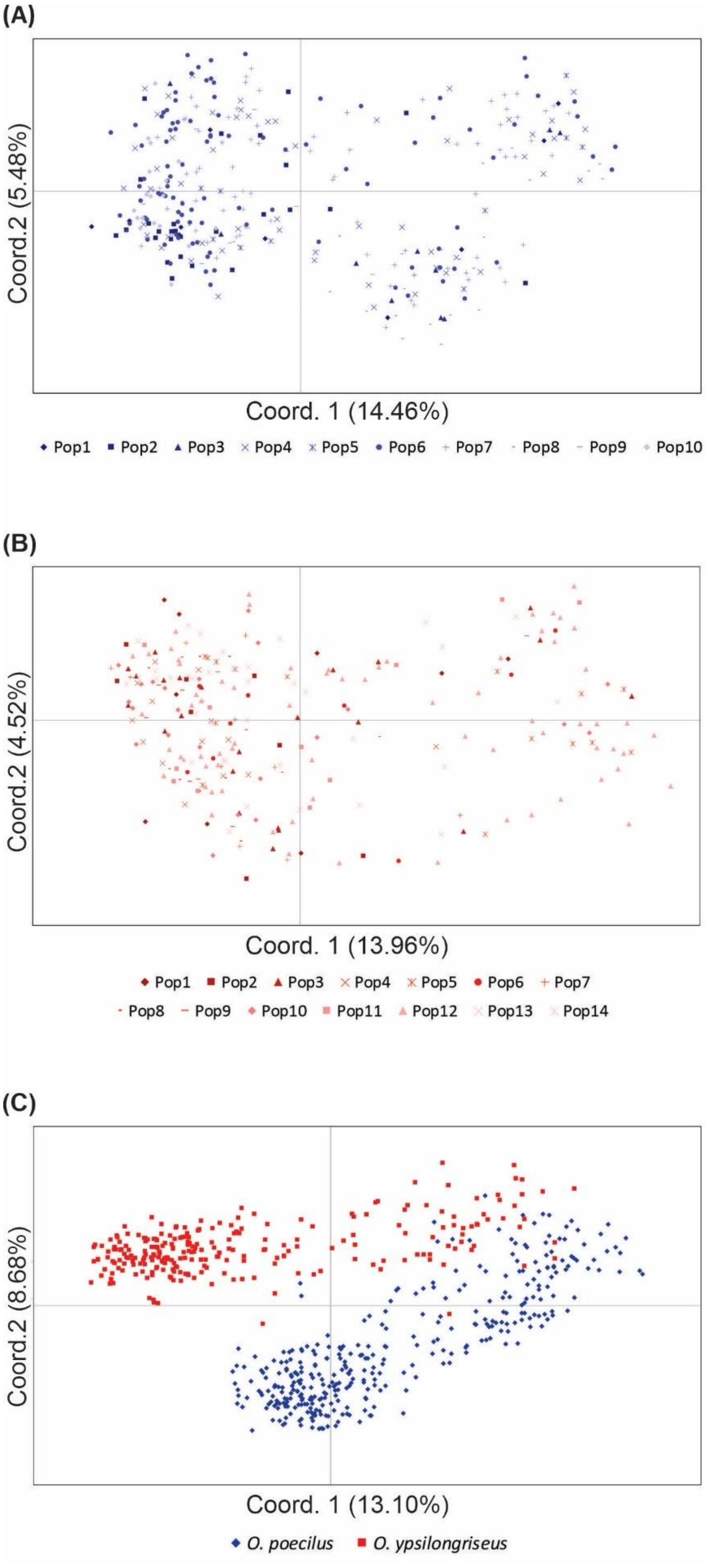

(A)
Coord.2 (5.48%)
Coord. 1 (14.46%)
Pop1
Pop2
Pop3
Pop4
Pop5
Pop6
Pop7
Pop8
Pop9
Pop10
(B)
Coord.2 (4.52%)
Coord. 1 (13.96%)
Pop1
Pop2
Pop3
Pop4
Pop5
Pop6
Pop7
Pop8
Pop9
Pop10
Pop11
Pop12
Pop13
Pop14
(C)
Coord.2 (8.68%)
Coord. 1 (13.10%)
O. poecilus
O. ypsilongriseus

Figure 2.

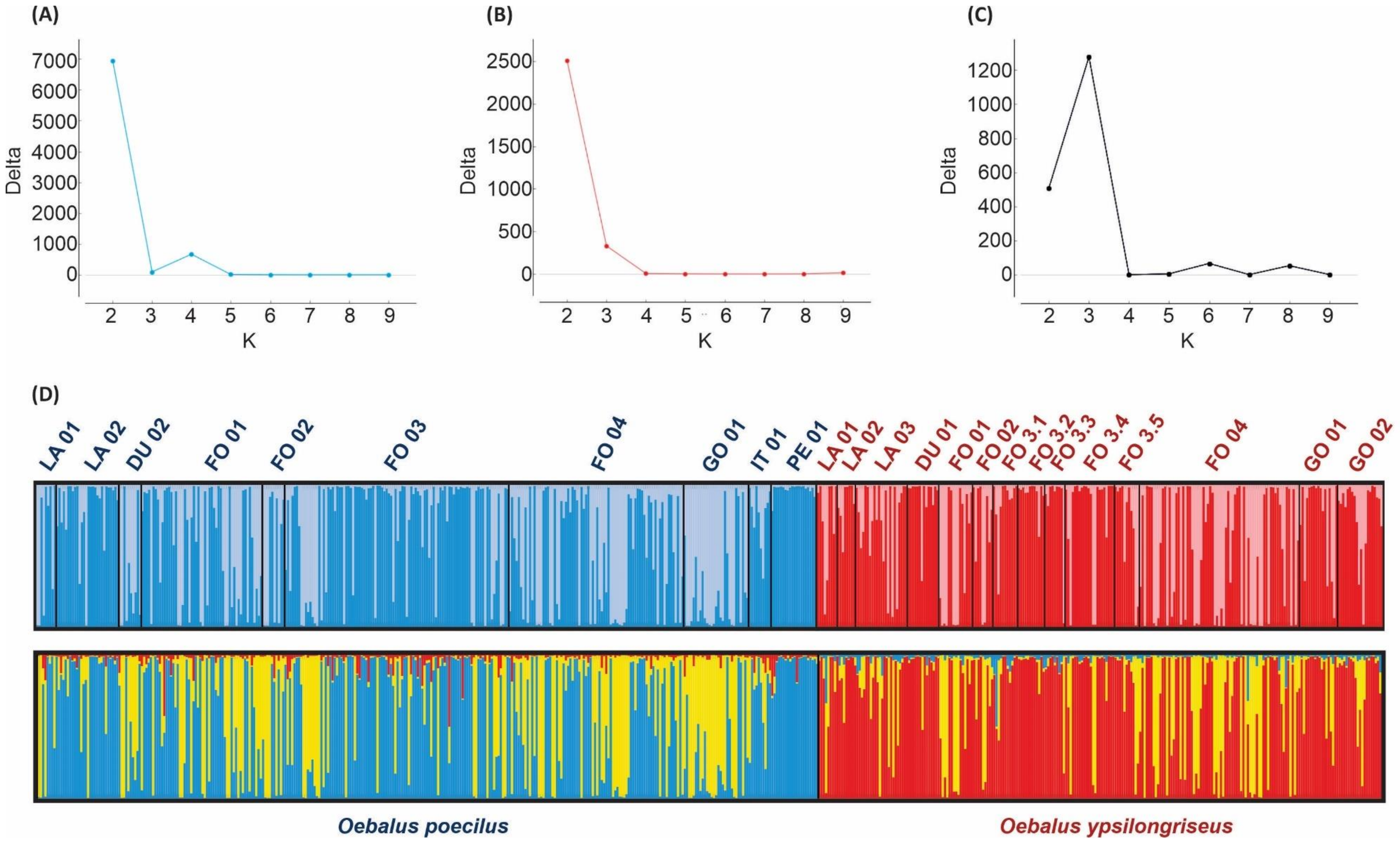
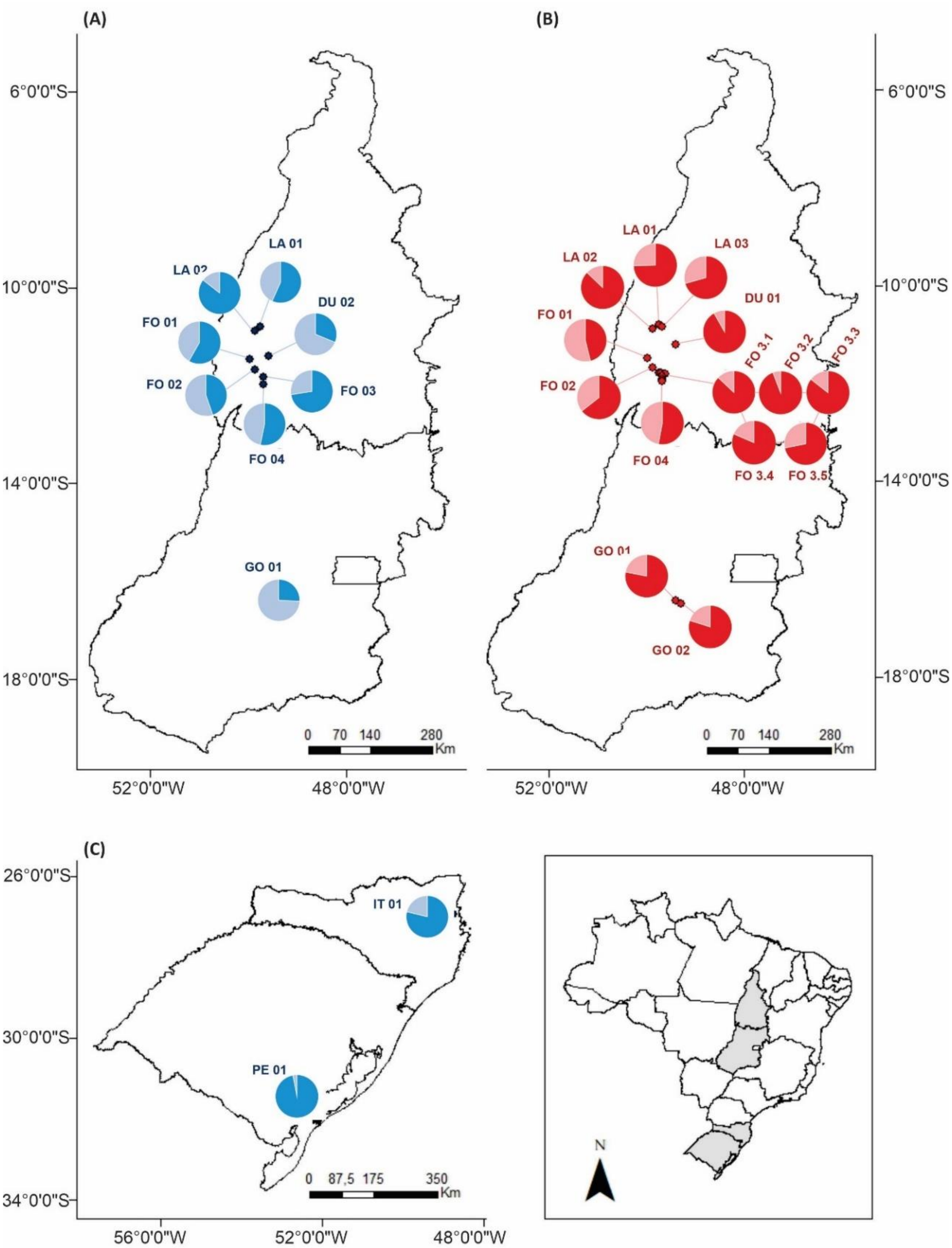

(A)
Delta
7000
6000
5000
4000
3000
2000
1000
0
2 3 4 5 6 7 8 9
K
(B)
Delta
2500
2000
1500
1000
500
0
2 3 4 5 6 7 8 9
K
(C)
Delta
1200
1000
800
600
400
200
0
2 3 4 5 6 7 8 9
K
(D)
LA 01
LA 02
DU 02
FO 01
FO 02
FO 03
FO 04
GO 01
IT 01
PE 01
LA 01
LA 02
LA 03
DU 01
FO 01
FO 02
FO 3.1
FO 3.2
FO 3.3
FO 3.4
FO 3.5
FO 04
GO 01
GO 02
Oebalus poecilus
Oebalus ypsilongriseus

Figure 3.


(A)
6°0'0"S
10°0'0"S
14°0'0"S
18°0'0"S
LA 01
LA 02
DU 02
FO 01
FO 02
FO 03
FO 04
GO 01
0 70 140 280
Km
52°0'0"W
48°0'0"W
(B)
LA 01
LA 02
LA 03
DU 01
FO 01
FO 02
FO 3.1
FO 3.2
FO 3.3
FO 3.4
FO 3.5
FO 04
GO 01
GO 02
0 70 140 280
Km
52°0'0"W
48°0'0"W
(C)
26°0'0"S
30°0'0"S
34°0'0"S
IT 01
PE 01
0 87,5 175 350
Km
56°0'0"W
52°0'0"W
48°0'0"W
N

4. To each his own? Niche specialization and potential occurrence of pest species of the rice stink bug complex

To each his own? Niche specialization and potential occurrence of pest species of the rice stink bug complex

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Abstract

The ongoing scenario of worldwide anthropogenic-mediated changes, including climate change, and the increasing influence of anthropogenic stressors, affect species distribution, dispersal and local competition, potentially leading to shifts in their dominance and prevalence. In the Neotropical region where the expansion in rice cultivation associated with the above-reported conditions, apparently favored the range expansion of species of the rice stink bugs or *Oebalus* complex. This in turn has important economic and management consequences for rice production. An important tool to unravel and predict native and invasive species distribution, particularly under changing climate conditions, is species distribution modeling. It is also a powerful tool for monitoring shifts in the distributions of closely related species, as is the case in the rice stink bug complex in the Americas. Here, we explored the most determinant and predictor variables of the rice stink bugs' occurrence and overlap, and modeled their potential distribution under the current climate scenario using ensemble projections. Limiting conditions of precipitation and temperature were most influential in niche determination of *Oebalus pugnax* in the U.S., and the sensibility to dry periods seems to be important in ecological niche specialization between the Neotropical species *O. ypsilongriseus* and *O. poecilus*. The four algorithms used to predict the distribution of the rice stink bugs exhibited high accuracy (AUC ≥ 0.95, TSS ≥ 0.88, COR ≥ 0.69), with random forest having the best fit in all projections. *Oebalus pugnax* appears to meet the conditions for establishing in South America, and aside some particularities, the three *Oebalus* species could potentially settle in the grain-producing regions in Meridional Europe, Equatorial and Central Africa, Asia, and Australia. Although distribution modeling using bioclimate variables provides valuable information on the habitat suitability and distribution of these species, future studies should determine the thermal and humidity thresholds of their development, and interspecific relationships. This will be necessary to better understand their distribution and to guide the development of suitable pest management programs.

Keywords: bioclimatic model, invasive pest species, niche shifts, species distribution modeling, species overlap.

Key message

- Occurrence shifts of rice stink bug species of the *Oebalus* complex have been observed in the Americas.
- Species distribution modeling was used to explore niche specialization and to predict habitat suitability of these species.
- Limiting conditions of precipitation and temperature were the most influential for *O. pugnax* occurrence.
- Sensibility to dry periods was critical in niche specialization between the Neotropical species *O. ypsilongriseus* and *O. poecilus*.
- Aside some particularities, these rice stink bugs possess the potential to settle in the major grain-producing regions worldwide.

Author contribution statement

JLV, TLC and RNCG conceived and designed the study. JAFB and RNCG provided the materials and tools. JLV and TLC performed the experiments and analyzed the data. JLV, GS, KM and RNCG structured and wrote the manuscript. All authors revised the material.

Introduction

It is an increasingly well-known statement that climatic and physical factors profoundly affect the distribution of native and invasive species (Musolin 2007; Biber-Freudenberger et al. 2016; Jung et al. 2016b). Native species may experience range shifts, i.e., expansion or decline, while invasive species could benefit from favorable conditions for its establishment, and subsequent spread (Hill et al. 2016b; Jung et al. 2016b; Kavallieratos et al. 2017b; Halsch et al., 2020). Regardless, the overall global costs of biological invasions, especially of pest species, are substantial, even if usually underestimated (Bradshaw et al. 2016). Curiously, whilst the direct costs derived from economic losses (e.g., crop losses) associated with biological invasions of pest species are a core concern and easily recognized, the indirect impacts of the invasion and even the range expansion of pest species remain poorly understood (Hawkins et al. 2015; McGeoch et al. 2015; Kumschick et al. 2016). The latter includes effects on species assemblages and community structure and function, which all depend on the drivers behind the establishment and dispersal processes of these species (Lockwood et al. 2013, Hill et al. 2016). The rice stink bug complex of pest species provides an important economic context to improve our understanding of the process of invasion, establishment, range expansion, and their consequences.

The rice stink bugs are oligophagous Neotropical species that feed on flowering plants belonging to the Poaceae (Ferreira et al. 2001b; Bhavanam et al. 2021). The main cultivated host is rice, and secondary crops include barley, corn, oats, rye, sorghum, and wheat (Ferreira et al. 2001b; Bhavanam et al. 2021). Besides that, weedy grasses and natural vegetation may serve as alternative hosts (Ferreira et al. 2001b; Bhavanam et al. 2021). Among the rice stink bug species, *Oebalus pugnax* (F.) (Hemiptera: Heteroptera: Pentatomidae) has a dominant distribution in the U.S., being reported in all main rice-growing regions east of the Rocky Mountains (Bhavanam et al. 2021). In contrast, *O. poecilus* (Dallas) has a Neotropical distribution, with a more significant impact on flooded rice fields in the southernmost regions of the Americas. Lastly, *O. ypsilongriseus* (DeGeer) was more recently documented as co-occuring with *O. poecilus* and was recently introduced in the United States, where its invasive potential was recognized (Cherry et al. 1998b; Vanweelden et al. 2020b). Despite the rice stink bugs' dispersion ability, reaching 255m in flight (Ferreira et al. 2001b; Barrigossi 2008b; Bhavanam et al. 2021), and their considerable economic impact on yield and grain quality (Barrigossi 2008b; Krinski and Foerster 2017b; Wilson and Stout 2020), the dominance and potential occurrence of these pests are barely investigated. Apparently, the neonicotinoid thiamethoxan does not play a fundamental role in mediating the distribution of rice stink bugs in the Neotropics (Vieira et al. 2021).

The ongoing global warming and other anthropogenic disturbances influence the niche idiosyncrasy of closely related species, but the main drivers remain unclear due to the multifaceted and ecologically complex interactions involved. Relying on the ecological niche, species distribution modeling (SDM), or also called ecological niche modeling (ENM), has been increasingly employed to recognize the distribution of native and invasive species under current and future climatic conditions, as well to identify high-risk regions (Kumar et al. 2014; Naimi and Araújo 2016; Tonnang et al. 2017; Barbet-Massin et al. 2018). Hence, SDM raises as a promising tool for monitoring species inter-relation such as in the current the scenario of rice crop expansion in the Americas, since it enables the characterization of environmental conditions suitable for the species involved and consequently the identification of suitable environments for their establishment and spread in a real spatial framework (Naimi and Araújo 2016). Correlative SDM methods (i.e., inductive) are flexible enough to incorporate many abiotic and biotic factors, and rely on the correlation of environmental variables and occurrence data. They do not require detailed knowledge of the pest's life history, but are still able to provide adequate information to allow the prediction of potentially suitable areas for the species occurrence (Kumar et al. 2014; Tonnang et al. 2017).

Reliable data on species' location and temporal distribution provide determinant information for describing their habitat specificities (or overlapping) using SDM approaches. Accordingly, response variables (i.e., predictors) used in these models should be carefully selected to avoid overfitting and artificially increasing the prediction accuracy, mainly because the bioclimatic variables are highly correlated dimensions (Peterson 2007; Dormann et al. 2013). To access the contribution of each response variable and project species potential distributions in space and time, artificial intelligence (AI), machine learning (ML), and ecological niche (EN), such as Bioclim, generalized linear models (GLM), maximum entropy (Maxent), Boosted Regression Tree (BRT), and random forest (RF) have been commonly employed (Tonnang et al. 2017; Araújo et al. 2019; Lantschner et al. 2019). However, the prediction results based on different algorithms differ significantly, making intricate the model selection. Ensemble models have been widely applied in this sense, minimizing the uncertainties and variabilities from single niche-based models and improving the precision of potential species distribution obtained from multiple models (Araújo and New 2007; Naimi and Araújo 2016; Araújo et al. 2019; Hao et al. 2019).

Realistically understanding the ecological niche of closely related pests, such as the rice stink bug complex in the Americas, is crucial for strategic decision-making and designing ecologically sound management programs. In addition, the identification of areas environmentally suitable for invasive species allows for the prevention or delay of possible invasions. Therefore, using three species of the *Oebalus* complex as a model (i.e., *O. pugnax, O. poecilus,* and *O. ypsilongriseus*), the objective of this study was: (i) to explore the most determinant predictor variables for these species' occurrence (and overlap); and (ii) to predict their habitat suitability under the current climate scenario using ensemble projections of the best fitting algorithms. Some overlap of predictors and occurrence is to be expected, but niche partitioning likely predominates, especially for *O. pugnax* as this already contrasts with that of the other two species based on their current respective distributions (Cherry et al. 1998, Ferreira et al. 2001, Santos et al. 2003).

Materials and methods

Data collection and pre-processing

Oebalus occurrence records

Geographic information on the presence of *Oebalus pugnax, O. poecilus*, and *O. ypsilongriseus* were obtained from online databases of species occurrence records and searching on published literature. The occurrence databases accessed were the Global Biodiversity Information Facility (GBIF; https://www.gbif.org), the Centre for Agriculture and Bioscience International (CABI, https://www.cabi.org), *Species*Link (https://specieslink.net), and the Illinois Museum of Zoology Collection (http://www.illinoisstatemuseum.org). The occurrence records present in published literature were obtained by searching with the keywords *Oebalus*, *Oebalus pugnax*, *Oebalus ypsilongriseus*, *Oebalus poecilus*, and rice stink-bugs, in Web of Science, Science Direct, Google, and PubMed. The last access to both platforms (i.e., species report and scientific publication) was in January 2022. Only information with verified taxonomic identification from studies published in peer-reviewed journals and from official institutions and databases were included in this study (Supplementary Table S1). Additionally, *O. ypsilongriseus* and *O poecilus* occurrence data were gathered in Brazil through field expeditions in collaboration with the Rice and Beans Research Center of the Brazilian Agricultural Research Corporation (Embrapa Arroz e Feijão) (Supplementary Table S1).

The spatial reference was set to a Universal Transverse Mercator (UTM) projection, and the GPS coordinates were determined to a global datum (WGS 84) and presented in decimal degrees. A total of 2713 georeferenced sites were recorded for *O. pugnax*, 349 for *O. ypsilongriseus*, and 229 for *O. poecilus*. To account for spatial bias and avoid model overfitting, the records of each species were filtered using the R package *spThin* (Boria et al. 2014). Duplicated records and those which were found within 10 km from each other were iteratively removed in this process. Following filtering, 833 records were obtained for *O. pugnax*, 121 for *O. ypsilongriseus*, and 71 for *O. poecilus,* which were used for modeling the habitat suitability (Supplementary

Table S1). To further eliminate potential errors, the latitude and longitude of each occurrence record were checked using the Google Earth platform (https://www.google.com/earth).

Predictor variables

A set of 19 bioclimatic variables (Table 1) obtained from WorldClim database version 2.1 (Fick and Hijmans 2017) was used to predict the potential distribution of *Oebalus* species. These bioclimatic variables comprise satellite raster grids of average monthly climate data for minimum, mean, and maximum temperature and precipitation. The variables describe a long-term mean of the current conditions (1970 to 2000), for instance, representing annual trends of temperature and precipitation, seasonality (e.g., yearly range), and extreme or limiting factors (e.g., conditions as the coldest and warmest month, and the wet and dry periods). A topographic variable represented by elevation data was also obtained from the WorldClim database (Table 1). A total of twenty variables were set at a spatial resolution of 5 arc-min for analysis, corresponding to approximately 9 km. The multicollinearity among these variables was scrutinized and eliminated in a stepwise model selection procedure described below.

Predictor variables selection

In species distribution modeling (SDM), the prediction can be compromised by volatility and instability in the model parameterization resulting from the collinearity between predictor variables (Dormann et al. 2013; Naimi and Araújo 2016). Accordingly, the Pearson correlation coefficient and the variance inflation factor (VIF) were calculated for each species to detect the multicollinearity effect. Variables with a correlation coefficient $|r| \geq 0.7$ were excluded from the model. The *vifstep* function of the *usdm* package in R software (Naimi and Araújo 2016) was used to perform the regressions and compute the VIF for the set of variables in each species and subsequently remove those with VIF > 10 through a stepwise process. The VIF index is relevant because it reflects the standard error inflation caused by the multicollinearity of the variables included in the model (Dormann et al. 2013). As a result, the correlated variables were expunged, retaining distinct sets of eleven uncorrelated variables for each species (Table 1). More straightforward and less dimensional environmental data sets improve model prediction, and its understanding (Peterson and Nakazawa 2008; Rödder and

Lötters 2009; Peterson 2011). Therefore, according to their relative importance, more variables were removed from the uncorrelated data sets in stepwise procedures until the relevant orthogonal features most suited for the model prediction in each *Oebalus* species were established. The assessments resulted in the selection of four relevant and uncorrelated variables for each species used in the final modeling (Table 1).

Species distribution models fitting

Each *Oebalus* species record was related to the respective set of selected predictor variables using the species distribution modeling (*sdm*) package in R software (Naimi and Araújo 2016). The *sdm* package has a selection of over twenty SDM methods combining different parallel executions and machine learning in an object-oriented, reproducible, and extensible approach framework (Naimi and Araújo 2016). Only the presence records were used for this study because it was unfeasible to determine the actual absence data from the field, and the lack of sighting is not a guarantee of absence (Jiménez-Valverde et al. 2011; Yackulic et al. 2013; Valavi et al. 2022). Additionally, the *sdm* package can generate and use pseudoabsence observation data to compensate for the lack of actual absence data and enable the use of presence-background methods (Naimi and Araújo 2016). Thus, the stink bugs were assumed to have the same probability of occurrence across the landscape; hence, every pixel had the same likelihood of being tagged as background pseudoabsence across the environmental and geographic space (Naimi and Araújo 2016). The *sdmdata* function was applied, generating 1000 pseudoabsence records that were used against the presence-only records of each species to calibrate the model.

Some of the most commonly used algorithms in species distribution were submitted to trial runs including bioclim, boosted regression trees (BRT), flexible discriminant analysis (FDA), generalized linear model (GLM), Maxent, random forests (RF), and support vector machine (SVM). Based on the area under the curve (AUC), true skill statistic (TSS), correlation index and deviance, BRT, Maxent, RF, and SVM were the algorithms chosen for predicting the habitat suitability of *Oebalus* species (Table 2). In BRT, consecutive trees from a random sub-sample of observations are built. For each successive tree, the number of times a variable is selected at each node is weighted by the squared improvement to the model obtained by each split on the dataset (Friedman 2002; Friedman and Meulman 2003; Elith et al. 2008). Maxent is one of the most widely used modeling tools to predict the distribution and ecological niche (Merow et al. 2013; Guisan et al. 2017). It is based on the principle of maximum entropy, which states the probability distribution that best represents the data is the one with the greatest entropy, i.e., the one which best reproduces the data (Phillips et al. 2006; Guisan et al. 2017). The RF, also widely applied (Guisan et al. 2017), is a supervised machine learning algorithm that consists of many decision trees in a forest that is used to predict the species distribution based on the class that generates the majority of votes (Breiman 2001; Guisan et al. 2017). Lastly, SVM is a supervised learning model that classify the study extent with regard to the species most likely to be present in each grid cell when trained on the coordinates of occurrence localities (Guo et al. 2005; Guisan et al. 2017).

The *sdm* function of the *sdm* package was run for the model calibration of all algorithms selected in each *Oebalus* species. The packages used by *sdm* in the parallel execution for the selected models and their corresponding syntax codes are presented in Table 2. Thereafter, the *predict* function was applied to forecast the habitat suitability under the current climate scenario resulting in an output for each species model separately (i.e., BRT, Maxent, RF, and SVM). Ensemble projections were employed to estimate the mean predictions of each *Oebalus* species distribution over the four selected algorithms. The *ensemble* function of the *sdm* package is an interesting tool that combines the output and uncertainty of the different models in one layer using the weighted or unweighted average of each pixel (Araújo and New 2007; Naimi and Araújo 2016; Araújo et al. 2019; Hao et al. 2019). Additionally, other aspects as the relative importance of each variable, which may vary across models, were also averaged. Hence, the ensemble modeling approach fits and maximizes model accuracy resulting in improved reliability and superior predictive performance (Araújo and New 2007; Naimi and Araújo 2016; Araújo et al. 2019; Hao et al. 2019). This approach is especially powerful in this situation, as the accuracy of the algorithms could differ among species, and strictly selecting a single model make it more difficult to make comparisons. The results of individual models in each species were harmonized under the ensemble model using the true skill statistics (TSS) weighted average approach. The habitat suitability ensembled under the current climate scenario were represented for the American continent and globally in each *Oebalus* species.

Validation of the models

The random data splitting method of *sdm* package was run to assess the accuracy of the *Oebalus* species distribution models, allowing for evaluating the contributions of variables in each of them. Model accuracy and validation were estimated using a ten-fold cross-validation approach (Naimi and Araújo 2016; Araújo et al. 2019). Additionally, automation was used to independently and randomly draw a 70% sample for calibration of the models, while the remaining 30% were used to validate their performances. The bootstrap minimizes the selection bias and overfitting in the model because it tests each data point only once, and then this point is used in the training set $k - 1$ times.

The performance of the models was evaluated using threshold-dependent statistics, such as true skills statistics (TSS), sensitivity, and specificity, as well as threshold-independent statistics represented by the area under the curve (AUC) of the receiver operating curves (ROC) (Marzban 2004; Phillips and Dudík 2008; Guisan et al. 2017). Sensitivity (rate of correctly predicted presence) and specificity (rate of correctly predicted absence) of the model's fitting are graphically represented by the ROC, and the area under this plot (AUC) indicates the overall accuracy of model performance. AUC values vary from 0 and 1, with 1 indicating perfect prediction by the model or optimal occurrence. TSS values close to +1 denotate optimal modeling or flawless ordering of observed presence versus its predicted occurrence, whilst values lower than zero ($TSS \leq 0$) represent poor or impossible occurrences (Marzban 2004). The parameters of the TSS variables are described in equations 1 to 3. Models with AUC and TSS values of ≥ 0.8 imply high performance and accurate prediction (Guisan et al. 2017):

(1) $TSS = (sensitivity + specificity) - 1$

where sensitivity represents the true presence rate and specificity is the true absence rate.

(2) $Sensitivity = a/(a+b)$

where a is the number of true presence and b is the number of records where the species was present, but the model predicts as absence.

(3) Specifity =d/(c+d)

where d is the number of true absence and b is the number of cells where species was absent, but the model predicts as presence.

Results

Variable selection and model development

Among the twenty variables obtained from Worldclim for each *Oebalus* species, ten variables per species were identified as the most independent, i.e., exhibiting the lowest VIF values (Table 1). These variables were not correlated among themselves nor to other variables (|r| ≤ 0.70) (Figure 1). Based on their importance and individual interactions (Figures 1 and 2a), four non-conflating variables were selected in a stepwise elimination procedure for each species model development (Figure 2b). Only isothermality (Bio3) and precipitation of the warmest quarter (Bio18) were selected among the three species (Figure 2b). Precipitation of the driest month (Bio14) was considered for *O. pugnax* and *O. ypsilongriseus*, annual precipitation (Bio12) for *O. ypsilongriseus* and *O. poecilus*, mean temperature of warmest quarter (Bio10) and elevation (ELEV) were selected only for *O. pugnax* and *O. poecilus*, respectively (Figure 2b). The main variables for the potential distribution of *O. pugnax* were Bio14 and Bio10, with 30.4% and 29.8% of contributions each (Figure 2b). Bio12 and Bio3 exhibited the highest importance for both *O. ypsilongriseus* (41.4% and 35.7%) and *O. poecilus* (40.5% and 33.3%) (Figure 2b).

Species distribution models and validation

ROC demonstrated a good fit between training and test datasets for the three *Oebalus* species with few variations between individual 10-fold bootstrap runs (Figure 3). BRT, Maxent, and RF consistently fit the indicated model stably, while also having relatively consistent predictions within replicates (Figure 3abc). On the other hand, SVM had the largest deviations of AUC for individual replicates, mainly in *O. ypsilongriseus* and *O. poecilus*, suggesting relative model instability (Figure 3d).

In fact, all models well predicted the occurrence of *Oebalus* species with high accuracy (Table 3; Figure 3). For instance, *O. pugnax* presented AUC ≥ 0.98, TSS ≥ 0.89, COR ≥ 0.90, and deviance between 0.20 and 0.63, *O. ypsilongriseus* had AUC ≥ 0.96, TSS ≥ 0.90, COR ≥ 0.75, and deviance between 0.16 and 0.33, and *O. poecilus*

AUC ≥ 0.95, TSS ≥ 0.88, COR ≥ 0.69, and deviance between 0.13 and 0.23 (Table 3; Figure 3). The RF algorithm had the highest accuracy for all three species, with test AUC values of 0.99, TSS ≥ 0.93, COR of ≥ 0.83, and deviance between 0.13 and 0.20 (Table 3; Figure 3). The lowest accuracy among the species varied, but in some cases was produced using BRT or SVM methods, depending on the species (Table 3; Figure 3). Additionally, the differences between attained AUC and TSS model accuracies ranged between 4% (0.04) for the random forest in *O. pugnax* and 9% (0.09) for BRT and Maxent in *O. pugnax* and *O. poecilus*, respectively (Table 3). A higher difference indicates disparities in threshold-dependent and -independent evaluation criteria.

Ensemble projection under the current climatic scenario

The ensemble projection of the four models (BRT, Maxent, RF, and SVM) accurately predicted the occurrence of *O. pugnax* (AUC = 0.940, COR = 0.932, deviance = 0.375, $P < 0.001$), *O. ypsilongriseus* (AUC = 0.993, COR = 0.868, deviance = 0.215, $P < 0.001$), and *O. poecilus* (AUC = 0.989, COR = 0.805, deviance = 0.172, $P < 0.001$) (Figures 4 and 5).

Under the current climatic scenario, where *O. pugnax* is widespread across the eastern region of the Rocky Mountains in the U.S. (Figure 4), this species is likely able to occur also in the Midwest, Southeast and South of Brazil, and in some regions of Bolivia, Paraguay, Argentina, and Uruguay (Figures 4 and 5). From a global perspective, this species is predicted to occur in some areas of Meridional Europe, in small regions at the east and west African coasts, and in areas of Kashmir in India, southeast China, Vietnam, Japan, and east Australia (Figure 5).

Oebalus ypsilongriseus, considered an invasive species in the US, has already a widespread occurrence on the American continent (Figure 4). It has a predicted niche distribution corresponding to an expansion including the Orinoco River plain in Colombia and Venezuela, some regions of Mexico, and some countries of Central America, like Cuba, Dominican Republic, Jamaica, and Puerto Rico (Figures 4 and 5). Also, it is able to occur along the Chilean coast (Figures 4 and 5). In general, *O. ypsilongriseus* showed the predicted ability to occur globally across equatorial climate zones (Figure 5). In Africa, these zones include Guinea, Sierra Leone, Liberia, Ivory Coast, Ghana, Nigeria, Cameroon, Equatorial Guinea, and Congo, spreading to tropical central regions such as the Central African Republic, Democratic Republic of

Congo, Southern Sudan, Sudan and Ethiopia (Figure 5). Besides, some suitable regions were identified in Tanzania, South Africa, and Madagascar (Figure 5). In Europe, the predicted distribution of *O. ypsilongriseus* was low and negligible (Figure 5). In Asia and Oceania, it is able to occur in the Kerala region of south India, Sri Lanka, Myanmar, Thailand, Malaysia, Indonesia, Philippines, Papua New Guinea, and in some pointed areas in Vietnam, Cambodia, Vanuatu, Fiji Islands, New Zealand, Tasmania and in the Australian east coast (Figure 5).

Considered a Brazilian native species, *O. poecilus* is reported in tropical zones in South and Central America (Figure 4), and is able to occur in some southern regions of the U. S., including the states of Louisiana, Mississippi, Alabama, Georgia, and Florida (Figures 4 and 5). Based on the predictions, it should be able to expand to some regions of Mexico, Central America (including Cuba, Jamaica, and Panamá), and in South America in some small portion of the Chilean coast (Figure 4). Similar to *O. ypsilongriseus*, *O. poecilus* is likely to expand in equatorial regions across the globe, however, on a minor scale (Figure 5). The suitable African areas include some regions in Guinea Bissau, Guinea, Sierra Leone, Liberia, Ivory Coast, Togo, Benin, Nigeria, Cameroon, Central African Republic, Tanzania, Mozambique, South Africa, and Madagascar (Figure 5). In Asia and Oceania, minor coastal regions of India, Sri Lanka, Bangladesh, Myanmar, Thailand, Cambodia, Laos, Vietnam, Malaysia, Indonesia, Philippines, Vanuatu, New Zealand, and the north and east coasts of Australia (Figure 5).

Discussion

SDMs have been successfully used to predict species' potential occurrence and dispersion in response to environmental changes (Tonnang et al. 2017; Lantschner et al. 2019). These models are based on the assumption that predictor variables (i.e., bioclimatic variables) are profuse delimitating the ecosystem and consequently in the pest species distribution (Jung et al. 2016), and become particularly more and more important under the current context of climate change and agriculture expansion. Performing SDM is even more interesting when it comes to a species complex that is capable of infesting a range of host plants of economic importance, in addition to contrasting cultivation systems, such as in the flooded and upland rice (Barrigossi 2008; Bhavanam et al. 2021). Inspired by this, the aim here was to investigate the ecological niche overlap of the three main species of the rice stink bug complex and predict their global habitat suitability under the current climate conditions. We expected some overlap of predictors and occurrence, but the main niche partitioning should be for *O. pugnax* as it already contrasts with the other two species based on their current respective distributions (Cherry et al. 1998, Ferreira et al. 2001, Santos et al. 2003). The first hypothesis was confirmed, and as for the second, *O. pugnax* exhibited greater potential for range expansion, not only in the Americas, but also worldwide, and this despite its apparently better adaptation to milder climate conditions.

Environmental predictors were selected by taking into account the climate and topography which could both influence species distribution. Climatic factors, mainly temperature and precipitation, are cornerstones that shape the development, reproduction, dispersion, and abundance of insects (Musolin 2007; Jung et al. 2016). In this sense, bioclimatic variables could represent descriptive annual trends (i.e., conditioning predictors) or restricting conditions (i.e., limiting predictors), which may bottleneck many taxa occurrences. Additionally, because most insects have diverse altitudinal regimes, topography can also have an impact on the presence of available food resources and local climatic conditions (Ackerly et al. 2010; Jackson et al. 2018), which in turn could hinder or encourage shifts in niche specialization of *Oebalus* species. Here, the overlap between correlated predictor variables was excluded, leaving non-redundant features which were selected in a stepwise elimination procedure, increasing the fit parsimony (Dormann et al. 2013; Naimi and Araújo 2016). Isothermality and precipitation during the warmest quarter were the only predictors selected for the three species. The former is an annual trend predictor, that sets the thermal range and thus could limit a species occurrence, while the second is a more, rapidly changing environmental variable which may be a major limiting condition for the occurrence of *Oebalus* species (Fick and Hijmans 2017).

The two main predictors for *O. pugnax* were precipitation during the driest month and the mean temperature during the warmest quarter, characterizing the limiting conditions of precipitation and temperature, respectively. *Oebalus pugnax* is currently reported in the east of the Rocky Mountains in the U.S., unlike the other *Oebalus* species that are reported only in tropical areas, and thus are apparently better adapted to milder temperature and drier conditions. In North America, the driest month occurs during the winter, coinciding with the diapause of the rice stink bugs (Ferreira et al.

2001; Santos et al. 2003; Bhavanam et al. 2021), and has also been observed for the soybean stink bug, *Euschistus heros* (Panizzi and Vivan 1997; Panizzi and Oliveira 1998). So, avoiding the impact of the driest conditions during their latency stage, could potentially explain the habitat suitability, but this requires further investigation. Moreover, the mean temperature of the warmest quarter seems to be a development threshold of a species better adapted to areas of marked seasonality. On the other hand, *O. ypsilongriseus* and *O. poecilus* were more susceptible to annual precipitation and isothermality, as their first and second selected main variables. These annual trends predictors corroborate with their co-existence in areas of low thermal variability, such as the Neotropical regions where these species occur in South America (Ferreira et al. 2001).

The distribution and dominance of *O. ypsilongriseus* and *O. poecilus* in rice fields have been a target of interest in the studies of these pest insect species (Ferreira et al. 2001). The neonicotinoid thiamethoxan does not seem to mediate the occurrence and prevalence of rice stink bug species (Vieira et al. 2021), and these two species exhibit similar genetic structure (Vieira et al. 2022). Here, however, precipitation during the driest month, which was the main predictor for *O. pugnax*, was the third in importance for *O. ypsilongriseus* and not selected for *O. poecilus.* In Brazil, where these last two species coexist, *O. ypsilongriseus* is more frequently reported in upland rice in the Midwest region and *O. poecilus* in irrigated rice in the South (Ferreira et al. 2001; Barrigossi 2008). In the Midwest (Köppen-Geiger classification Aw), the annual temperature varies little and the two marked seasons are based on the amount of precipitation, unlike the South region (Köppen-Geiger classification Cfa), which has seasons defined by temperature and almost constant annual precipitation (https://pt.climate-data.org/). These results raise suspicions about the importance of some bioclimatic factors, such as the susceptibility to limited precipitation during dry periods, which could define the ecological niche specialization between the Neotropical species of rice stink bugs. In addition, the recent invasion of *O. ypsilongriseus* in the U.S. (Cherry et al. 1998; Vanweelden et al. 2020), seems to indicate that this invasive species has an intermediary position in the *Oebalus* complex distribution with a higher niche plasticity.

Using the respective set of selected predictors, each species model produced accurate ordering of sensitivity and specificity with low variation among test and training mean curves. Model instability is deprecated as it indicates a decrease in the dependability for niche prediction of species, especially where high precision and accuracy are desirable (Marzban 2004; Felton et al. 2021). Only SVM models produced test curves with notable variation. However, prediction skewness was not significant since the model attained acceptable ordering of sensitivity and specificity, far from the one-to-one mid-point interaction line on the ROC models (Kajita et al. 2017; Gao and Tian 2021). Besides that, all the models exhibited high performance in simulating the occurrence of the species, considering that the AUC and TSS values obtained were higher than the desirable value of ≥ 0.8. Notably, the algorithm RF exhibited the best fit amongst the four predictive models in each *Oebalus* species, confirming its robustness in predicting the distribution and habitat suitability of many species (Kampichler et al. 2010).

Expected variation between models is explained by different algorithms using different mathematical equations and approaches for prediction (Naimi and Araújo 2016). Consequently, different results are produced. Here, the differences observed were consistent among the species and with previous studies that have used multiple SDMs to predict habitat suitability (Hao et al. 2019; Azrag et al. 2022; Makori et al. 2022). With the aim of providing a more robust prediction and reducing uncertainty, various models and their set of averages can be ensembled to run the prediction. Indeed, the ensemble projection provided higher predictive power and accuracy than one model when using the weighted average of each pixel in each model to predict the habitat suitability of the species (Araújo and New 2007; Naimi and Araújo 2016; Hao et al. 2019).

All regions where the three species are currently reported were identified in the ensemble models as areas with a high probability of incidence, corroborating the accuracy of the projections. The habitat suitability of *O. pugnax* predicted here*,* together with its current co-occurrence with *O. ypsilongriseus* in Florida (Bhavanam et al. 2021) suggests that *O. pugnax* might meet the conditions for its establishment in South America, particularly in Uruguay, Argentina, Paraguay, and in Brazilian South and Midwest regions. Although it is not known how the three species could coexist (e.g., competing for resources), their establishment in these regions could potentially impact rice and wheat production. Beyond that, *O. pugnax* ensembled prediction suggests it could settle in grain-producing regions in Meridional Europe, in areas of rice production in Asia, mainly the China Southeast, and in the Australian East, where wheat, sorghum, and rice are produced (FAO, 2020). On the other hand, *O. ypsilongriseus* could potentially affect equatorial regions, where rice is predominantly important (FAO, 2020), such as Equatorial and Central Africa and South-Eastern Asia. And finally, *O. poecilus* seems to have low niche plasticity, which the only focus of concern from a global point of view would be its introduction in the U.S. or areas of Equatorial Africa and Asia.

Other critical factors that also determine the distribution of insect species, such as the presence of host plants (primary and secondary) as well as the occurrence of natural enemies, and both their abundances, were not included in our models. Nor the interspecific relationships arising from these species' coexistence, like the competition for resources or even potential hybridization, were explored, as they remain unclear (Ferreira et al. 2001; Bhavanam et al. 2021; Vieira et al. 2022). Nonetheless, modeling species distribution with bioclimate variables provides valuable information on the habitat suitability and distribution of the species, guiding decision-making and development of management strategies (Estay et al. 2009; Biber-Freudenberger et al. 2016; Tonnang et al. 2017; Trębicki et al. 2017). The range of hosts, cultivated and non-cultivated crops, suggests that over time, those species have stochastically adapted to shifting vegetation density and diversity (Ferreira et al. 2001; Bhavanam et al. 2021). This adaptation could inform the dispersion and distribution of suitable niches across different agroecological and agro-climatic zones, with subtle differences within given zones. Future studies should determine the thermal and humidity development thresholds and the interspecific relationship of these *Oebalus* species in order to better understand its ecology and distribution.

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Compliance with ethical standards

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Table 1. Bioclimatic and elevation variables obtained from the Worldclim database and their computed variance inflation factor (VIF). The values in bold correspond to the uncorrelated variables used in variables selection for the *Oelabus'* distribution models.

			VIF		
Variable	**Description**	**Unit**	**Opug**	**Oy**	**Op**
BIO1	Annual mean temperature	°C	4.17e+03	4.37e+03	4.09e+03
BIO2	Mean diurnal range (mean of monthly (max temp – min temp))	°C	**2.08e+01**	**2.04e+01**	**2.08e+01**
BIO3	Isothermality (bio2/bio7) (×100)	°C	**1.06e+01**	**9.76e+00**	**9.23e+00**
BIO4	Temperature seasonality (standard deviation ×100)	°C	1.61e+03	1.63e+03	1.57e+03
BIO5	Maximum temperature of warmest month	°C	4.50e+14	4.50e+14	4.50e+14
BIO6	Minimum temperature of coldest month	°C	6.43e+14	6.44e+14	6.43e+14
BIO7	Temperature annual range (bio5-bio6)	°C	1.43e+14	1.43e+14	1.43e+14
BIO8	Mean temperature of wettest quarter	°C	**5.65e+01**	**5.78e+01**	**5.97e+01**
BIO9	Mean temperature of driest quarter	°C	**2.91e+01**	**2.98e+01**	**2.94e+01**
BIO10	Mean temperature of warmest quarter	°C	**1.04e+04**	1.12e+04	**1.05e+04**
BIO11	Mean temperature of coldest quarter	°C	8.90e+03	8.86e+03	8.48e+03
BIO12	Annual precipitation	mm	8.96e+01	**9.98e+01**	**9.30e+01**
BIO13	Precipitation of wettest month	mm	**1.27e+02**	1.21e+02	8.87e+01
BIO14	Precipitation of driest month	mm	**9.09e+01**	**9.05e+01**	**1.06e+02**
BIO15	Precipitation seasonality (coefficient of variation)	mm	3.23e+00	**3.04e+00**	2.91e+00
BIO16	Precipitation of wettest quarter	mm	1.95e+02	2.05e+02	1.71e+02
BIO17	Precipitation of driest quarter	mm	1.27e+02	1.27e+02	1.43e+02
BIO18	Precipitation of warmest quarter	mm	**8.78e+00**	**7.29e+00**	**7.67e+00**
BIO19	Precipitation of coldest quarter	mm	**5.29e+00**	**4.92e+00**	**4.45e+00**
ELEV	Elevation derived from the srtm data	km	**4.43e+00**	**4.43e+00**	**4.52e+00**

Table 2. Ecological niche modeling methods selected and their corresponding syntax R codes used to predict the habitat suitability of rice stink bugs *Oebalus*.

Modeling method	**R Package used**	**Syntax code in 'sdm' package**	**Reference**
Boosted regression trees	Gbm	BRT	Elith et al. 2008
Maximum entropy	Dismo	Maxent	Phillips et al. 2006
Random forest	randomForest	RF	Breiman 2001
Support vector machine	e1071	SVM	Guo et al. 2005

Table 3. The accuracy assessment of the four selected methods to predict the habitat suitability of each *Oebalus* species using different evaluation metrics - area under the curve (AUC), correlation (COR), true skill statistic (TSS), and the deviance.

Species	**Methods**	**AUC**	**COR**	**TSS**	**Deviance**
O. pugnax	BRT	0.98	0.90	0.89	0.63
	Maxent	0.98	0.90	0.89	0.54
	RF	0.99	0.95	0.95	0.20
	SVM	0.98	0.92	0.91	0.29
O. ypisilongriseus	BRT	0.98	0.77	0.90	0.33
	Maxent	0.97	0.75	0.91	0.25
	RF	0.99	0.87	0.94	0.16
	SVM	0.96	0.83	0.91	0.23
O. poecilus	BRT	0.98	0.74	0.89	0.23
	Maxent	0.98	0.69	0.91	0.21
	RF	0.99	0.83	0.93	0.13
	SVM	0.95	0.69	0.88	0.23

Figure Captions

Fig. 1. Pearson correlation matrix representing collinearity among the predictor variables used in the habitat suitability models of the *Oebalus* rice stink bugs. Non-significant correlations ($\alpha = 0.05$) are left blank, while blue color indicates positive and red color indicates negative correlation. Color intensity of ellipses indicates the correlation extent and its slope represents the direction of the correlation among variables with those facing right denoting positive correlation.

Fig. 2. Relative importance of uncorrelated variables (a), and selected variables (b) used for predicting habitat suitability of the rice stink bugs *Oebalus* (mean ± 95% CI).

Fig. 3. Receiver operating curves (ROC) of the algorithms BRT (a), Maxent (b), RF (c) and SVM (d), used to predict the habitat suitability of *Oebalus* rice stink bugs. The red and the dark blue curves represent smoothed mean area under the curve (AUC) for the training and testing data, respectively, while the light blue curves represent the ten-fold replication of the training data set for each model. The black dotted lines display the one-to-one mid-point interaction between sensitivity and specificity for each model.

Fig. 4. America's habitat suitability for *Oebalus* rice stink bug species under current climate conditions predicted using the ensemble modeling. Green, yellow, and red colors indicate low, moderate, and high suitability, respectively. The dots represent the current distribution of the three *Oebalus* species.

Fig. 5. Global habitat suitability for *Oebalus* rice stink bug species occurrence under current climate conditions predicted using the ensemble modeling. Green, yellow, and red colors indicate low, moderate, and high suitability, respectively.

Fig. 1.

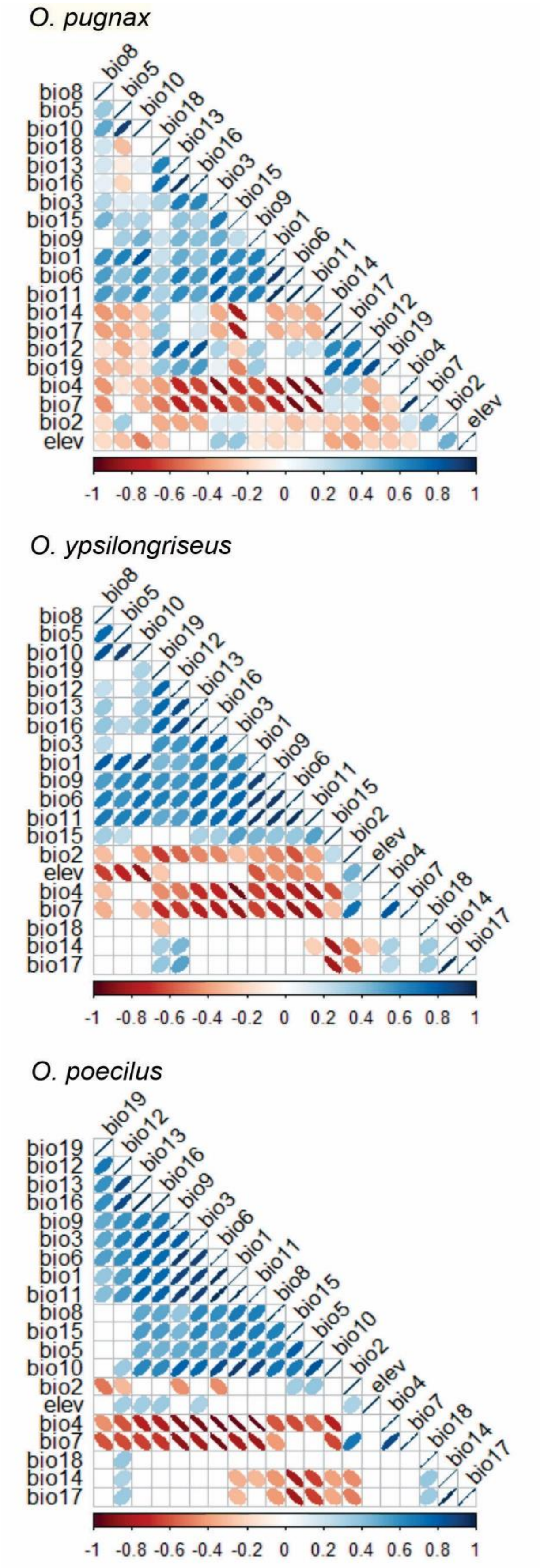

O. pugnax
bio8
bio5
bio10
bio18
bio13
bio16
bio3
bio15
bio9
bio1
bio6
bio11
bio14
bio17
bio12
bio19
bio4
bio7
bio2
elev
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
O. ypsilongriseus
bio8
bio5
bio10
bio19
bio12
bio13
bio16
bio3
bio1
bio9
bio6
bio11
bio15
bio2
elev
bio4
bio7
bio18
bio14
bio17
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
O. poecilus
bio19
bio12
bio13
bio16
bio9
bio3
bio6
bio1
bio11
bio8
bio15
bio5
bio10
bio2
elev
bio4
bio7
bio18
bio14
bio17
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1

Fig.2.

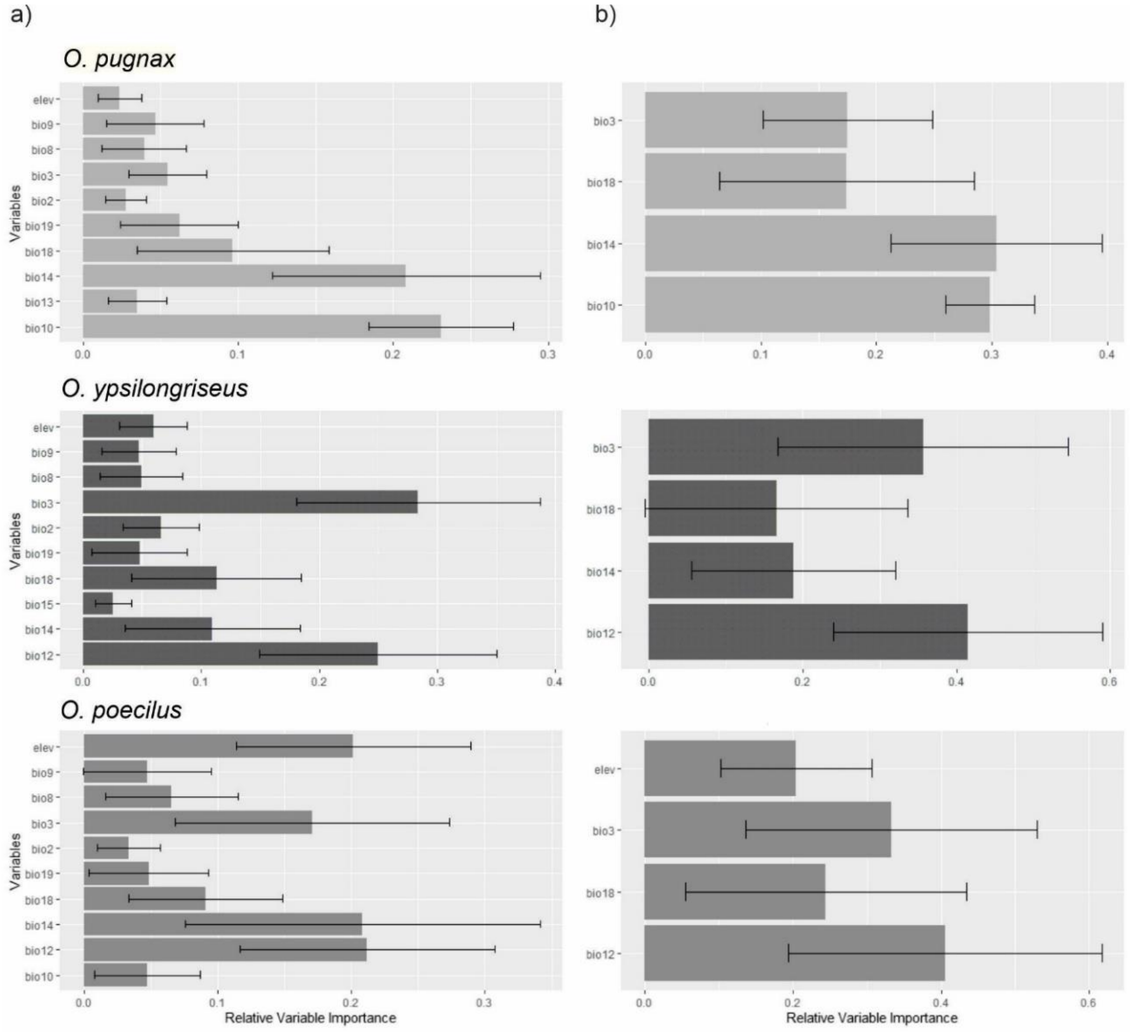

a)
b)
O. pugnax
elev
bio9
bio8
bio3
bio2
bio19
bio18
bio14
bio13
bio10
Variables
0.0
0.1
0.2
0.3
bio3
bio18
bio14
bio10
0.0
0.1
0.2
0.3
0.4
O. ypsilongriseus
elev
bio9
bio8
bio3
bio2
bio19
bio18
bio15
bio14
bio12
Variables
0.0
0.1
0.2
0.3
0.4
bio3
bio18
bio14
bio12
0.0
0.2
0.4
0.6
O. poecilus
elev
bio9
bio8
bio3
bio2
bio19
bio18
bio14
bio12
bio10
Variables
0.0
0.1
0.2
0.3
Relative Variable Importance
elev
bio3
bio18
bio12
0.0
0.2
0.4
0.6
Relative Variable Importance

Fig. 3.

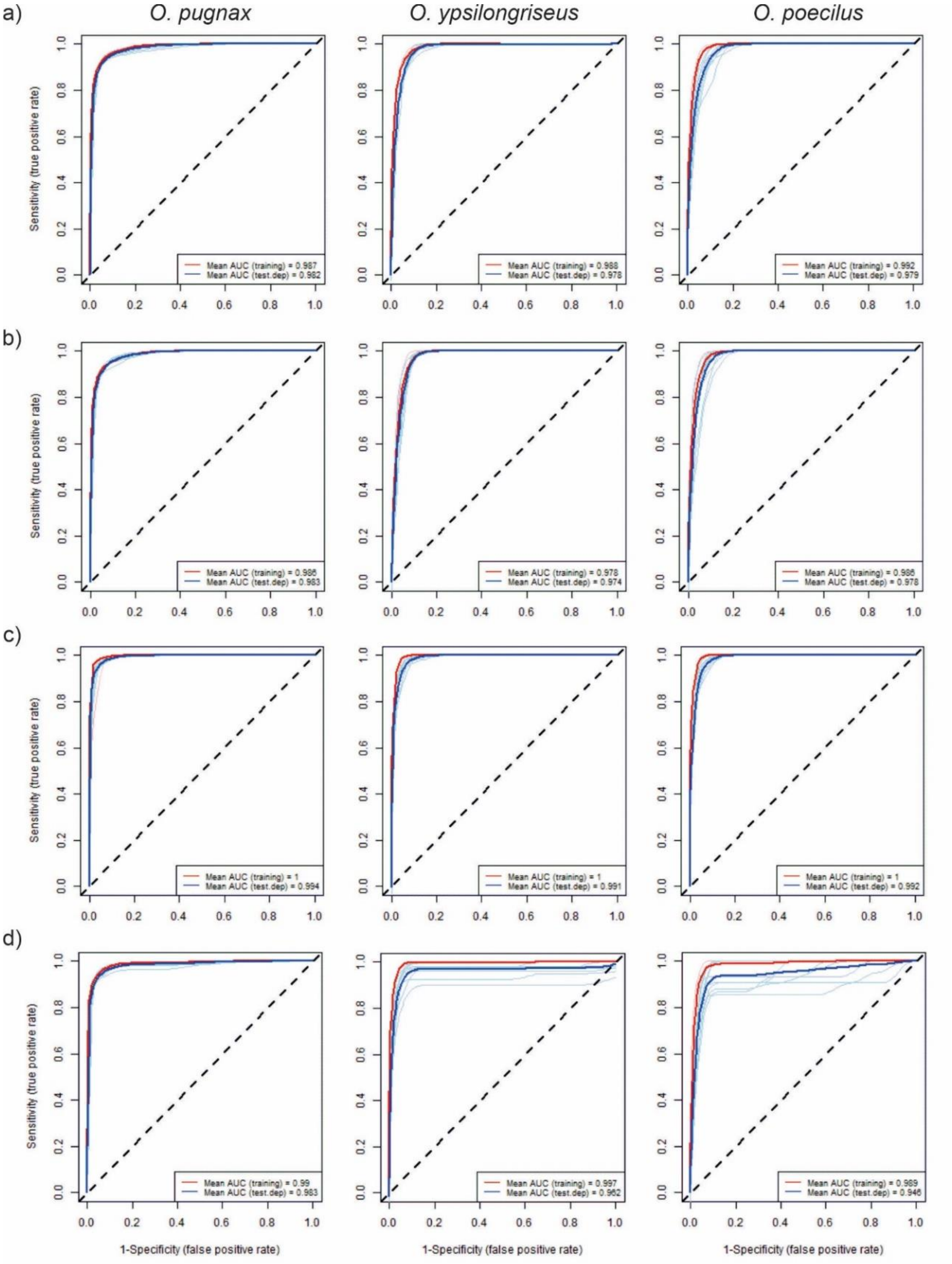

a)
O. pugnax
O. ypsilongriseus
O. poecilus
Sensitivity (true positive rate)
Mean AUC (training) = 0.987
Mean AUC (test.dep) = 0.982
Mean AUC (training) = 0.988
Mean AUC (test.dep) = 0.978
Mean AUC (training) = 0.992
Mean AUC (test.dep) = 0.979
b)
Mean AUC (training) = 0.986
Mean AUC (test.dep) = 0.983
Mean AUC (training) = 0.978
Mean AUC (test.dep) = 0.974
Mean AUC (training) = 0.986
Mean AUC (test.dep) = 0.978
c)
Mean AUC (training) = 1
Mean AUC (test.dep) = 0.994
Mean AUC (training) = 1
Mean AUC (test.dep) = 0.991
Mean AUC (training) = 1
Mean AUC (test.dep) = 0.992
d)
Mean AUC (training) = 0.99
Mean AUC (test.dep) = 0.983
Mean AUC (training) = 0.997
Mean AUC (test.dep) = 0.962
Mean AUC (training) = 0.989
Mean AUC (test.dep) = 0.946
0.0
0.2
0.4
0.6
0.8
1.0
1-Specificity (false positive rate)

Fig. 4.

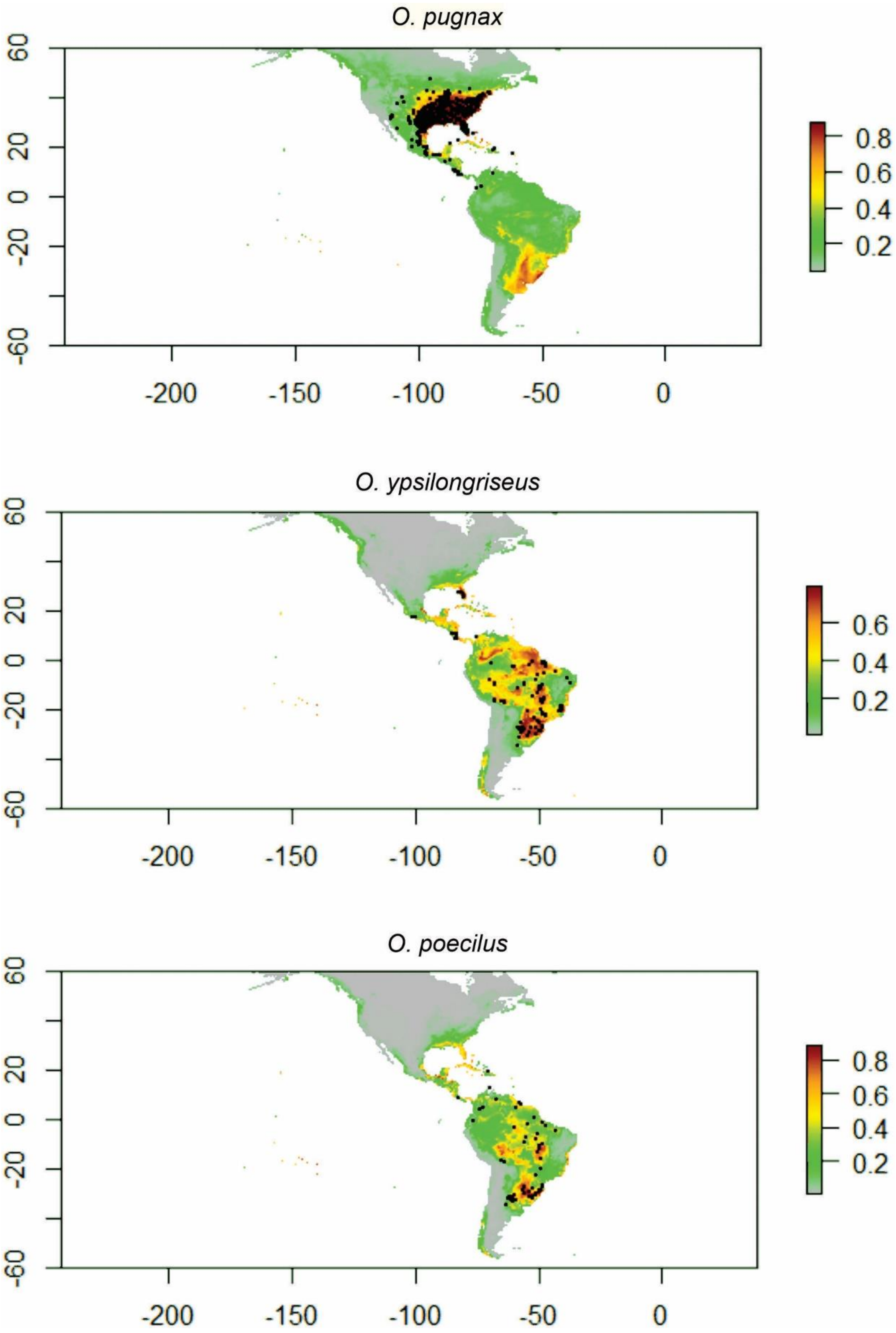
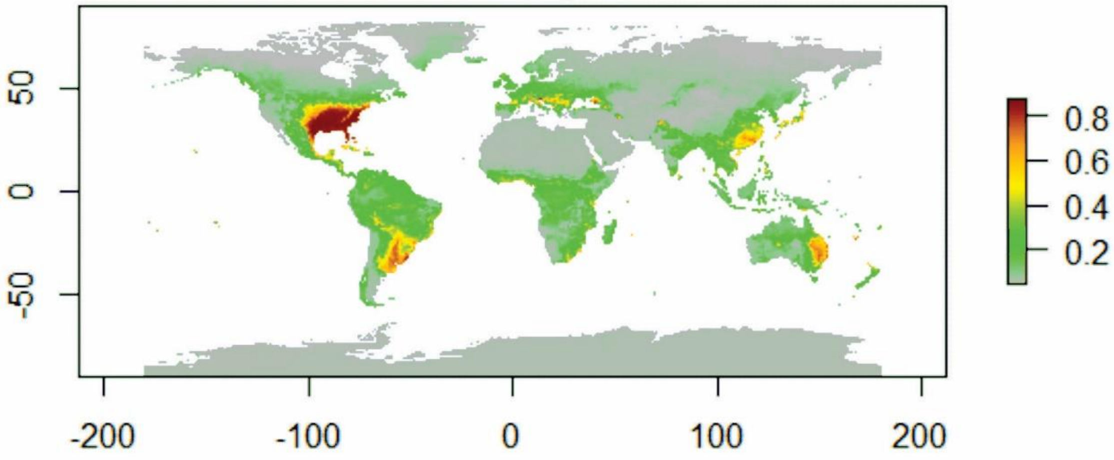
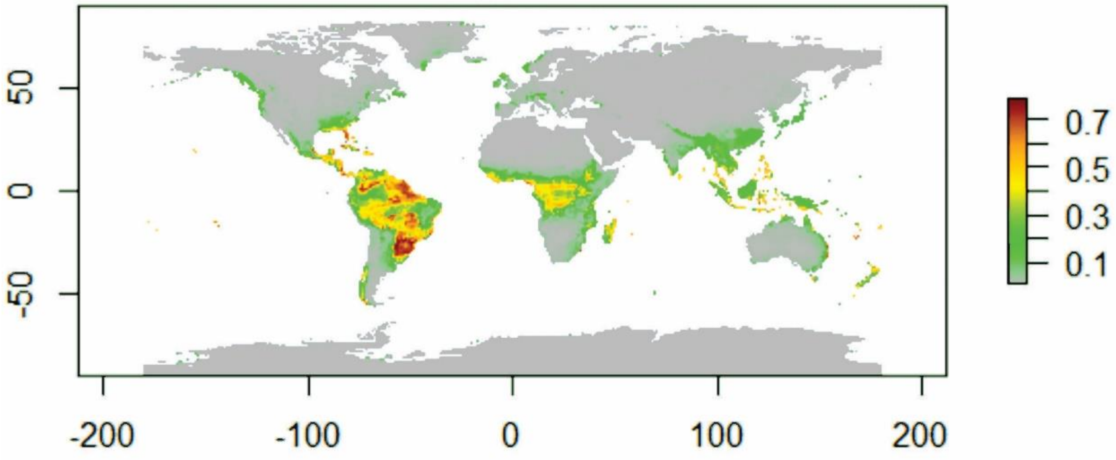

O. pugnax
60
20
0
-20
-60
-200
-150
-100
-50
0
0.8
0.6
0.4
0.2
O. ypsilongriseus
60
20
0
-20
-60
-200
-150
-100
-50
0
0.6
0.4
0.2
O. poecilus
60
20
0
-20
-60
-200
-150
-100
-50
0
0.8
0.6
0.4
0.2

Fig. 5.

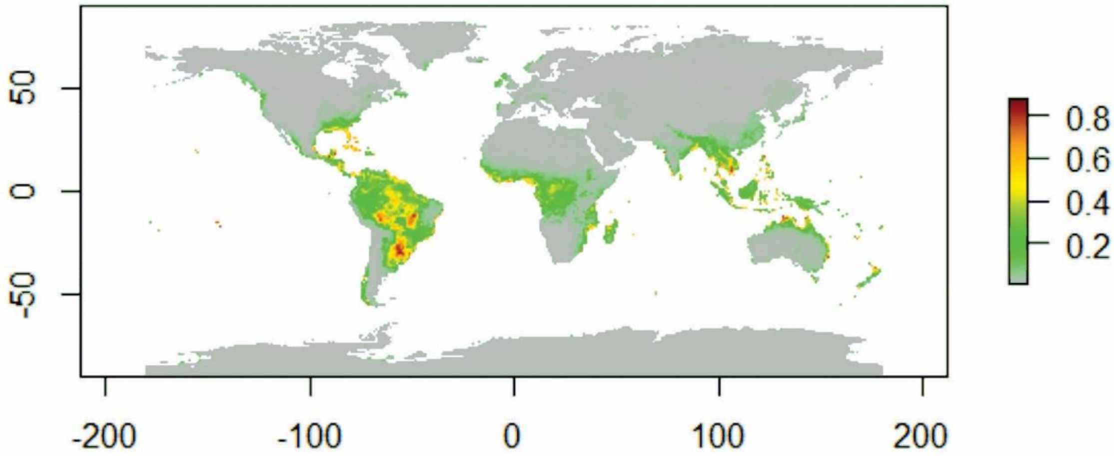
O. pugnax



O. ypsilongriseus



O. poecilus



General Conclusions

Under the current climate change scenario, the reported co-occurrence of the Neotropical rice stink bugs *O. poecilus* and *O. ypsilongriseus* provides a context in which apparent area expansion and invasions are taking place, allowing a species complex establishment. Understanding some factors mediating the distribution and overlap of these two rice pest species was the primary objective of this thesis. To fulfill this goal, (i) the thiamethoxam resistance ratio and its potential risk of control failure, (ii) the genetic diversity and population genetic structure, and (iii) the most related variables in the niche characterization of these species, were all unraveled.

Accordingly, the main aspects of the biology of *O. poecilus* and *O. ypsilongriseus*, their current pest status, and some strategies applied to their management were summarized in light of the first Chapter, while in the second Chapter, populations from both species were screened for thiamethoxam resistance within rice fields in central Brazil. The insecticide potency and efficacy tested presented comparable values between *O. poecilus* and *O. ypsilongriseus*, and both species also exhibited similar resistance levels. Contrary to the initial expectation, this insecticide does not seem to be involved in eventual shifts in species dominance, and thus could not explain the increasing occurrence of *O. ypsilongriseus*. Thiamethoxam resistance was detected in nearly half of the populations of *O. poecilus* and about a third of *O. ypsilongriseus*, but at low levels (< ten-fold). As a consequence, the risk of control failure with thiamethoxam due to insecticide resistance was also rather low. The spatial dependence identified in this survey was significant for both species and phenomena (i.e., thiamethoxam resistance and control failure), likely reflecting the local pattern of insecticide use.

The genetic diversity and population structure of the rice stink bugs were accessed in the third Chapter, aiming to understand their relative occurrence, potential prevalence, and the likelihood of range expansion. Analyzing the populations of these species, both stink bugs showed similar levels of diversity and structuring, which was not expected as *O. ypsilongriseus* is regarded as in recent expansion. Additionally, *O. poecilus* was the only one that exhibited a significant correlation between genetic and geographic distances. Therefore, the number of introduction events of *O. ypsilongriseus* in new areas and its origin are questions that deserve answers from future studies. Comparing the stink bug species, the genetic structure differed, where

84% of the overall genetic variability was identified within species, and three genetic groups were recognized through the Bayesian approach. The presence of an intermediary genetic group that overlaps both species of rice stink bugs may indicate additional adaptation and range expansion, which also should be investigated further in future research.

As the thiamethoxam resistance and the population genetics did not provide all the expected answers, in the fourth Chapter the main predictor variables for the rice stink bug's niche occurrence and overlap were explored. In this Chapter, the addition of a third species of economic importance in the U. S. rice fields, *Oebalus pugnax,* was fundamental to understanding and selecting the variables that explain the co-occurrence of *O. ypsilongriseus* with *O. poecilus* in South America and with *O. pugnax* in the U.S. In this sense, limited rainfall and temperature were identified as the most influential conditions in niche determination of *O. pugnax* in the U.S., while sensibility to dry periods seems to be important in ecological niche specialization between the Neotropical species *O. ypsilongriseus* and *O. poecilus*. From the main predictor variables selected, the species' potential distribution could be analyzed under the current climate scenario through the species distribution modeling (*sdm*) tool. The four algorithms used in this modeling showed high statistic accuracy, with random forest presenting the best fit in all projections. *O. pugnax* appears to meet the conditions needed to establish itself in South America, and the three species could potentially also establish themselves in grain-producing regions in Meridional Europe, Equatorial and Central Africa, Asia, and Australia. Although distribution modeling using bioclimate variables provides valuable information on the habitat suitability and distribution of a species, future research should focus on determining (i) the thermal and moisture development thresholds and (ii) the interspecific relationship among *Oebalus* species, aiming to better understanding their biology and ecology, thereby recognizing their potential prevalence and co-occurrence in different regions.

Undoubtedly, there are still many gaps in the study of *O. poecilus* and *O. ypsilongriseus* that needs to be investigated, given the growing importance of these species in the face of climate change and rice cultivation expansion. Here, the focus of the study was to understand some factors mediating the distribution and overlap of these species. As mentioned before, the initial suspicion was the resistance to the main insecticide used in their control, thiamethoxam, playing an important role in determining species distribution. However, thiamethoxam resistance nor the genetic divergence presented a plausible justification, considering that both species showed similar levels of resistance, genetic diversity, and population structuring. On the other hand, the phenotypic plasticity (or its limitation) observed in the SDM of the rice stink bug species indicated that sensitivity to drier periods is an essential component in its distribution and dominance in Neotropical regions. Therefore, by combining estimates of control failure likelihood, insecticide resistance, and spatiotemporal dynamics with population genetics and species distribution modeling, this study opens the door to geographically based decision-making in the management of two historically neglected species.

Appendix

Supplementary material – Chapter 3

S1. Primers' reference.

Code	Sequence (5'-3')	Annealing temp (°C)	Amplification product	Reference
CI01	$(CA)_{10}^{a}$	56	yes	Palma et al. 2015
CI02	$(CA)_{10}T$	56	yes	Palma et al. 2015
CI03	$(CA)_{10}G$	57	yes	Palma et al. 2015
CI04	$(CA)_{10}C$	54	yes	Palma et al. 2015
CI05	$(GACA)_{4}$	54	yes	Palma et al. 2015
CI06	$(GGAT)_{4}$	55	no	Palma et al. 2015
CI07	$(CT)_{4}(CA)_{5}$	55	yes	Palma et al. 2015
UBC807	$(AG)_{8}T$	52	no	Fedrigo et al. 2016
UBC808	$(AG)_{8}C$	50	yes	Fedrigo et al. 2016
UBC809	$(AT)_{8}T$	55	no	Fedrigo et al. 2016
UBC810	$(GA)_{8}T$	52	no	Kil et al. 2012 Fedrigo et al. 2016
UBC811	$(GA)_{8}C$	53	yes	Fedrigo et al. 2016
UBC813	$(CT)_{8}T$	50	no	Fedrigo et al. 2016
UBC814	$(CT)_{8}^{a}$	50	no	Fedrigo et al. 2016
UBC815	$(CT)_{8}G$	53	no	Fedrigo et al. 2016
UBC817	$(CA)_{8}^{a}$	52	no	Fedrigo et al. 2016
UBC820	$(GT)_{8}T$	52	no	Fedrigo et al. 2016
UBC822	$(TC)_{8}^{a}$	55	no	Fedrigo et al. 2016
UBC823	$(TC)_{8}C$	55	no	Fedrigo et al. 2016
UBC824	$(TC)_{8}G$	50	no	Fedrigo et al. 2016
UBC826	$(AC)_{8}C$	52	no	Fedrigo et al. 2016
UBC827	$(AC)_{8}G$	53	yes	Fedrigo et al. 2016
UBC828	$(TG)_{8}^{a}$	50	yes	Fedrigo et al. 2016
UBC880	$(GGAGA)_{3}$	52	no	Kil et al. 2012 Fedrigo et al. 2016

S2. Sequences used to run the BLAST analysis.

Species	Sample ID	Baeysian clustering	Sequence
OP	23.4	OP	GGGAATGTTGGTTAAGATCGGGTCTCCTCCTCCTGAAGGGTCAAAAATGATGTATTAAAGTTTCGATCGGTTAATAGTATAGTGATAGCCCCAGCTAGCACTGGAAGGGATAATAATAGTAATAGAGCTGTAATTCCAACTGATCATACAAATAAAGGGATTCGTTCAGGGATTATCCCTGCGGGTCGTATATTGATAATAGTAGAAATGAAGTTTACTGCTCCTAGAATTGAGGATACTCCAGCTAAGTGAAGTGAAAAAATTGCTAAATCTACGGATGCTCCTCTATGGGATAAGTTTCTTGATAAAGGAGGATAAACGGTTCATCCAGTTCCAGCTCCAGATTCTGCTAGTCTTCTTATTATTAATAATGTTAATGAAGGAGGTAATAGTCAAAATCTTATATTATTTATTCGAGGGAATGCCATATCAGGAGCTCCAATTATTAAAGGTACTAATCAGTTTCCGAATCCCCCAATTATAATTGGCATTACTATGAAGAAAATTATTACAAATGCATGAGCTGTTACGATTACGTTATAAATTTGATCATCTCCAATGAATCTTCCTGGTTGTCCTAATTCAATTCGAATGATTAATCTTATAGCGGATCCCACTATTCCAGCTCACATCCCGAATATAAAATATAATGTTCCAATATCTTTATGATTTGTTGAC
	20.1	3rd group	GGGAAGGTTGGTTAAGATTGGGTCTCCTCCTCCTGAAGGGTCAAAAAATGATGTATTAAAGTTTCGATCGGTTAATAGTATAGTGATAGCTCCAGCTAGCACTGGAAGGGATAATAATAGTAATAGAGCTGTAATTCCAACTGATCATACAAATAAAGGGATTCGTTCAGGGATTATCCCTGCGGGTCGTATATTGATAATAGTAGAAATGAAGTTTACTGCTCCTAGAATTGAGGATACTCCAGCTAAGTGAAGTGAAAAAATTGCTAAATCTACGGATGCTCCTCTATGGGATAAGTTTCTTGATAAAGGAGGATAAACGGTTCATCCAGTTCCGGCTCCAGATTCTGCTAGTCTTCTTATTATTAATAATGTTAATGAAGGAGGTAATAGTCAAAATCTTATATTATTTATTCGAGGGAATGCCATATCAGGAGCTCCAATTATTAAAGGTACTAATCAGTTTCCGAATCCCCCAATTATAATTGGCATTACTATGAAGAAAATTATTACAAATGCATGAGCTGTTACGATTACGTTATAAATTTGATCATCTCCAATGAATCTTCCTGGTTGTCCTAATTCAATTCGAATGATTAATCTCATAGCGGATCCCACTATTCCAGCTCACATCCCGAATATAAAATATAATGTTCCAATATCTTTATGATTTGTTGAAC
	33.1	3rd group	GGGAAGGTTGGTATAAGATCGGGTCTCCTCCTCCTGAAGGGTCAAAAAATGATGTATTAAAGTTTCGATCGGTTAATAGTATAGTGATAGCTCCAGCTAGCACTGGAAGGGATAATAATAGTAATAGAGCTGTAATTCCAACTGATCATACAAATAAAGGAATTCGTTCAGGGATTATCCCTGCGGGTCGTATATTGATAATAGTAGAAATGAAGTTTACTGCTCCTAGAATTGAGGATACTCCAGCTAAGTGAAGTGAAAAAATTGCTAAATCTACGGATGCTCCTCTATGGGATAAGTTTCTTGATAAAGGAGGATAAACGGTTCATCCAGTTCCAGCTCCAGATTCTGCTAGTCTTCTTATTATTAATAATGTTAATGAAGGAGGTAATAGTCAAAATCTTATATTATTTATTCGAGGGAATGCCATATCAGGAGCTCCAATTATTAAAGGTACTAATCAGTTTCCGAATCCCCCAATTATAATTGGCATTACTATGAAGAAAATTATTACAAATGCATGAGCTGTTACGATTACGTTATAAATTTGATCATCTCCATGAATCTTCCTGGTTGTCCTAATTCAATTCGAATGATTAATCTCATAGCGGATCCCACTATTCCAGCTCACATCCCGAATATAAAATACAATGTTCCAATATCTTTA

Species	Sample ID	Baeysian clustering	Sequence
	46.3	3rd group	GATCGGGTCTCCTCCTCCTGAAGGGTCAAAAAATGATGTATTAAAGTTTCGATCGGTTAATAATATAGTGATAGCTCCAGCTAGCACTGGAAGGGATAATAATAGTAATAGAGCTGTAATTCCAACTGATCATACAAATAAAGGGATTCGTTCAGGGATTATCCCTGCGGGTCGTATATTGATAATAGTAGAAATGAAGTTTACTGCTCCTAGAATTGAGGATACTCCAGCTAAGTGAAGTGAAAAAATTGCTAAATCTACGGATGCTCCTCTATGGGATAAGTTTCTTGATAAAGGAGGATAAACGGTTCATCCAGTTCCAGCTCCAGATTCTGCTAGTCTTCTTATTATTAATAATGTTAATGAAGGAGGTAATAGTCAAAATCTTATATTATTTATTCGAGGGAATGCCATATCAGGAGCTCCAATTATTAAAGGTACTAATCAGTTTCCGAATCCCCCAATTATAATTGGCATTACTATGAAGAAAATTATTACAAATGCATGAGCTGTTACGATTACGTTATAAATTTGATCATCTCCAATGAATCTTCCTGGTTGTCCTAATTCAATTCGAATGATTAATCTCATAGCGGATCCCACTATTCCAGCTCACATCCCGAATATAAAATATAATGTTCCAATATCTTTAT
	56.7	3rd group	GATCGGGTCATCCTCCTCCTGAAGGGTCAAAAAATGATGTATTAAAGTTTCGATCGGTTAATAGTATAGTGATAGCCCCAGCTAGCACTGGAAGGGATAATAATAGTAATAGAGCTGTAATTCCAACTGATCATACAAATAAAGGGATTCGTTCAGGGATTATCCCTGCGGGTCGTATATTGATAATAGTAGAAATGAAGTTTACTGCTCCTAGAATTGAGGATACTCCAGCTAAGTGAAGTGAAAAAATTGCTAAATCTACGGATGCTCCTCTATGGGATAAGTTTCTTGATAAAGGAGGATAAACGGTTCATCCAGTTCCAGCTCCAGATTCTGCTAGTCTTCTTATTATTAATAATGTTAATGAAGGAGGTAATAGTCAAAATCTTATATTATTTATTCGAGGGAATGCCATATCAGGAGCTCCAATTATTAAAGGTACTAATCAGTTTCCGAATCCCCCAATTATAATTGGCATTACTATGAAGAAAATTATTACAAATGCATGAGCTGTTACGATTACGTTATAAATTTGATCATCCCCAATGAATCTTCCTGGTTGTCCTAATTCAATTCGAATGATTAATCTCATAGCGGATCCCACTATTCCAGCTCACATCCCGAATATAAAATATAATGTTCCAATATCTTTA
OY	39.3	OY	GGGAAGTTGGTTAAATAGGATCTCCCCCTCCAGATGGGTCAAAGAATGATGTATTGAAGTTTCGATCAGTTAGTAATATTGTAATAGCTCCTGCTAATACAGGTAATGATAATAATAAAAGTAATGCAGTGATTCCAACTGATCATACGAATAAAGGGATTCGTTCTGGAATCATTCCTGCTGGTCGTATATTAATGATTGTTGAGATGAAGTTTACCGCTCCTAGAATTGAAGATACACCTGCTAAATGAAGTGAAAAAATTGCTAAGTCTACAGATGCTCCTCTATGTGAAAGGTTTCTTGATAAAGGAGGGTATACTGTTCATCCAGTTCCAGCTCCTGATTCTGCTAGACTGCTTATCATTAATAATGTTAGTGAAGGAGGTAATAATCAGAATCTTATATTATTTATTCGAGGGAATGCTATGTCTGGTGCTCCAATTATTAAAGGAACTAGTCAATTTCCAAATCCTCCAATTATAATTGGCATTACTATAAAGAAAATTATTACGAAGGCGTGAGCTGTTACGATTACGTTATAAATTTGATCATCTCCAATGAATCTTCCAGGTTGGCCTAATTCAATACGAATGATTAATCTTATAGCGGATCCCACTATCCCAGCTCATATTCCAAATATGAAGTATAGTGTTCCAATATCTTTA
	15.1	3rd group	GGGAAGGTTGGTAAAGATCGGGTCTCCTCCTCCTGAAGGGTCAAAAAATGATGTATTAAAGTTTCGATCGGTTAATAGTATAGTGATAGCTCCAGCTAGCACTGGAAGGGATAATAATAGTAATAGAGCTGTAATTCCAACTGATCATACAAATAAAGGGATTCGTTCAGGGATTA

Species	Sample ID	Baeysian clustering	Sequence
			TCCCTGCGGGTCGTATATTGATAATAGTAGAAATGAAGTTTACTGCTCCTAGAATTGAGGATACTCCAGCTAAGTGAAGTGAAAAAATTGCTAAATCTACGGATGCTCCTCTATGGGATAAGTTTCTTGATAAAGGAGGATAAACGGTTCATCCAGTTCCAGCTCCAGATTCTGCTAGTCTTCTTATTATTAATAATGTTAATGAAGGAGGTAGTAGTCAAAATCTTATATTATTTATTCGAGGGAATGCCATATCAGGAGCTCCAATTATTAAAGGTACTAATCAGTTTCCGAATCCCCCAATTATAATTGGCATTACTATGAAGAAAATTATTACAAATGCATGAGCTGTTACGATTACGTTATAAATTTGATCATCTCCAATGAATCTTCCTGGTTGTCCTAATTCAATTCGAATGATTAATCTCATAGCGGATCCCACTATTCCAGCTCACATCCCGAATATAAAATATAATGTTCCAATATCTTTA
	25.3	3rd group	GGGAAGTTGGTTAAATAGGATCTCCCCCTCCAGATGGATCAAAGAATGATGTATTGAAGTTTCGATCAGTTAGTAATATTGTAATAGCCCCTGCTAATACAGGTAATGATAATAATAAAAGTAATGCGGTGATTCCAACTGATCATACAAATAAAGGGATTCGTTCTGGAATTATTCCTGCTGGTCGTATATTAATGATTGTTGAGATGAAGTTTACCGCTCCTAGGATTGAAGATACACCTGCTAAATGAAGTGAAAAAATTGCTAAGTCTACAGATGCTCCTCTATGTGAAAGGTTTCTTGATAAAGGAGGGTATACTGTTCATCCAGTTCCAGCTCCTGATTCTGCTAGACTGCTTATCATTAATAATGTTAGTGAAGGAGGTAATAATCAGAATCTTATATTATTTATTCGAGGGAATGCTATGTCTGGTGCTCCAATTATTAAAGGAACTAGTCAATTTCCAAATCCTCCAATTATAATTGGCATTACTATAAAGAAAATTATTACGAAGGCGTGAGCTGTTACGATTACGTTATAAATTTGATCATCTCCAATGAATCTTCCAGGTTGGCCTAATTCAATACGAATGATTAATCTTATAGCGGATCCCACTATTCCAGCTCATATTCCAAATATGAAGTATAGTGTTCCAATATCTTTA
	32.2	3rd group	GGGAAGTTGGTTAAATAGGATCTCCCCCTCCAGATGGGTCAAAGAATGATGTATTGAAGTTTCGATCAGTTAGTAATATTGTAATAGCTCCTGCTAATACAGGTAATGATAATAATAAAAGTAATGCAGTGATTCCAACTGATCATACGAATAAAGGGATTCGTTCTGGAATCATTCCTGCTGGTCGTATATTAATGATTGTTGAGATGAAGTTTACCGCTCCTAGAATTGAAGATACACCTGCTAAATGAAGTGAAAAAATTGCTAAGTCTACAGATGCTCCTCTATGTGAAAGGTTTCTTGATAAAGGAGGGTATACTGTTCATCCAGTTCCAGCTCCTGATTCTGCTAGACTGCTTATCATTAATAATGTTAGTGAAGGAGGTAATAATCAGAATCTTATATTATTTATTCGAGGGAATGCTATGTCTGGTGCTCCAATTATTAAAGGAACTAGTCAATTTCCAAATCCTCCAATTATAATTGGCATTACTATAAAGAAAATTATTACGAAGGCGTGAGCTGTTACGATTACGTTATAAATTTGATCATCTCCAATGAATCTTCCAGGTTGTCCTAATTCAATACGAATGATTAATCTTATAGCGGATCCCACTATCCCAGCTCATATTCCAAATATGAAGTATAGTGTTCCAATATCTTTATATTTGTGTT
	62.2	3rd group	GGGAAGTTGGTTAAATAGGATCTCCCCCTCCAGATGGGTCAAAGAATGATGTATTGAAGTTTCGATCAGTTAGTAATATTGTAATAGCTCCTGCTAATACAGGTAATGATAATAATAAAAGTAATGCAGTGATTCCAACTGATCATACGAATAAAGGGATTCGTTCTGGAATCATTCCTGCTGGTCGTATATTAATGATTGTTGAGATGAAGTTTACCGCTCCTAGAATTGAAGATACACCTGCTAAATGAAGTGAAAAAATTGCTAAGTCTACAGATGCTCCTCTATGTGAAAGGTTTCTTGATAAAGGAGGGTATACTGTTCATCCAGTTCCAGCTCCTGATTCTGCTAGAC

Species	Sample ID	Baeysian clustering	Sequence
			TGCTTATCATTAATAATGTTAGTGAAGGAGGTAATAATCAGAATCTTATATTATTTATTCGAGGGAATGCTATGTCTGGTGCTCCGATTATTAAAGGAACTAGTCAATTTCCAAATCCTCCAATTATAATTGGCATTACTATAAAGAAAATTATTACGAAGGCGTGAGCTGTTACGATTACGTTATAAATTTGATCATCTCCAATGAATTTTCCAGGTTGGCCTAATTCAATACGAATGATTAATCTTATAGCGGATCCCACTATCCCAGCTCATATTCCAAATATGAAGTATAGTGTTCCAATATCTTTA

Supplementary material – Chapter 4

S1. Occurrence records thinned

Species	Latitude	Longitude	References	Country
Opug	17.08621	-61.80270	GBIF	Antigua and Barbuda
	25.06709	-77.97494	GBIF	Bahamas
	42.26368	-83.06634	GBIF	Canada
	4.19921	-74.63241	GBIF	Colombia
	3.53945	-76.31904	GBIF	Colombia
	9.90000	-83.68333	Illinois Museum	Costa Rica
	10.30000	-84.81660	Illinois Museum	Costa Rica
	10.76310	-85.33440	GBIF	Costa Rica
	8.56288	-83.49907	GBIF	Costa Rica
	8.67910	-83.56671	GBIF	Costa Rica
	10.16822	-85.37366	GBIF	Costa Rica
	10.33497	-85.24638	GBIF	Costa Rica
	10.42767	-85.10301	GBIF	Costa Rica
	10.71833	-85.14472	GBIF	Costa Rica
	10.83641	-85.61549	GBIF	Costa Rica
	10.96254	-85.49524	GBIF	Costa Rica
	22.58630	-83.83950	GBIF	Cuba
	18.58404	-69.83274	GBIF	Dominican Republic
	18.83422	-69.88894	GBIF	Dominican Republic
	19.19054	-69.43148	GBIF	Dominican Republic
	13.83568	-89.28066	Illinois Museum	El Salvador
	14.33484	-87.18985	GBIF	Honduras
	16.41000	-92.41000	Illinois Museum	Mexico
	16.64660	-93.94914	GBIF	Mexico
	16.70077	-92.79624	GBIF	Mexico
	20.21752	-98.00051	GBIF	Mexico
	20.71132	-99.81078	GBIF	Mexico
	23.75330	-99.08334	GBIF	Mexico
	24.67325	-99.77059	GBIF	Mexico
	24.86046	-99.80594	GBIF	Mexico
	25.53627	-100.31683	GBIF	Mexico
	25.64111	-100.26158	GBIF	Mexico
	26.13917	-100.18348	GBIF	Mexico
	27.18777	-99.44004	GBIF	Mexico
	27.35444	-108.81222	GBIF	Mexico
	31.10260	-111.37690	GBIF	Mexico
	22.76161	-102.61428	GBIF	Mexico
	16.71590	-94.10760	GBIF	México
	16.76722	-96.78056	GBIF	México
	16.83222	-91.49500	GBIF	México
	17.26194	-96.86472	GBIF	México
	17.57444	-97.40778	GBIF	México
	17.76722	-96.95083	GBIF	México
	18.06056	-97.06972	GBIF	México
	19.60056	-96.37500	GBIF	México
	19.75722	-96.41528	GBIF	México
	20.29281	-102.71658	GBIF	México
	21.16417	-99.36000	GBIF	México
	21.17361	-87.48194	GBIF	México
	22.06056	-100.49639	GBIF	México
	22.90889	-99.34306	GBIF	México
	23.08194	-99.16222	GBIF	México
	8.37294	-82.27191	GBIF	Panama
	25.17222	-80.37278	GBIF	USA
	25.39046	-80.63440	GBIF	USA
	25.60859	-80.31835	GBIF	USA
	25.75338	-80.49733	GBIF	USA
	25.76080	-80.62867	GBIF	USA
	25.77870	-80.30810	GBIF	USA
	25.80514	-80.85310	GBIF	USA
	25.85286	-97.38547	GBIF	USA
	25.90187	-81.22280	GBIF	USA
	25.89617	-97.47690	GBIF	USA
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	26.05611	-98.03635	GBIF	USA
	26.05690	-97.60893	GBIF	USA
	26.06943	-98.14623	GBIF	USA
	26.07723	-97.20674	GBIF	USA
	26.10007	-80.23292	GBIF	USA
	26.12612	-97.95783	GBIF	USA
	26.14021	-80.58445	GBIF	USA
	26.15481	-97.81568	GBIF	USA
	26.17346	-98.38263	GBIF	USA

Species	Latitude	Longitude	References	Country
	26.18192	-80.12601	GBIF	USA
	26.18682	-97.63846	GBIF	USA
	26.23081	-80.91762	GBIF	USA
	26.23159	-98.21122	GBIF	USA
	26.23421	-97.36516	GBIF	USA
	26.26725	-80.17342	GBIF	USA
	26.29454	-97.81822	GBIF	USA
	26.30930	-98.15831	GBIF	USA
	26.33794	-98.34360	GBIF	USA
	26.38818	-80.11710	GBIF	USA
	26.42839	-82.10238	GBIF	USA
	26.48513	-97.77586	GBIF	USA
	26.51494	-99.11654	GBIF	USA
	26.54044	-98.04616	GBIF	USA
	26.57386	-98.70929	GBIF	USA
	26.65279	-81.84655	GBIF	USA
	26.84816	-97.77611	GBIF	USA
	26.91353	-82.04126	GBIF	USA
	26.96745	-80.79773	GBIF	USA
	27.00578	-80.97275	GBIF	USA
	27.04422	-82.23593	GBIF	USA
	27.13033	-82.45315	GBIF	USA
	27.18700	-81.33800	GBIF	USA
	27.22156	-97.75616	GBIF	USA
	27.24847	-98.15435	GBIF	USA
	27.31096	-97.71113	GBIF	USA
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	27.35009	-82.16119	GBIF	USA
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	27.47349	-97.28653	GBIF	USA
	27.47751	-82.34442	GBIF	USA
	27.51990	-97.89605	GBIF	USA
	27.53428	-99.43845	GBIF	USA
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	27.58758	-97.45449	GBIF	USA
	27.59554	-82.55232	GBIF	USA
	27.62057	-97.22272	GBIF	USA
	27.62058	-97.22271	GBIF	USA
	27.64781	-98.21197	GBIF	USA
	27.66938	-97.32877	GBIF	USA
	27.69752	-98.79495	GBIF	USA
	27.70314	-97.47124	GBIF	USA
	27.75916	-82.22950	GBIF	USA
	27.79057	-97.66899	GBIF	USA
	27.80058	-97.39638	GBIF	USA
	27.81417	-81.54779	GBIF	USA
	27.81933	-97.08186	GBIF	USA
	27.85369	-97.21501	GBIF	USA
	27.86610	-97.60883	GBIF	USA
	27.88060	-98.02637	GBIF	USA
	27.94225	-99.50747	GBIF	USA
	27.97543	-97.67571	GBIF	USA
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	28.06748	-82.40285	GBIF	USA
	28.09946	-97.55583	GBIF	USA
	28.10324	-97.69083	GBIF	USA
	28.10833	-97.37787	GBIF	USA
	28.13524	-97.26111	GBIF	USA
	28.13583	-96.98758	GBIF	USA
	28.13908	-98.60347	GBIF	USA
	28.15112	-82.46148	GBIF	USA
	28.15475	-81.56216	GBIF	USA
	28.16887	-81.70384	GBIF	USA
	28.18898	-100.09290	GBIF	USA
	28.20498	-97.90352	GBIF	USA
	28.24861	-97.30834	GBIF	USA
	28.31038	-96.80150	GBIF	USA
	28.31697	-98.54931	GBIF	USA
	28.32472	-99.40671	GBIF	USA
	28.36610	-80.85690	GBIF	USA
	28.40088	-81.49374	GBIF	USA
	28.44830	-81.21970	GBIF	USA
	28.44908	-96.51578	GBIF	USA
	28.45365	-96.40844	GBIF	USA
	28.45521	-81.72873	GBIF	USA
	28.47862	-98.34683	GBIF	USA
	28.49490	-81.34078	GBIF	USA
	28.49664	-96.84307	GBIF	USA
	28.51086	-81.07023	GBIF	USA
	28.53830	-81.21250	GBIF	USA

Species	Latitude	Longitude	References	Country
	28.57081	-81.58046	GBIF	USA
	28.60190	-81.79140	GBIF	USA
	28.61812	-80.81009	GBIF	USA
	28.61618	-82.38593	GBIF	USA
	28.64415	-81.31140	GBIF	USA
	28.65523	-97.38692	GBIF	USA
	28.66330	-81.20860	GBIF	USA
	28.69360	-100.38697	GBIF	USA
	28.70778	-96.21722	GBIF	USA
	28.70914	-100.49952	GBIF	USA
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	28.75130	-82.28280	GBIF	USA
	28.79000	-99.76000	GBIF	USA
	28.79199	-81.77938	GBIF	USA
	28.80285	-97.04846	GBIF	USA
	28.80801	-81.34388	GBIF	USA
	28.81161	-96.90256	GBIF	USA
	28.87161	-96.21810	GBIF	USA
	28.91407	-95.57836	GBIF	USA
	28.92146	-99.09518	GBIF	USA
	28.93378	-95.30845	GBIF	USA
	28.94691	-100.62653	GBIF	USA
	28.95383	-98.50155	GBIF	USA
	29.02979	-98.79611	GBIF	USA
	29.04578	-95.47540	GBIF	USA
	29.05767	-81.36711	GBIF	USA
	29.05817	-95.22916	GBIF	USA
	29.08586	-97.28655	GBIF	USA
	29.18380	-95.45027	GBIF	USA
	29.21234	-94.95458	GBIF	USA
	29.21457	-103.23069	GBIF	USA
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	29.26524	-81.02586	GBIF	USA
	29.31523	-94.81850	GBIF	USA
	29.33147	-100.38627	GBIF	USA
	29.39786	-98.37923	GBIF	USA
	29.41635	-95.10007	GBIF	USA
	29.45281	-98.47739	GBIF	USA
	29.42468	-94.95818	GBIF	USA
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	29.49367	-81.74377	GBIF	USA
	29.49544	-99.71271	GBIF	USA
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	29.55601	-95.58762	GBIF	USA
	29.56173	-82.19218	GBIF	USA
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	29.61093	-98.54325	GBIF	USA
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	29.62038	-98.67510	GBIF	USA
	29.62142	-95.03313	GBIF	USA
	29.65918	-94.10218	GBIF	USA
	29.65088	-98.07924	GBIF	USA
	29.65344	-95.46110	GBIF	USA
	29.67271	-96.27059	GBIF	USA
	29.68081	-85.21672	GBIF	USA
	29.68155	-98.34270	GBIF	USA
	29.69794	-98.57954	GBIF	USA
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	29.70476	-96.76106	GBIF	USA
	29.72110	-82.38858	GBIF	USA
	29.71284	-95.33747	GBIF	USA
	29.72304	-95.67976	GBIF	USA
	29.72356	-99.08869	GBIF	USA
	29.72896	-90.73462	GBIF	USA
	29.74522	-99.55505	GBIF	USA
	29.75056	-95.77972	GBIF	USA
	29.76640	-95.45239	GBIF	USA
	29.76872	-98.96616	GBIF	USA
	29.77025	-93.64929	GBIF	USA
	29.78800	-98.41900	GBIF	USA

Species	Latitude	Longitude	References	Country
	29.79133	-98.66195	GBIF	USA
	29.80258	-96.99645	GBIF	USA
	29.81329	-95.61786	GBIF	USA
	29.82361	-97.29370	GBIF	USA
	29.83580	-94.38480	GBIF	USA
	29.85227	-98.49323	GBIF	USA
	29.86186	-95.15945	GBIF	USA
	29.88556	-100.99417	GBIF	USA
	29.88610	-96.91248	GBIF	USA
	29.90355	-97.96967	GBIF	USA
	29.90915	-95.66963	GBIF	USA
	29.92312	-95.56102	GBIF	USA
	29.94378	-90.21735	GBIF	USA
	29.94926	-94.25689	GBIF	USA
	29.95830	-95.16505	GBIF	USA
	29.98701	-90.08933	GBIF	USA
	29.99838	-96.87906	GBIF	USA
	29.99909	-90.41230	GBIF	USA
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	30.01661	-103.24487	GBIF	USA
	30.02872	-95.30092	GBIF	USA
	30.03534	-95.65103	GBIF	USA
	30.04640	-99.14262	GBIF	USA
	30.04799	-94.33574	GBIF	USA
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Species	Latitude	Longitude	References	Country
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	38.81002	-91.35352	GBIF	USA
	38.80901	-89.93869	Illinois Museum	USA
	38.82132	-77.22482	GBIF	USA
	38.83963	-77.01139	GBIF	USA
	38.86190	-84.08697	GBIF	USA
	38.87194	-88.44611	Illinois Museum	USA
	38.88820	-77.69674	GBIF	USA
	38.89028	-82.59645	GBIF	USA
	38.88041	-90.20015	GBIF	USA
	38.91255	-92.28522	GBIF	USA
	38.92997	-80.79331	GBIF	USA
	38.93079	-77.37103	GBIF	USA
	38.93483	-76.93809	GBIF	USA
	38.93618	-74.94464	GBIF	USA
	38.96590	-77.18369	GBIF	USA
	38.97000	-90.43139	GBIF	USA
	38.97471	-77.04851	GBIF	USA
	39.02912	-76.79727	GBIF	USA
	39.03423	-76.45320	GBIF	USA
	39.04503	-75.52817	GBIF	USA
	39.04696	-76.11805	GBIF	USA
	39.05904	-78.06307	GBIF	USA
	39.05921	-77.24854	GBIF	USA
	39.10239	-76.61499	GBIF	USA
	39.12360	-76.80715	GBIF	USA
	39.13534	-95.43889	GBIF	USA
	39.14538	-78.26497	GBIF	USA
	39.15514	-84.50127	GBIF	USA
	39.16323	-76.48362	GBIF	USA
	39.16365	-75.49594	GBIF	USA
	39.19846	-77.02732	GBIF	USA
	39.21280	-77.54071	GBIF	USA
	39.23826	-76.86693	GBIF	USA
	39.24435	-76.59669	GBIF	USA
	39.27402	-89.89361	Illinois Museum	USA
	39.29143	-84.68439	GBIF	USA
	39.29952	-90.60357	Illinois Museum	USA

Species	Latitude	Longitude	References	Country
	39.31983	-99.81249	GBIF	USA
	39.42160	-76.54144	GBIF	USA
	39.43295	-76.76276	GBIF	USA
	39.43766	-75.71425	GBIF	USA
	39.44584	-74.41073	GBIF	USA
	39.44891	-91.05358	GBIF	USA
	39.55758	-77.00123	GBIF	USA
	39.56374	-75.72883	GBIF	USA
	39.56698	-75.90174	GBIF	USA
	39.59557	-79.95490	GBIF	USA
	39.60788	-74.20175	GBIF	USA
	39.61040	-75.16474	GBIF	USA
	39.62039	-75.55326	GBIF	USA
	39.67692	-79.37659	GBIF	USA
	39.69925	-75.10338	GBIF	USA
	39.82898	-74.99153	GBIF	USA
	39.84767	-75.19729	GBIF	USA
	39.93317	-75.43766	GBIF	USA
	39.95716	-75.01676	GBIF	USA
	39.98612	-75.19982	GBIF	USA
	40.00237	-74.82197	GBIF	USA
	40.02076	-75.94607	GBIF	USA
	40.04884	-89.74036	GBIF	USA
	40.06028	-106.80750	Illinois Museum	USA
	40.07626	-75.23448	GBIF	USA
	40.08626	-88.51608	GBIF	USA
	40.11836	-88.21108	GBIF	USA
	40.11323	-87.83275	GBIF	USA
	40.13877	-75.06596	GBIF	USA
	40.30209	-90.05114	GBIF	USA
	40.33028	-89.93111	GBIF	USA
	40.35878	-91.41879	GBIF	USA
	40.50842	-74.21422	GBIF	USA
	40.55604	-74.11278	GBIF	USA
	40.60344	-73.92524	GBIF	USA
	40.62989	-74.96921	GBIF	USA
	40.71161	-73.16620	GBIF	USA
	40.71520	-88.00630	Illinois Museum	USA
	40.71519	-74.05431	GBIF	USA
	40.77618	-73.31022	GBIF	USA
	40.78370	-73.92534	GBIF	USA
	40.78998	-73.78725	GBIF	USA
	40.94168	-73.47789	GBIF	USA
	41.06844	-74.30213	GBIF	USA
	41.11361	-89.19444	Illinois Museum	USA
	41.18208	-73.89321	GBIF	USA
	41.29118	-73.59697	GBIF	USA
	41.41309	-73.86508	GBIF	USA
	41.60232	-71.25033	GBIF	USA
	41.64183	-87.11457	GBIF	USA
	41.76219	-72.18015	GBIF	USA
	42.01500	-89.32501	GBIF	USA
	42.03382	-93.60288	GBIF	USA
	42.06094	-87.67273	GBIF	USA
	42.08708	-71.17730	GBIF	USA
	42.14248	-87.78935	GBIF	USA
	42.33169	-70.89644	GBIF	USA
	42.45174	-87.80478	GBIF	USA
	42.48987	-96.39001	GBIF	USA
	42.62505	-87.82063	GBIF	USA
	43.59164	-79.51214	GBIF	USA
	47.19150	-95.17700	Illinois Museum	USA
	34.37697	-77.63991	GBIF	USA
	28.06311	-97.87220	GBIF	USA
	26.66890	-80.63122	Cherry et al. 1998	USA
	30.23919	-92.34568	Blackman et al. 2015	USA
	29.73476	-94.61096	Espino et a. 2008	USA
	32.37802	-100.85210	Espino et a. 2008	USA
	29.57138	-95.80941	Espino et a. 2008	USA
	28.93109	-96.51501	Espino et a. 2008	USA
	32.76507	-94.39115	Espino et a. 2008	USA
	35.78100	-76.35700	Reisig et al. 2013	USA
	31.47950	-83.52254	Bundy & McPherson 2000	USA

Species	Latitude	Longitude	References	Country
	30.76385	-84.48584	Bundy & McPherson 2000	USA
	34.47549	-91.41844	Rashid et al. 2006	USA
	33.42078	-90.90703	Awuni et al. 2015	USA
	33.29245	-90.94995	Awuni et al. 2015	USA
	33.88000	-90.84882	Awuni et al. 2015	USA
	33.52059	-90.69575	Awuni et al. 2015	USA
	34.28900	-79.73840	Reay-Jones 2010	USA
	34.07460	-80.11790	Reay-Jones 2014	USA
	26.64217	-80.47756	VanWeelden et al. 2020	USA
	31.56969	-83.29822	Tillman et al. 2016	USA
	31.63114	-83.19047	Tillman et al. 2019	USA
	28.62618	-81.35964	GBIF	USA
	9.05000	-69.75000	Illinois Museum	Venezuela
Oy	-34.78817	-58.39761	GBIF	Argentina
	-31.37020	-58.11796	GBIF	Argentina
	-28.03000	-58.04000	GBIF	Argentina
	-27.86670	-55.65000	GBIF	Argentina
	-27.61177	-56.85359	GBIF	Argentina
	-27.57337	-56.67871	GBIF	Argentina
	-27.37509	-58.68431	GBIF	Argentina
	-17.34332	-63.83745	GBIF	Bolivia
	-17.08349	-65.18409	GBIF	Bolivia
	-16.87298	-64.01740	GBIF	Bolivia
	-16.20274	-67.79354	GBIF	Bolivia
	-16.48407	-68.10938	GBIF	Bolivia
	-30.01886	-51.23888	Species Link	Brazil
	-29.13198	-56.52482	Species Link	Brazil
	-27.67606	-48.48622	GBIF	Brazil
	-27.41329	-51.22983	Species Link	Brazil
	-27.37037	-53.39484	Species Link	Brazil
	-25.21860	-50.04120	Species Link	Brazil
	-25.12521	-50.11559	GBIF	Brazil
	-23.37213	-51.95359	GBIF	Brazil
	-22.94796	-46.99475	GBIF	Brazil
	-22.34295	-48.77384	GBIF	Brazil
	-22.29128	-48.12131	GBIF	Brazil
	-22.06465	-46.98821	Species Link	Brazil
	-21.23814	-48.34290	GBIF	Brazil
	-20.47000	-49.21000	GBIF	Brazil
	-20.51627	-54.56186	GBIF	Brazil
	-17.77441	-48.77291	GBIF	Brazil
	-15.86066	-47.91483	GBIF	Brazil
	-15.76907	-47.85393	GBIF	Brazil
	-15.67461	-47.85112	GBIF	Brazil
	-14.66585	-52.36746	GBIF	Brazil
	-9.96076	-67.87119	GBIF	Brazil
	-9.85997	-56.06809	GBIF	Brazil
	-9.73545	-67.69006	GBIF	Brazil
	-9.59760	-55.93187	GBIF	Brazil
	-9.53061	-37.30007	GBIF	Brazil
	-7.52869	-38.49492	GBIF	Brazil
	-3.01693	-60.15728	GBIF	Brazil
	-2.96355	-59.92102	GBIF	Brazil
	-7.96566	-69.95293	GBIF	Brazil
	-0.74315	-48.01642	GBIF	Brazil
	-11.40327	-58.70047	GBIF	Brazil
	-1.15694	-48.13071	GBIF	Brazil
	-7.77023	-51.13067	GBIF	Brazil
	-1.19000	-47.31198	GBIF	Brazil
	-6.08429	-50.17664	GBIF	Brazil
	-2.98845	-47.36497	GBIF	Brazil
	-5.87829	-50.48059	GBIF	Brazil
	-1.51039	-48.04638	GBIF	Brazil
	-2.23474	-53.85391	GBIF	Brazil
	-1.41576	-48.41974	GBIF	Brazil
	-1.36378	-48.23573	GBIF	Brazil
	-1.35513	-48.32750	GBIF	Brazil
	-2.93101	-47.21584	GBIF	Brazil
	-0.86084	-48.13088	GBIF	Brazil
	-1.16945	-47.80143	GBIF	Brazil
	-5.51809	-47.50243	GBIF	Brazil
	-0.86797	-52.53278	GBIF	Brazil

Species	Latitude	Longitude	References	Country
	-30.03333	-51.38333	Klein et al. 2013	Brazil
	-28.68972	-49.43194	Campos et al. 2009	Brazil
	-28.93472	-49.36306	Campos et al. 2009	Brazil
	-10.21278	-48.36028	Fragoso et al	Brazil
	-13.28339	-50.17023	Couto et al.	Brazil
	-29.63333	-54.05000	Pasini et al. 2019	Brazil
	-5.03927	-42.78957	et al. 2019	Brazil
	-26.94389	-48.75861	Hickel & Oliverira 2020	Brazil
	-28.93139	-49.49778	Hickel & Oliverira 2020	Brazil
	-30.05000	-50.86667	Acosta et al. 2017	Brazil
	-31.14500	-54.19583	Ricalde et al. 2015	Brazil
	-20.99361	-41.61111	Vélez et al. 2020	Brazil
	-20.51583	-41.18583	Vélez et al. 2020	Brazil
	-20.39500	-41.08028	Vélez et al. 2020	Brazil
	-20.13778	-41.13861	Vélez et al. 2020	Brazil
	-19.82861	-40.66556	Vélez et al. 2020	Brazil
	-19.68583	-40.28861	Vélez et al. 2020	Brazil
	-19.67667	-40.38778	Vélez et al. 2020	Brazil
	-19.49222	-40.76250	Vélez et al. 2020	Brazil
	-19.56917	-40.99389	Vélez et al. 2020	Brazil
	-18.56667	-40.72333	Vélez et al. 2020	Brazil
	-18.48889	-40.82917	Vélez et al. 2020	Brazil
	-18.59111	-40.89167	Vélez et al. 2020	Brazil
	-11.68677	-49.86214	Vieira	Brazil
	-11.21523	-49.38393	Vieira	Brazil
	-11.39840	-49.57863	Vieira	Brazil
	-11.96168	-49.69759	Vieira	Brazil
	-11.85534	-49.68663	Vieira	Brazil
	-11.78763	-49.75825	Vieira	Brazil
	-10.92140	-49.86177	Vieira	Brazil
	-10.80594	-49.75203	Vieira	Brazil
	-11.45729	-49.93210	Vieira	Brazil
	-29.78984	-57.01985	Vieira	Brazil
	-16.50381	-49.28296	Vieira	Brazil
	-16.43614	-49.39581	Vieira	Brazil

Species	Latitude	Longitude	References	Country
	9.63000	-74.91222	GBIF	Colombia
	-1.07544	-69.51375	GBIF	Colombia
	8.56694	-83.49226	GBIF	Costa Rica
	8.67910	-83.56671	GBIF	Costa Rica
	8.75900	-83.28300	GBIF	Costa Rica
	9.10008	-83.12448	GBIF	Costa Rica
	9.51935	-82.95628	GBIF	Costa Rica
	9.63336	-83.00343	GBIF	Costa Rica
	10.40126	-84.04931	GBIF	Costa Rica
	10.46641	-84.11639	GBIF	Costa Rica
	10.53955	-83.50648	GBIF	Costa Rica
	10.61046	-84.99497	GBIF	Costa Rica
	10.64405	-83.74201	GBIF	Costa Rica
	10.88327	-85.41354	GBIF	Costa Rica
	10.89381	-84.78885	GBIF	Costa Rica
	17.54333	-99.91056	GBIF	Mexico
	17.55000	-101.28833	GBIF	Mexico
	-25.36725	-57.16654	GBIF	Paraguay
	25.50817	-80.50406	GBIF	USA
	25.76272	-80.49844	GBIF	USA
	25.77882	-80.30816	GBIF	USA
	26.07648	-80.31082	GBIF	USA
	27.19723	-82.48316	GBIF	USA
	27.38865	-81.08327	GBIF	USA
	27.18284	-81.35208	GBIF	USA
	26.66890	-80.63122	Cherry et al. 1998	USA
	26.64217	-80.47756	VanWeelden et al. 2020	USA
Op	-34.98606	-62.98843	GBIF	Argentina
	-33.44694	-60.79376	GBIF	Argentina
	-33.15751	-59.34817	GBIF	Argentina
	-32.87720	-60.67367	GBIF	Argentina
	-32.86730	-61.15417	GBIF	Argentina
	-31.87268	-62.71908	GBIF	Argentina
	-31.73228	-60.47010	GBIF	Argentina
	-31.67454	-60.75451	GBIF	Argentina
	-31.43129	-60.91839	GBIF	Argentina
	-31.21350	-61.61387	GBIF	Argentina
	-30.66755	-59.97172	GBIF	Argentina
	-27.38511	-55.59280	GBIF	Argentina
	12.55778	-70.05341	GBIF	Aruba

Species	Latitude	Longitude	References	Country
	-17.34272	-63.84020	GBIF	Bolivia
	-16.91667	-65.36667	GBIF	Bolivia
	-31.33333	-54.09972	Species Link	Brazil
	-30.30242	-51.29940	Species Link	Brazil
	-30.01886	-51.23888	Species Link	Brazil
	-29.31369	-49.75179	Species Link	Brazil
	-29.13198	-56.52482	Species Link	Brazil
	-27.68397	-48.50236	GBIF	Brazil
	-26.75478	-48.72918	GBIF	Brazil
	-23.05891	-50.98712	GBIF	Brazil
	-20.47000	-49.21000	GBIF	Brazil
	-9.59758	-55.93131	GBIF	Brazil
	-3.09041	-59.99175	GBIF	Brazil
	-2.39396	-54.73186	GBIF	Brazil
	-30.10357	-51.32667	GBIF	Brazil
	-32.10500	-52.19008	GBIF	Brazil
	-30.67818	-51.41298	GBIF	Brazil
	-1.36219	-48.37893	GBIF	Brazil
	-2.93101	-47.21584	GBIF	Brazil
	0.90289	-52.00501	GBIF	Brazil
	-1.95632	-54.73481	GBIF	Brazil
	-7.77023	-51.13067	GBIF	Brazil
	-1.46787	-48.44181	GBIF	Brazil
	-6.08429	-50.17664	GBIF	Brazil
	-4.87716	-43.36004	GBIF	Brazil
	-16.44611	-49.42639	Ferreira et al. 2002	Brazil
	-7.12917	-55.38917	Krinski & Foerster 2017	Brazil
	-30.00000	-51.45000	Albuquerque 1993	Brazil
	-28.68972	-49.43194	Campos et al. 2009	Brazil
	-28.93472	-49.36306	Campos et al. 2009	Brazil
	-10.21278	-48.36028	Fragoso et al	Brazil
	-13.28339	-50.17023	Couto et al.	Brazil
	-29.63333	-54.05000	Pasini et al. 2019	Brazil
	-28.23333	-52.40000	Bianchi et al. 2018	Brazil
	-5.03927	-42.78957	Silva et al. 2019 - Silva et al. 2021	Brazil
	-26.94556	-48.76167	Hickel & Oliverira 2020	Brazil
	-28.93139	-49.49778	Hickel & Oliverira 2020	Brazil
	-30.05000	-50.86667	Acosta et al. 2017	Brazil
	-11.97993	-49.68315	Vieira	Brazil
	-11.48611	-49.98250	Vieira	Brazil
	-11.68677	-49.86214	Vieira	Brazil
	-11.39840	-49.57863	Vieira	Brazil
	-10.92140	-49.86177	Vieira	Brazil
	-10.80594	-49.75203	Vieira	Brazil
	-11.87516	-49.70312	Vieira	Brazil
	-11.78720	-49.75695	Vieira	Brazil
	-11.80873	-49.62501	Vieira	Brazil
	-30.08020	-55.69759	Vieira	Brazil
	-31.81180	-52.46922	Vieira	Brazil
	4.11810	-73.68340	GBIF	Colombia
	4.91845	-72.37394	GBIF	Colombia
	19.30068	-70.19942	GBIF	Dominican Republic
	-0.67435	-76.39735	GBIF	Equador
	4.86778	-58.93742	GBIF	Guyana
	6.46389	-57.75694	Sutherland & Baharally 2002	Guyana
	8.37275	-82.27179	GBIF	Panamá
	5.94095	-57.00038	GBIF	Suriname
	7.87882	-67.46335	GBIF	Venezuela