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RESEARCH

Melanagromyza vignalis Spencer (Diptera: Agromyzidae): a new invasive pest impacting cowpea, *Vigna unguiculata* (L.) Walp., in Brazil

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Abstract The pod fly *Melanagromyza vignalis* Spencer (Diptera: Agromyzidae) has posed a major constraint to cowpea cultivation because it damages pods and seeds, thus representing a pest of significant economic importance. This study aimed to clarify the identity of the pod fly responsible for damage to cowpea crops in the North and Northeast regions of Brazil. Specimens collected from cowpea production areas in the states of Pará, Paraíba, and Pernambuco were identified as *M. vignalis* through morphological and molecular analyses. The species is native to the African continent, and this report constitutes the first record for South America. The larvae damage developing grains, rendering them unfit for consumption. Species identification represents the first step toward implementing appropriate control strategies for this pest in Brazil.

Keywords Alien species · COI barcoding · Cowpea pest · Pod fly · Taxonomy

Introduction

The genus *Melanagromyza* Hendel comprises more than 300 described species (Boucher, 2010; Xuan et al., 2023). Most of them are characterized by larvae with an endophytic habit in stems or shoots, although some develop in roots, seeds, and capitula (Boucher, 2010). In the Neotropical region, approximately 93 species are currently known, a number that is

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certainly underestimated considering the likely undiscovered diversity of *Melanagromyza* in the region (Boucher & Pollet, 2021; Spencer, 1983; Spencer & Stegmaier, 1973). Species of *Melanagromyza* interact with a wide range of plant families, including Convolvulaceae, Solanaceae, Fabaceae, Asteraceae, Boraginaceae, and Brassicaceae (Boucher, 2010).

Among the economically important species are *M. caerula* (Malloch), whose larvae infest seeds of *Ipomoea batatas* (L.) Lam. (Convolvulaceae); *M. tomaterae* Steyskal and *M. caucensis* Steyskal, which attack tomato (*Solanum lycopersicum* L.) (Solanaceae) stems; *M. phaseolivora* Spencer, which develops in common bean pods (*Phaseolus vulgaris* L.) (Fabaceae) (Galván et al., 2002; Spencer, 1973; Spencer & Stegmaier, 1973); *M. sojae* (Zehntner) and *M. obtusa* (Malloch), recorded on soybean (*Glycine max* (L.) Merr.) and common bean (*P. vulgaris*) in South America (Martínez-Alava et al., 2016; Czepak et al., 2018), respectively; and *M. vignalis* Spencer, an important pest in African and the Middle Eastern countries, responsible for yield losses exceeding 50% in cowpea [*Vigna unguiculata* (L.) Walp.] under conventional cultivation (Çıkman & Uygun, 2003; Parh, 1999).

In Brazil, cowpea cultivation is concentrated mainly in the North and Northeast regions, with an estimated production of 488.4 thousand tons in the 2024/25 growing season, corresponding to approximately 66% of national production (Freire Filho et al., 2011; CONAB, 2025). However, a yet unidentified *Melanagromyza* species has caused severe damage to pods and grains of the crops, resulting in yield losses and raising concern among farmers in these regions (Canal Agro, 2023; Sinimbu, 2023).

Given the economic relevance of cowpea in Brazil and the scarcity of taxonomic information on the pod fly attacking this crop, the present study aims to clarify, through morphological and molecular analyses, the identity of the species infesting cowpea in the North and Northeast regions of the country. Aspects related to infestation and the damage caused by this pest species are also discussed.

Material and methods

Specimen collection

Adults and immature stages of the pest species were collected in three states in Northern and Northeastern Brazil (Fig. 1). In the Northern region, 52 specimens were collected in the municipality of Belém, Pará (PA), in infested cowpea pods of three varieties (BRS Marataoã, BRS Pajeú and BRS Rouxinol) in the experimental area of Embrapa Amazônia Oriental

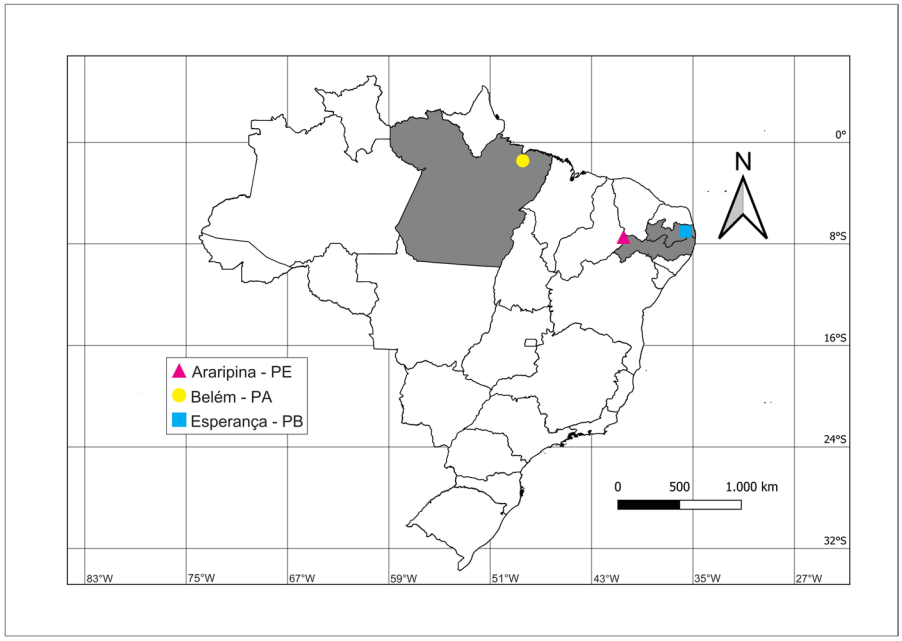


Fig. 1 Sites where occurrences of *Melanagromyza vignalis* Spencer, 1959 have been recorded in Brazil

(1°26′31″S, 48°26′44″W, 24 m) in August 2022. Pods of *V. unguiculata* with infestation symptoms were collected and placed in glass jars covered with voile fabric at 25 °C, ± 80% RH, until adult emergence. In the Northeast region, 55 specimens were collected in the municipality of Araripina, Pernambuco (PE), at the experimental station of the Instituto Agronômico de Pernambuco (7°27′31″S, 40°25′02″W, 831 m) in April 2023, and 18 specimens in the municipality of Esperança, Paraíba (PB) (6°58′51.1″S, 35°54′40.1″W, 625 m) in February 2025. Samples of adults, larvae, and puparia were sent to the Insect Taxonomy Laboratory of the Department of Entomology and Acarology, "Luiz de Queiroz" College of Agriculture (ESALQ/USP), for species identification.

Morphological study

The study of puparia was conducted using a light microscope, and the structures were compared with the descriptions of Spencer (1959, 1973). Adults were examined under a stereomicroscope for taxonomic identification. Identification at the genus level was based on the key of Boucher (2010), whereas identification at the species level was performed using the key and descriptions of Spencer (1959, 1973). Male genitalia were prepared according to Shi and Gaimari (2015), which consists of removing the abdomen and placing it in a 10% potassium hydroxide (KOH) solution for 5–7 min, followed by rinsing in water and transferring to glycerin for structure examination. Subsequently, the genitalia of each specimen were transferred to a plastic microtube containing glycerin, which was attached to the same pin as the respective specimen. The genitalia were examined using a Leica DM1000 microscope equipped with a camera lucida for illustrations. Terminology of the male genitalia followed Shi and Gaimari (2015).

Images of the wing and puparium were captured using a Leica DFC 450 camera mounted on a Leica M205 C stereomicroscope. The puparium was examined using a JEOL JSM-IT300 scanning electron microscope. For this purpose, the dried puparium, sputter-coated using a Balzers SCD 050 equipment, was mounted and fixed with double-sided carbon tape on a metal stub. The photographic plates were produced using CorelDRAW 18. Illustrations of the genitalia were vectorized using Inkscape v1.4.2 and edited with GIMP 2.10.36. Voucher specimens are deposited in the "Luiz de Queiroz" Entomology Museum (MELQ) under accession numbers ESALQENT001793–ESALQENT001804.

Molecular analysis

A fragment of the *cytochrome c oxidase I* (COI) gene was used; amplification was performed using the primers LCO1490 and HCO2198 (Folmer et al., 1994) and LEP-F1 and LEP-R1 (Hebert et al., 2004). We genotyped seven specimens, five from Araripina (PE) and two from Belém (PA). Three specimens from Pará state had issues with amplification and sequencing, most likely due to DNA integrity. It is also noteworthy that samples from Paraíba state were received after genotyping and were characterized only morphologically. DNA extraction was performed using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI), following the manufacturer's protocol. PCR reactions were carried out in a final volume of 25 µL, containing 80 ng of total DNA, 1.5 mM/µL $MgCl_2$, 2.5 mM/µL dNTPs, 0.5 pmol/µL of each primer, 1 U of Platinum Taq DNA Polymerase (Sinapse™, Carlsbad, CA, USA), and 10×Taq DNA buffer. The PCR cycling conditions consisted of an initial denaturation at 95 °C for 2 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 40 s, and a final extension at 72 °C for 10 min.

Subsequently, PCR products were checked by agarose gel electrophoresis (1.5% w/v) and photodocumented. After amplification, samples were purified with 0.33 µL of Exo + SAP (Cellco Biotec™, São Carlos, SP, BR), 0.67 µL of ultrapure water, together with 10 µL of PCR product, incubated at 37 °C for 30 min and then at 80 °C for 15 min. Next, 2.5 µL of primer was added. The prepared samples were sequenced using the Sanger method at the Center for Agricultural Biotechnology, Department of Biological Sciences, "Luiz de Queiroz" College of Agriculture (CEBETEC-ESALQ-USP). The resulting sequences were checked for integrity and edited in BioEdit 7.2 (Informer Technologies, Inc.). The generated sequences were deposited in the National Center for Biotechnology Information (NCBI) databases (PQ514039–PQ514045).

To infer genetic distances and relationships among genotyped *Melanagromyza* specimens collected from cowpea in Brazil, as well as sequences from

public databases, we employed the Kimura 2-parameter model (Kimura, 1980; Tamura et al., 2021) and Bayesian inference using MrBayes (Nguyen et al., 2015), as detailed below. These specimens were compared with 17 highly similar Agromyzidae sequences (> 98% similarity) from the Barcode of Life Data Systems (BOLD; BIN: BOLD: ADA3039). Specifically, the dataset includes one *M. vignalis* sequence from Guinea, one Agromyzidae sequence from Trinidad and Tobago, and 15 Agromyzidae sequences from various African countries. Additionally, we included sequences of other *Melanagromyza* species from GenBank (NCBI), namely *M. aenea* (Meigen), *M. aeneoventris* (Fallén), *M. angeliciphaga* Spencer, *M. chaerophylli* Spencer, *M. lappae* (Loew), *M. nigrissima* Spencer, *M. pubescens* Hendel (Roslin et al., 2021), *M. chalcosoma* Spencer, *M. cleomae* Spencer, *M. minimoides* Spencer, *M. virens* (Loew) (Scheffer et al., 2007), *M. metallica* (Thomson) (BOLD: Barcode of Life Data System, 2023), *M. obtusa* (Ashfaq et al., 2018), *M. sojae* (Arnemann et al., 2016), and *M. virginiensis* Spencer (Hebert et al., 2016). The accession numbers for these sequences in BOLD and NCBI are provided in Fig. 2 and Supplementary Table 1.

A dataset of 40 sequences was aligned with ClustalW and trimmed to 658 bp in MEGA 11 (Tamura et al., 2021). Due to the limited number of *Melanagromyza* sequences, sequences that did not span the entire target COI region were retained in the analyses, as they provide meaningful information for species identification. Intra- and interspecific genetic distances were calculated using Kimura's two-parameter model (Kimura, 1980; Tamura et al., 2021). The genetic relationships among the *Melanagromyza* sequences generated in this study were inferred from a Bayesian tree constructed with MrBayes (Nguyen et al., 2015) for 10 million generations. The most appropriate model, selected by Mr. Modelblock, was GTR + I + G (Kalyaanamoorthy et al., 2017). After the analyses were completed, 25% of the initial trees were discarded as burn-in. *Ophiomyia similata* (Malloch) was used to root the tree.

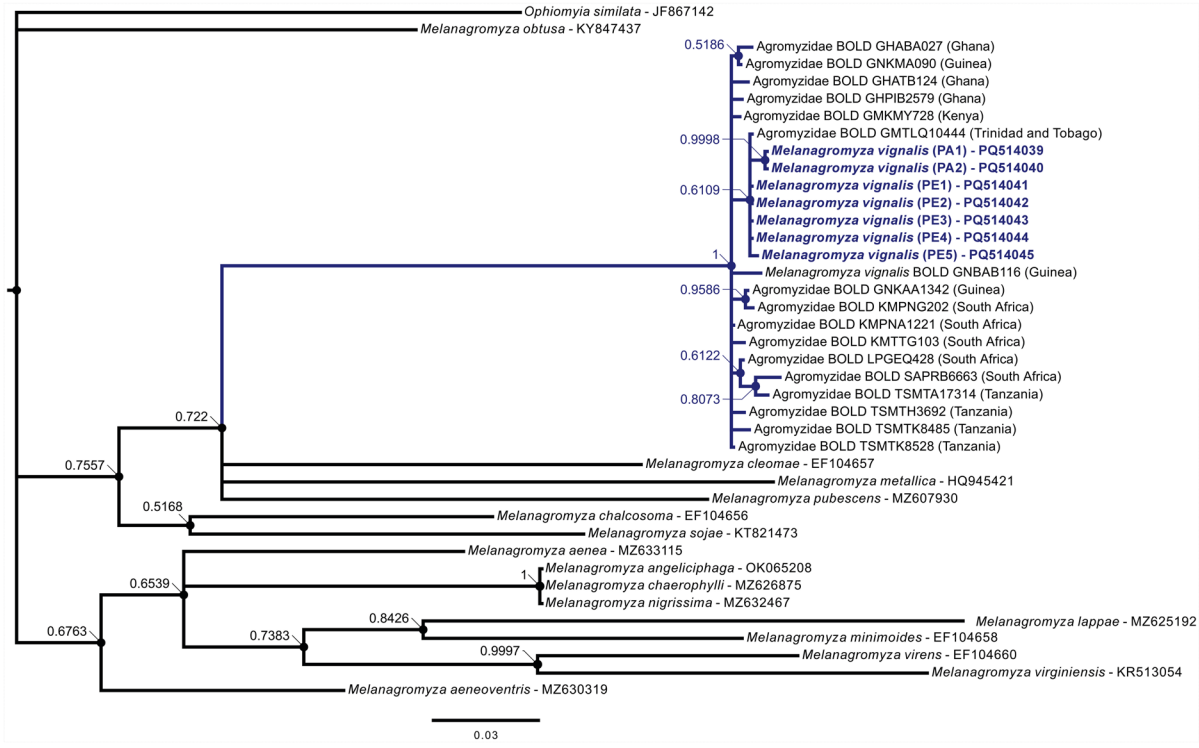


Fig. 2 Bayesian consensus tree of *Melanagromyza* species, highlighting the clade of *M. vignalis* and related South American and African specimens (blue lines)

Results

A total of 125 adult pod flies from Belém (PA), Araripina (PE), and Esperança (PB) were examined morphologically and identified as *Melanagromyza vignalis* Spencer, 1959 (Fig. 3a). The record of *M. vignalis* infesting cowpea pods and grains in Brazil, in Pará (PA), Pernambuco (PE), and Paraíba (PB) states (Fig. 1), represents the first record of the species in South America. Adults of *M. vignalis* associated with cowpea exhibit a dark metallic green coloration (Fig. 3a), hyaline wings, up to 2.7 mm in length, and vein C strongly extending to vein M_{1+2} (Fig. 3b). The pod fly can be distinguished from other *Melanagromyza* species by characteristics of the male genitalia (Fig. 4) and the puparium (Fig. 5).

Male genitalia Epandrium setulose (Fig. 4a). Surstylus truncated at the apex, bearing five short, robust spines, adjacent to a cluster of three slender, elongated apical setae (Fig. 4a). Cerci setulose, subovoid, approximately twice as long as wide (Fig. 4a). Hypandrium Y-shaped, with the inner margin weakly sclerotized at the apex and a short apical apodeme measuring about one-quarter of the total length (Fig. 4a). Ejaculatory apodeme well developed and asymmetric, with a dark medial region; blade flat, fan-shaped, narrowing into a short peduncle, with a single small latero-basal process (Fig. 4b). Ejaculatory hood transverse and slender, with broad extremities directed upward (Fig. 4b). Phallapodeme straight, approximately 1.5 times longer than the hypandrium (Fig. 4c). Aedeagus with a membranous layer; basiphallus asymmetric, U-shaped, with the right lateral portion elongated and forming a membranous extension, and the left lateral portion short and weakly delimited; phallapodeme with an acuminate apex in ventral view; mesophallus tubular, rotated to the right and forming a median extension toward the apex of the distiphallus (Fig. 4d); distiphallus forming lobes partially fused medially, with the posterior portion globose, similar in size to the basiphallus and as wide as long, whereas the distal portion forms a pair of projections that are initially broadened and become acuminate toward the apical region (Fig. 4c and d).

The puparium of *M. vignalis* is longer than wide, brown in color; the anal segment bears two divergent protuberances (Fig. 5a, b), which at their apex show the scars of the posterior spiracles from the last larval instar (Fig. 5c), conspicuous due to their dark coloration at the tips of the protuberances (Fig. 5a). At the apex of each protuberance, there is an elliptical structure composed of up to 12 scars of spiracular openings arranged around a central scar (Fig. 5c).

DNA barcoding sequence analyses suggest that the Brazilian cowpea genotyped specimens belong to the *M. vignalis* taxon, as they closely match the sequence of a *M. vignalis* specimen from Guinea, supporting the morphological identification. Intraspecific pairwise genetic distances (K2P) among Brazilian sequences (PA and PE states) varied from 0.0000 to 0.0045, while the distances between Brazilian sequences and the Guinea sequence ranged

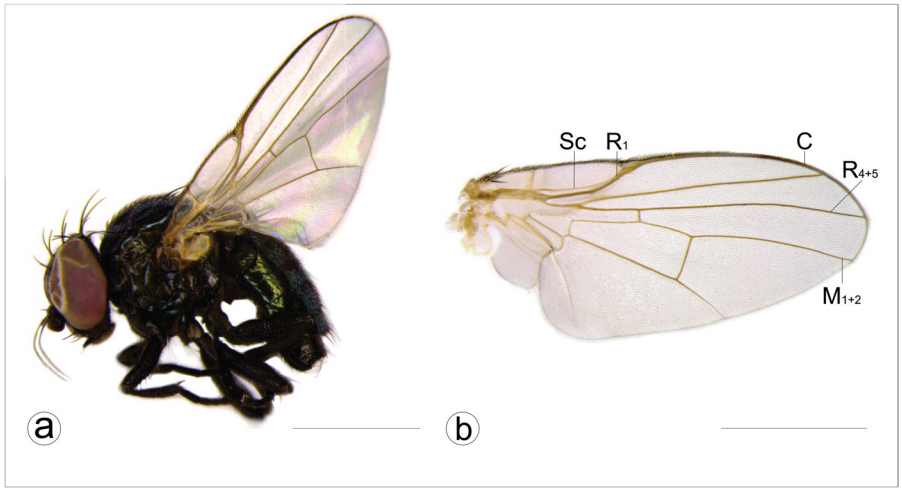


Fig. 3 *Melanagromyza vignalis* Spencer, 1959. **a**. Adult, lateral view, **b**. Wing. Scale bar = 1 mm

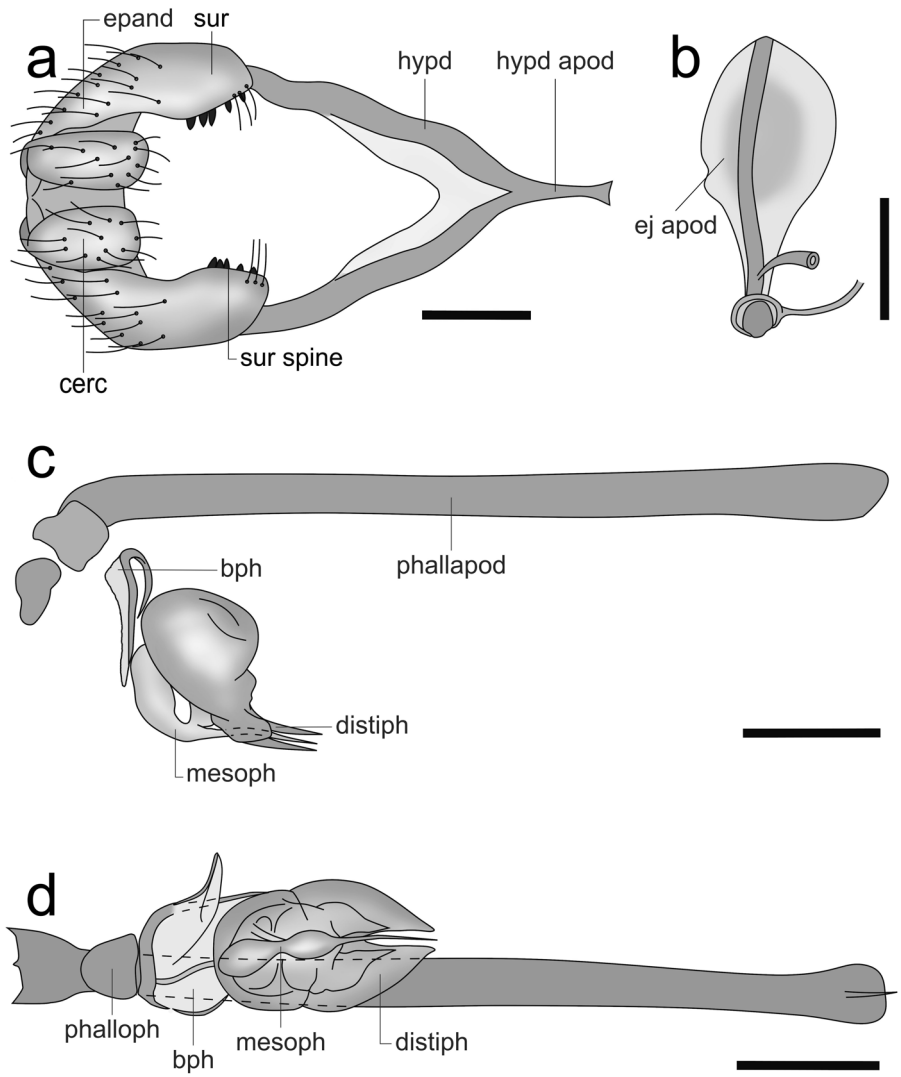


Fig. 4 *Melanagromyza vignalis* Spencer, 1959. Male genitalia. **a**. Epandrial complex, ventral view. **b**. Sperm pump, lateral view. **c**, **d**. Phallic complex, lateral and ventral views. Scale bar = 0.1 mm. Abreviations: bph, basiphallus; cerc, cercus; distiph, distiphallus; ej apod, ejaculatory apodeme; epand, epandrium; hypd – hypandrium; hypd apod, hypandrial apodeme; mesoph, mesophallus; phallapod, phallapodeme; phalloph, phallophorus; sur, surstylus; sur spine, surstylus spines

from 0.0138 to 0.0169 (see Supplementary Table 1). Furthermore, the genetic distance between the Trinidad and Tobago specimens and Brazilian sequences ranged from 0.0000 to 0.0030, while the distance from African continent sequences to Brazilian ones ranged from 0.0061 to 0.0155 (considered conspecific here). In contrast, interspecific distances among *Melanagromyza* species varied from 0.1474 to 0.2019 when compared with the Brazilian cowpea specimens (Supplementary Table 1). The Bayesian tree (Fig. 2) clustered the Brazilian specimens, the Guinean sequence of *M. vignalis*, and also the Trinidad and Tobago, and African Agromyzidae sequences into a single clade, supporting the morphological data from Brazilian specimens identified as *M. vignalis*.

Discussion

The identification of *M. vignalis* from Brazil was based on a combined approach, utilizing both morphological characteristics (Spencer, 1959, 1973; Tavares & Baker, 2008) and COI molecular data (Folmer et al., 1994; Hebert et al., 2003). The species identification relied on detailed morphological analysis of key diagnostic features, such as male genitalia. These findings were sustained by DNA barcoding, as the Brazilian specimens grouped with *M. vignalis* from Guinea (the species' native range), thereby supporting the morphological identification. Therefore, this study presents the first record of *M. vignalis* in Brazil (South America), offers an updated

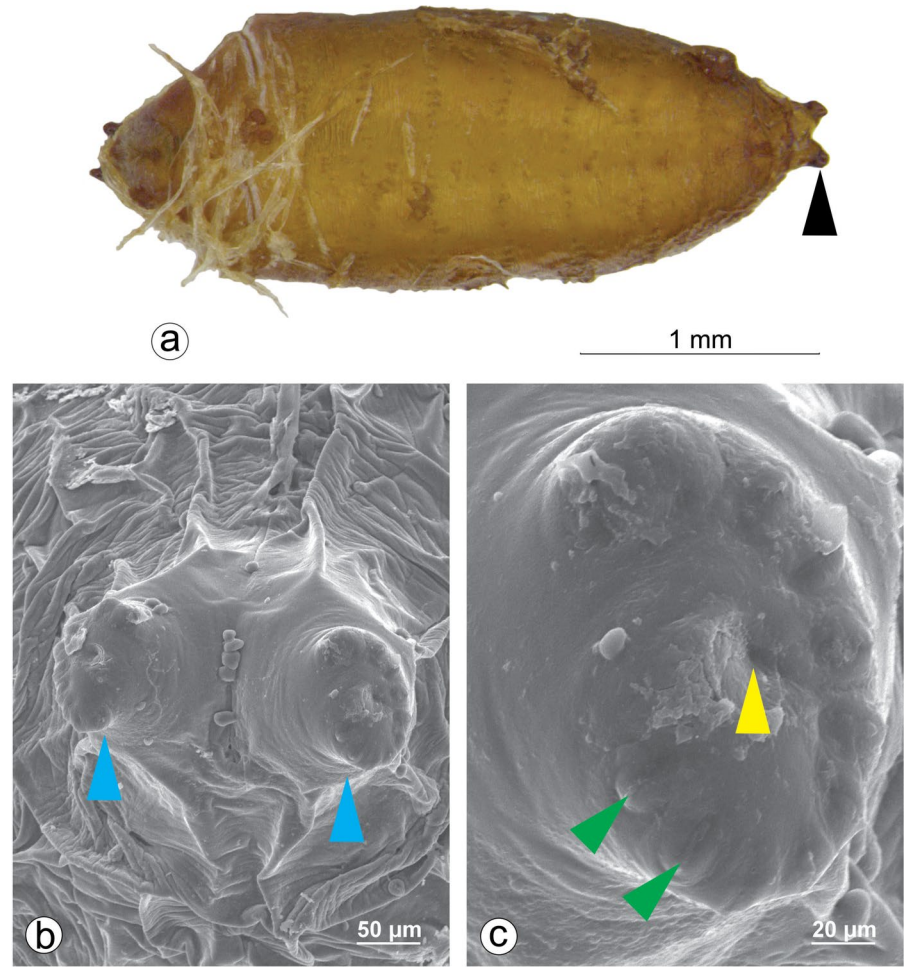


Fig. 5 *Melanagromyza vignalis* Spencer, 1959. **a**. Puparium, ventral view, anal protuberance, posterior spiracular processes with brown apex (black arrow). **b**. Divergent spiracular processes (blue arrow). **c**. Protuberance with scars from the spiracular openings (green arrow) and central scar (yellow arrow)

morphological description of male genitalia of the species, and provides a mitochondrial marker suitable for rapid and reliable species identification.

Based on the results, we propose that the pod fly sequences from cowpea in Brazil belong to *M. vignalis*. This conclusion is supported by DNA barcoding variation below 0.04, a threshold commonly used for intraspecific variation in Diptera (Meier et al., 2006; Renaud et al., 2012; Sinclair & Gresens, 2008). The observed intraspecific variation ranged from 0.0000 to 0.0169, within the typical range for intraspecific variation, indicating natural polymorphisms among individuals within the same gene pool (Boucher & Savage, 2022). Conversely, genetic divergence among different *Melanagromyza* species ranged from 0.1474 to 0.2019, well above species thresholds, reflecting accumulated mutations over millions of years of reproductive isolation (Boucher & Savage, 2022; Zhang & Bu, 2022). Additionally, the Bayesian analysis supports the hypothesis that the Brazilian, Trinidad and Tobago, and African specimens belong to the same species, as indicated by the bootstrap value (Fig. 2). The specimen from Trinidad and Tobago is genetically more similar to the Brazilian specimens than to the African specimens, which could indicate a bridgehead invasion effect from Brazil to Trinidad and Tobago (see Fig. 2), or vice versa. However, more data are needed to draw any conclusions in that regard.

The occurrence of *M. vignalis* in the North and Northeast regions of Brazil, which concentrate the largest share of the national cowpea production (CONAB, 2025), compromises grain yields due to the damage caused by this pest and calls for attention from plant protection authorities to monitor the species in other cowpea-producing states. Until now,

the distribution of *M. vignalis* had been restricted to the African continent, with infestation records on *V. unguiculata* in South Africa, Mali, Nigeria, Kenya, Senegal, and Sierra Leone (Spencer, 1973). However, Spencer (1973) also reported its occurrence on *Glycine* sp., reinforcing the potential phytosanitary risk posed by *M. vignalis* to soybean crops. There are also records of *M. vignalis* associated with other hosts from different botanical families in Turkey, including cotton (*Gossypium hirsutum* L.), pumpkin (*Cucurbita moschata* L.), zucchini (*Cucurbita pepo* L.), tomato (*Solanum* sp.), and amaranth (*Amaranthus* sp.) (Çıkman & Uygun, 2003). Nevertheless, these records should be treated with caution, as they differ from the infestation pattern of *M. vignalis* on cowpea and from the oligophagous habit typical of *Melanagromyza* species.

Infestation of cowpea by *M. vignalis* is characterized by oviposition on the pod and larval development within the seeds. Oviposition occurs on pods that are still developing when they are green and tender. After hatching, the larvae penetrate the pod through a small gallery opened by the mandibles of the cephalopharyngeal skeleton, reaching the grains, where the most severe damage occurs (Fig. 6a–c). Pods and grains infested by the larvae show dark spots at the points of penetration and internal galleries (Fig. 6a), compromising grain development and quality. Last-instar larvae open an exit gallery for adult emergence on the inner portion of the grain (Fig. 6c) and at the pod margin (Fig. 6d). Puparia are formed inside the grain (Fig. 6c) or within the pod between the grains (Fig. 6a, d), where they remain protected until adult emergence. In mature (dry) pods and seeds, the damage results in grains that are unfit for consumption and commercialization (Fig. 6b–c), thereby reducing crop productivity.

The feeding habit of *M. vignalis* larvae in cowpea pods in West Africa was briefly reported by Spencer (1973). Among the seven *Melanagromyza* species known to share this habit (Spencer, 1990), *M. vignalis* is morphologically most similar to *M. obtusa*, which has not been recorded in Brazil, due to the metallic green coloration of the adults and the development of the immature stages in bean pods of *P. vulgaris*. However, according to Spencer (1973), the two species differ in the shape of the aedeagus, which is asymmetric in *M. vignalis* and symmetric in *M. obtusa*. In addition, there are currently no records of *M. obtusa* infesting pods and grains of *V. unguiculata*.

The endophytic habit of *M. vignalis* poses an additional challenge for its management, as it reduces the

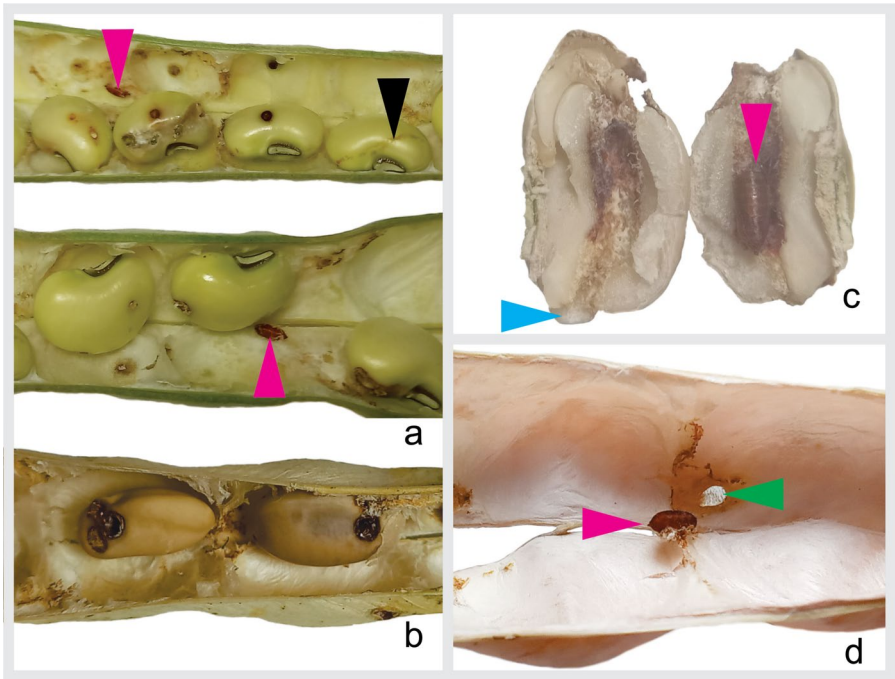


Fig. 6 Symptoms of attack by *Melanagromyza vignalis* Spencer, 1959 on *Vigna unguiculata* (L.) Walp. in Brazil: **a**. Damage to grains with galleries (black arrow) and puparium inside pods (pink arrow). **b**. Symptoms in mature grains. **c**. Larval gallery within the grain (blue arrow) and puparia (pink arrow). **d**. Puparium inside the pod (pink arrow) and adult emergence hole (green arrow)

effectiveness of contact insecticides. There are no recorded tests involving systemic insecticides, highlighting an important gap regarding the potential of these phytosanitary products for pest management and reinforcing the need for studies evaluating their efficacy. Although there are records of biological control by parasitoids and entomopathogenic fungi acting on larvae of *Melanagromyza* species (Bapfubusa et al., 1990; Salgado-Neto et al., 2018), the literature on chemical control remains scarce and outdated, with only Parh (1999) reporting attempts to control the pest using contact insecticides in Africa.

Introduced pest species are among the leading causes of agricultural losses, potentially threatening the food security of entire regions (Cook et al., 2011). The pod fly *M. vignalis* was originally described from specimens collected in Mali and Sierra Leone (Spencer, 1959), both in West Africa, which is also the center of origin of the cowpea *V. unguiculata*, its primary host (Panzeri et al., 2022). It is plausible that the species has dispersed to countries such as Turkey (Çıkman & Uygun, 2003) and Brazil through the international trade of seeds and plant material, facilitated by globalization (Venette & Hutchison, 2021). However, the low genetic divergence between Trinidad and Tobago and Brazilian specimens prompts questions regarding the source of the Brazilian invasion (see Fig. 2). In contrast, the genetic similarity observed between South American and African specimens reinforce the hypothesis that the species was introduced from its native center to South America (Fig. 2). Further research is needed to determine whether these populations stem from a single earlier introduction or multiple recent incursions. Climatic similarities between tropical Brazilian areas and sub-Saharan Africa, the absence of specialized natural enemies, the availability of *V. unguiculata* as a suitable host, and the internal movement of plant material have likely favored the spread of *M. vignalis* and its establishment in the North and Northeast regions of Brazil.

Therefore, this study provides a starting point for future research on *M. vignalis*, a pest associated with cowpea in Brazil. Accurate species identification, achieved through morphological methods and the COI genetic marker, represents a fundamental step toward advancing other areas of knowledge within integrated management programs for the pod fly in Brazilian cowpea crops.

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Author contributions LSD, ACSN, and MS conceived the research and, together with RAZ, ALL, and FH, wrote and edited the manuscript. MCE and NJSM identified *Melanagromyza vignalis* specimens, performed the morphological study. LSD, MS and NJSM prepared the plates. FH performed the molecular analyses. MS took the photographs of *M. vignalis*. LSD, AFC, and JBM supervised the fieldwork and specimen processing. All authors participated in the research and approved the final version of the manuscript.

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Data availability Data generated and retrieved for molecular analysis can be obtained from public repositories utilizing their respective accession or identification numbers.

Declarations

Authorization This publication was authorized by the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA; document No. 416/2025/DSV/SDA/MAPA).

Ethical approval Not applicable.

Conflicts of interest The authors declare no competing interests.

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