Investigating *Liriomyza* (Diptera: Agromyzidae) Populations From Northeastern Brazil: mtDNA Analyses of the Global Pests *L. sativae* and *L. huidobrensis*

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Subject Editor: Kelley Tilmon

Received 6 August 2021; Editorial decision 6 December 2021

Abstract

Species of *Liriomyza* Mik (Diptera: Agromyzidae) occur worldwide and are economically important leafminers. However, populations of some pest species, although very similar morphologically, show highly divergent mtDNA sequences, suggesting that nominal species are in fact complexes of cryptic species. This study focuses on two globally invasive pests, *L. huidobrensis* (Blanchard) and *L. sativae* Blanchard, already known to be highly destructive in Brazilian crops, although only a few studies on morphological and genetic divergences of them have been made. A total of 63 sequences of the mitochondrial cytochrome oxidase I (COI) and cytochrome oxidase II (COII) from Brazilian populations of *L. huidobrensis* and *L. sativae* collected from six crops (gypsophila, chrysanthemum, melon, watermelon, tomato, and onion) in Northeastern Brazil were generated to investigate their genetic structure together with available sequences from the Americas, Europe, Asia, Africa, and Australia. Genetic structure was not found to be correlated to neither host plant nor geographical locality. *Liriomyza huidobrensis* showed an overall low intraspecific global genetic divergence in both genes. On the other hand, high intraspecific divergences for *L. sativae* and its phylogenetic position confirm a divergent clade currently found only in Brazil and suggest it may be a global complex of cryptic species. Considering the possibility of cryptic species (in the latter case), we provided detailed redescriptions of these Brazilian populations for future studies and local management of these global pests. Finally, our results also revealed a new synonym herein proposed, *L. strigosa* Spencer as a junior synonym of *L. huidobrensis*.

Key words: cytochrome oxidase, leafminer, Brazilian population, pea leafminer, morphology

*Liriomyza* Mik (Diptera, Agromyzidae) is a diverse genus of Phytomyzinae, with 456 species worldwide distributed (ITIS 2016). Boucher (2010) recorded 85 species in the Neotropical Region, with this number to be updated to 114 in 2019 (Sousa et al. in prep.). *Liriomyza* species are leaf mining flies recorded from a large number of host plants (Benavent-Corai et al. 2005), and this genus has the largest number of host plants recorded among agromyzids (Scheffer et al. 2007). Most species are monophagous or oligophagous (Boucher 2010), however, some are polyphagous and considered potential agricultural pests.

A molecular phylogenetic analysis supported *Liriomyza* as a monophyletic genus (Scheffer et al. 2007). Most species have frons, postpronotum and notopleuron yellow, with a broad yellow medial stripe on scutellum, strongly contrasting with the dark brown to black background, reclinate orbital setulae, a costal vein extending to vein M₁, usually a well-developed vein dm-cu, a surstylus that is separate from the subependrial sclerite, a stridulatory organ sometimes visible in males, and apical section of the ejaculatory duct swollen (Lonsdale 2011). Lonsdale (2011) considered that only the last character could reliably diagnose *Liriomyza*. Some other important diagnostic characters of this genus are: inner process of...
pregonite always lacking and surstyli of various shapes, fully reduced or completely fused with epandrium (Zlobin 1996). Other similar genera, including *Haplopoedes* Steyskal, *Phytoliriomyza* Hendel, and *Metapomyna* Enderlein, together with *Liriomyza* form a monophyletic group (Lonsdale 2011). *Galiomyza* Spencer was recently synonymized with *Liriomyza* due to the presence of an apically swollen and pigmented ejaculatory duct, considered a unique and consistent synapomorphy (Lonsdale 2017).

*Liriomyza huidobrensis* (Blanchard) and *L. sativae* Blanchard are two of the seven species considered polyphagous and are important examples of potential agricultural pests worldwide (Spencer 1973, Lonsdale 2011). *Liriomyza huidobrensis* was first described from Argentina, mining leaves on *Cineraria* sp. (Asteraceae) in Buenos Aires (Blanchard 1926), but has already been recorded from 365 species of 49 botanical families (Weintraub et al. 2017). This species is globally known as a pest species, however, it has been eradicated or currently controlled by natural enemies in some parts of the world. In South and Central America and Africa it is still a serious agricultural pest. Newly established populations can be devastating, and *L. huidobrensis* is currently the most destructive pest among agromyzids (Scheffer et al. 2014). *Liriomyza huidobrensis* and *L. langeri* Frick are closely related and were once considered synonyms (Spencer 1973). Both species are morphologically very similar and it is not always possible to distinguish them only by external morphology; although analyses of the barcode region of mitochondrial cytochrome oxidase I has improved species identification (Weintraub et al. 2017). *Liriomyza sativae* was described from alfalfa as a host plant in La Pampa, Argentina (Blanchard 1938), but occurs in many host plants (Spencer 1973). Its coloration is very varied, closely resembling other species, such as *L. brassicace* (Riley), *L. eupatori* (Kaltenbach), *L. belantii* Spencer, *L. sabazae* Spencer, some morphs of *L. langeri* (Lonsdale 2011), and its sister species *L. trifoli* (Burgess) (Scheffer and Lewis 2006).

Seventeen species of *Liriomyza* have been recorded from Brazil (Sousa et al. unpublished). Nevertheless, both *L. huidobrensis* and *L. sativae* have been recorded as the most severe *Liriomyza* pests on Cucurbitaceae, Fabaceae, Solanaceae, and Asteraceae (Esposito et al. 1992, Costa-Lima et al. 2010, Araujo et al. 2013, Ferreira et al. 2017, Parish et al. 2017). Other species have few host crop records, such as, *L. trifoli* on coffee (Silva et al. 2015) and *L. brassicace* on Brassicaceae (Parish et al. 2017). Despite their economic importance in Brazil, little is known about their biology, host plants, as well as, their genetic structure. For example, only recently, an analysis of partial cytochrome c oxidase subunit I sequences revealed a divergent lineage of *L. sativae* in Brazil (Parish et al. 2017).

Phylogenetic studies with DNA sequences have been conducted for *Liriomyza* identifications and population surveys (Scheffer 2000, Scheffer and Lewis 2005). In some of the pest species studied (e.g., *L. cicerina* (Rondani), *L. sativae*, and *L. trifoli*), high mtDNA sequence divergence was found among populations, and authors suggest that they represent potential cryptic species (Scheffer and Lewis 2005, 2006; Pérez-Alquicilla et al. 2018; Carapelli et al. 2018). Other molecular methods were also used to discriminate leafminer species, such as Polymerase Chain Reaction-Restriction Fragment-Length Polymorphism (PCR-RFLP) (Scheffer et al. 2001, Kox et al. 2005) and Muplex PCR method (Miura et al. 2004, Nakamura et al. 2013).

The objective of our study was to investigate the genetic structure of *L. huidobrensis* and *L. sativae* from six cultivated crops in Northeastern Brazil, based on cytochrome oxidase subunit I and subunit II. The use of both markers was needed to compare our results to previously published works that used different genes or gene regions. Our results indicate that genetic structure was not found to be correlated to neither host plant nor geographical locality. Furthermore, comparisons of Brazilian populations of both species with populations from other regions of the world were conducted (which has never been conducted for *L. huidobrensis*) resulting in an overall low intraspecific global genetic divergence in *L. huidobrensis* and confirmation of a divergent clade of *L. sativae* currently found only in Brazil.

### Methods

#### Crops and Study Area

Mined leaves were collected in Mossoró (Rio Grande do Norte), Guaraciaba do Norte and São Benedito (Ceará), and Juazeiro (Bahia) in Northeastern Brazil (Table 1). *Liriomyza* specimens were reared from leaves from six crops: gypsophila (*Gypsophila paniculata* L.) (Fig. 1a), chrysanthemum (*Chrysanthemum morifolium* Ramat.) (Fig. 1b), melon (*Cucumis melo* L.), (Fig. 1c), watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) (Fig. 1d), tomato (*Solanum lycopersicum* L.) (Fig. 1e), and onion (*Allium cepa* L.) (Fig. 1f). Both *Liriomyza huidobrensis* and *L. sativae* were recorded in these crops and localities by Sousa et al. (2021).

#### Specimen Sampling

Leaves with mines were removed from all crops and transported to the laboratory, where they were placed in plastic containers covered with organza mesh until adult emergence. Adults were then mounted, labeled, and identified. For the morphological identification, taxonomic keys were used (Boucher 2010; Lonsdale 2011, 2017). Male and female terminalia were clarified in KOH 10%, prepared in acetic acid, 95–70% ethanol, and placed on temporary slides for dissection and analysis. Larvae were mounted on permanent slides with butyl acetate. High resolution images of adults were made using Leica video camera DFC450C attached to a Leica M205 C stereoscope with the Programa Leica Application Suite software version 4.8.0. Digital images of the larval structures, pupae, and male terminalia were made using an optical microscope NIKON ECLIPSE E200 MV R, with the software Zen 2 (version 2.0). Terminology followed Cumming and Wood (2017), with some exceptions: ‘ori’ and ‘ors’

| Locality                          | Crop          | Variety                  | Geographical coordinates |
|-----------------------------------|---------------|--------------------------|--------------------------|
| Mossoró, Rio Grande do Norte State| Melon         | Goldex                   | S04°03′53.7″ W040°53′34.0″ – 32m |
|                                   | Watermelon    | Quetzali                 | S04°53′43.2″ W037°21′36.7″ – 36m |
| Guaraciaba do Norte, Ceará State  | Tomato        | Paron, Janaina           | S04°03′53.7″ W040°53′43.4″ – 865m |
| São Benedito, Ceará State         | Chrysanthemum | Ómega time golden, Sunny Reagan | S04°03′53.6″ W040°53′43.4″ – 889m |
| Juazeiro, Bahia State             | Gypsophila    | Dynamic love             | S04°03′50.5″ W040°53′14.5″ – 855m |
|                                   | Onion         | –                        | S09.36731° W40.40468° |

Table 1. Cultivated crops in Northeastern Brazil where *Liriomyza* specimens were reared.
as in Lonsdale (2011). Material was deposited in Museu Nacional, UFRJ (MNRJ) and Embrapa Agroindústria Tropical (EMBRAPA) collections. DNA vouchers were deposited at MNRJ collection.

**Molecular Data**

A total of 13 specimens of *L. huidobrensis* (6–7 per cultivar) and 18 specimens of *L. sativae* (4–6 per cultivar) had their genomic DNA extracted (see Table 2). DNA extractions from the head and thorax of adult *Liriomyza* males preserved in 95% ethanol were performed. For DNA extraction, the DNeasy blood & tissue kit (QIAGEN) was used following a modified protocol from the supplier, without maceration of the specimen. The intact abdomen of the specimens and exoskeleton of head and thorax after DNA extraction were preserved as voucher material.
Previously published papers on *Liriomyza* species have used both COI and COII mtDNA markers, thus a comparison of genetic divergences and structuring between species was difficult. Therefore, we have decided to sequence all markers previously used for the two species. Two contiguous regions of the mitochondrial cytochrome oxidase I (COI) were amplified, the COI near the 3′ end (COI-5′-TAGTGCAATGAA-TL2-J-3037, Takiya et al. 2006) and 5′-TTGATTTT-TTAGTGCAATGAA (TL2-J-3037, Takiya et al. 2006) for COI-3′-TTCATTGCACTAATCTGCCATACTA (TL2-N-3014, Simon et al. 1994) and 5′-TTGATTTT-TTAGTGCAATGAA (TL2-J-3037, Takiya et al. 2006) for COII.

Table 2. Species identification, sampling site, host plant, specimen voucher codes, and GenBank accession numbers of sequences generated herein for the three gene fragments studied. Haplotype (H) numbers are given in relation to networks shown in Figs. 12 and 13.

| Species          | Sampling site | Host plant | Voucher      | COI-5′ | COI-3′ | COII  |
|------------------|---------------|------------|--------------|--------|--------|-------|
| *L. huidobrens*  | São Benedito, CE | Gypsophila | ENT3953 MT511771 (HA.1) | MT560562 (HB.1) | MT560841 (HC.3) |
|                  |               | Gypsophila | ENT3954 MT511769 (HA.1) | –       | –      | –     |
|                  |               |           | ENT4233 MT511767 (HA.1) | –       | MT560843 (HC.3) |
|                  |               |           | ENT4234 MT511765 (HA.1) | MT560567 (HB.1) | –      | –     |
|                  |               |           | ENT4235 MT511766 (HA.1) | MT560563 (HB.1) | MT560844 (HC.3) |
|                  |               |           | ENT4236 MT511768 (HA.1) | MT560569 (HB.1) | MT560835 (HC.3) |
|                  |               |           | ENT4237 – | MT560570 (HB.1) | MT560842 (HC.3) |
|                  |               |           | ENT3954 – | MT560565 (HB.1) | MT560838 (HC.3) |
|                  |               |           | ENT4248 MT511764 (HA.1) | –       | MT560836 (HC.3) |
|                  |               |           | ENT4249 MT511762 (HA.1) | MT560564 (HB.1) | MT560839 (HC.3) |
|                  |               |           | ENT4250 MT511770 (HA.1) | MT560568 (HB.1) | MT560840 (HC.3) |
|                  |               |           | ENT4251 MT511763 (HA.1) | MT560571 (HB.1) | MT560837 (HC.3) |
|                  |               |           | ENT4252 – | MT560566 (HB.1) | –      | –     |
|                  | Guaraciaba do Norte, CE | Tomato | ENT3952 MT511757 (SA.1) | MT560578 (SB.1) | –      | –     |
|                  |               |           | ENT4238 MT511750 (SA.1) | MT560580 (SB.1) | –      | –     |
|                  |               |           | ENT4239 MT511752 (SA.2) | MT560577 (SB.2) | –      | –     |
|                  |               |           | ENT4240 – | MT560585 (SB.1) | –      | –     |
|                  | Mossoró, RN | Melon | ENT3950 MT511758 (SA.1) | MT560574 (SB.1) | –      | –     |
|                  |               |           | ENT4228 MT511747 (SA.1) | –       | –      | –     |
|                  |               |           | ENT4229 MT511760 (SA.2) | MT560573 (SB.2) | –      | –     |
|                  |               |           | ENT4231 MT511756 (SA.1) | MT560575 (SB.1) | –      | –     |
|                  |               |           | ENT4232 MT511759 (SA.1) | MT560576 (SB.1) | –      | –     |
|                  |               |           | ENT4233 MT511746 (SA.1) | MT560582 (SB.1) | –      | –     |
|                  |               |           | ENT4234 MT511748 (SA.2) | MT560579 (SB.2) | –      | –     |
|                  |               |           | ENT4235 MT511749 (SA.1) | –       | –      | –     |
|                  |               |           | ENT4236 MT511761 (SA.1) | MT560584 (SB.1) | –      | –     |
|                  | Juazeiro, BA | Onion | ENT3963 MT511754 (SA.1) | MT560581 (SB.3) | –      | –     |
|                  |               |           | ENT4244 MT511755 (SA.1) | MT560572 (SB.1) | –      | –     |
|                  |               |           | ENT4245 MT511753 (SA.1) | –       | –      | –     |
|                  |               |           | ENT4246 MT511745 (SA.1) | –       | –      | –     |

PCR products of the correct size were sent to Macrogen (South Korea) for purification and Sanger sequencing of both DNA strands.

Complementary electrophorograms were assembled in Geneious v.6.1.8 (Biomatters Ltd.) and consensus sequences edited. Correct target region and taxon were confirmed using BLASTn (Genbank). Sequences generated in the present study (Table 2) were all aligned together with available sequences of *Liriomyza* species and *Melanagromyza sojae* Zehntner (outgroup) (KT597923) deposited in GenBank (Supp Tables 1–3 [online only]) using as reference a complete mitochondrial sequence of *L. huidobrens* (JN570505) in Geneious v.6.1.8. From this alignment, three aligned matrices were constructed, one for each fragment, COI-5′ (470 bp of 926 sequences), COI-3′ (775 bp of 799 sequences), and COII (629 bp of 184 sequences).

Neighbor-joining and genetic distances were calculated using MEGA X v.10.0.5 (Kumar et al. 2018). Genetic distances between sequences were estimated using uncorrected distances (p distances) for fragment COI-3′ and COII or distances modeled by Kimura-2-parameter (K2P) for COI-5′ (given that most literature use this substitution model). Neighbor-joining trees with bootstrap support with 500 pseudoreplicates were calculated for the three separate alignments, following the same models used for the genetic distance calculations. Bayesian phylogenetic analyses were conducted on the separate matrices with MrBayes 3.2.6 (Ronquist et al. 2012) with 4 independent runs of 4 chains for 50 million generations sampling every 5,000. Convergence and parameter mixing were evaluated using Tracer 1.71 (Rambaut...
et al. 2018). All markers were modeled under GTR+I+G based on AIC comparisons of likelihood scores of 24 different models with PartitionFinder 2.1.1 (Lanfear et al. 2016).

To evaluate genetic structuring among populations of focal species from different countries in the world, matrices of COI-5′ (26 and 181 sequences), COI-3′ (93 and 288 sequences), and COII (52 sequences of L. huidobrensis) including only L. huidobrensis or L. sativae, respectively, were constructed based on the above-mentioned ones. Haplotype networks of these three markers for each species were constructed using the TCS network method (Clement et al. 2002) with PopART v.4.8.4 (Leigh and Bryant 2015). Phylogenetic trees and haplotype networks were edited in Adobe illustrator CS6 v.16.0.0 (Adobe Systems Inc.).

**Nomenclature**

This paper has been registered in Zoobank (www.zoobank.org), the official register of the International Commission on Zoological Nomenclature. The LSID (Life Science Identifier) number of the publication is urn:lsid:zoobank.org:pub:44890547-034A-4A05-942A-7DC726D756D7

**Results**

**Molecular Analyses**

A total of 30 new sequences were generated for L. huidobrensis from Brazil, 10 of COI-5′, 10 of COI-3′, and 10 of COII, and a total of 33 new sequences of L. sativae from Brazil, 18 of COI-5′ and 15 of COI-3′ (Table 2). Considering only sequences generated in the present study, intraspecific distances of L. huidobrensis samples were 0% for all markers analyzed (COI-5′, COI-3′, and COII). For L. sativae, intraspecific distances were 0–0.8% (mean = 0.1) for COI-5′ and 0–0.4% (mean = 0.4) for COI-3′.

On a global scale, intraspecific distances for L. huidobrensis ranged from 0–1.1% for COI-5′ (Table 3), 0–2.0% for COI-3′ (Table 3), and 0–0.9% for COII (Table 3); while interspecific distances from L. huidobrensis compared to all other available Liriomyza species ranged from 6.0–22.1% for COI-5′ (Table 4), 4.6–20.8% for COI-3′ (Table 5), and 4.7–16.8% for COII (Table 6). For L. sativae, intraspecific distances ranged from 0–6.9% for COI-5′ (Table 3), 0–9.6% for COI-3′ (Table 3), and 0–1.7% for COII (Table 3); while interspecific distances from L. sativae to available Liriomyza species ranged from 6.1–21.3% for COI-5′ (Table 4), 7.5–18.7% for COI-3′ (Table 5), and 7.4–16.8% for COII (Table 6).

Bayesian analyses showed similar results between species groups for three fragments of COI and COII genes (Figs. 2–4) (see complete trees in Supp Fig. T1–T3 [online only]). Prior posterior probabilities and NJ bootstrap values for Liriomyza species were 1.00 and 96–100%, in most cases, respectively. Exceptions to this high clade support were for L. sativae with 0.98 and 76% in the COI-3′ and 76% in the COI-5′ analyses. Present analyses confirmed the identification of Brazilian specimens as belonging to both species treated in this study, because they were each recovered in clades with conspecifics. Haplotype networks of L. huidobrensis showed a single Brazilian haplotype for COI-5′ (10 sequences) shared with 2 sequences from Indonesia, with low levels of genetic differentiation from other sampled populations from Canada, and other countries in Africa and Asia (Fig. 5a). Similarly, a single Brazilian haplotype for COI-3′ (11 sequences) was found shared with sampled populations from Canada, Netherlands, South Africa, and countries in Asia (Fig. 5b) and also a unique Brazilian haplotype for COII (10 sequences), with few genetic differences from populations from Canada, Netherlands, Guatemala, and other countries in South America and Asia (Fig. 5c).

Haplotype networks for L. sativae revealed six different Brazilian haplotypes for COI-5′ (123 sequences), with low levels of genetic differentiation between them (Fig. 6a). However, these haplotypes show significant differences from the sampled populations of Australia, Mexico, and other countries from Asia. The COI-3′ revealed five different haplotypes from Brazil (23 sequences) and also suggested a fairly high genetic differentiation of Brazil populations from haplotypes found in Australia, Egypt, and countries throughout the Americas and Asia (Fig. 6b).

Both phylogenetic results (Figs. 2 and 3) and haplotype networks (Fig. 6) show the division of L. sativae into the four major clades previously proposed (Scheffer and Lewis 2005, Parish et al. 2017), L. sativae-A, L. sativae-B, L. sativae-E, and L. sativae-W. Herein we corroborate the Brazilian clade, L. sativae-B, which

**Table 3.** Range, mean ± standard deviation (SD) intraspecific pairwise sequence divergences expressed as percentages for Liriomyza species for COI-5′ (K2P), COI-3′ (uncorrected), and COII (uncorrected)

| Species         | COI-5′ | COI-3′ | COII  |
|-----------------|-------|-------|-------|
| L. asclepiads   | 0.2–0.8 (0.5 ± 0.2) | –     | –     |
| L. baptissae    | 0–17.6 (6.3 ± 7.5) | –     | –     |
| L. brassicaceae | 0–2.5 (0.3 ± 0.3)  | 0–1.7 (0.3 ± 0.4) | –     |
| L. bryoniae     | 0–0.3 (0.1 ± 0.1)  | 0     | 0–0.1 (0 ± 0) |
| L. chenopodii   | 0–0.5 (0.3 ± 0.3)  | 0–1.6 (0.3 ± 0.5) | 0–0.1 (0.1 ± 0) |
| L. chinensis    | 0–1.7 (0.4 ± 0.5)  | –     | –     |
| L. cicerina     | 0–16.5 (7.9 ± 6.8) | –     | –     |
| L. fricki       | 0–4.1 (1.3 ± 1.0)  | –     | –     |
| L. heliantichi  | 0–2.9 (0.8 ± 0.9)  | –     | –     |
| L. huidobrensis | 0–1.1 (0.4 ± 0.3)  | 0–2.0 (0.1 ± 0.2) | 0–0.9 (0.2 ± 0.3) |
| L. kenti        | 0–0.5 (0.2 ± 0.2)  | –     | –     |
| L. langei       | 0–1.4 (0.4 ± 0.3)  | 0–1.9 (0.4 ± 0.4) | 0–1.1 (0.2 ± 0.2) |
| L. philadelphivona | 0.2–0.2 (0.2 ± 0.2) | –     | –     |
| L. sativae      | 0–6.9 (2.6 ± 2.6)  | 0–9.6 (2.9 ± 3.5) | 0–7.3 (1.1 ± 2.0) |
| L. septentrionalis | 0–0.8 (0.3 ± 0.2) | –     | –     |
| L. sylvatica    | 0     | 0     | –     |
| L. trifoli      | 0–5.1 (0.7 ± 1.3)  | 0–7.4 (0.8 ± 1.5) | 0–0.4 (0.1 ± 0.1) |
| L. trifoliarum  | 0–0.5 (0.2 ± 0.2)  | –     | –     |
Table 4. Range, mean, and standard deviation of COI-5′ interspecific sequence divergences (K2P) expressed as percentages for *L. huidobrensis* and *L. sativae*

| Species       | *L. huidobrensis* | *L. sativae* |
|---------------|-------------------|-------------|
| *L. asclepiads* | 15.4–16.9 (mean = 16.6 ± 0.3) | 14.8–18.5 (mean = 17.4 ± 0.8) |
| *L. baptisiae* | 16.1–19.2 (mean = 17.7 ± 0.4) | 15.5–21.3 (mean = 18.9 ± 0.8) |
| *L. brassicae* | 15.6–19.5 (mean = 17.7 ± 0.4) | 13.2–17.8 (mean = 15.4 ± 0.3) |
| *L. bryoniae* | 6.0–6.8 (mean = 6.4 ± 0.1) | 9.9–14.2 (mean = 12.8 ± 0.7) |
| *L. chenopodii* | 13.8–15.2 (mean = 14.4 ± 0.3) | 10.0–13.3 (mean = 12.0 ± 0.6) |
| *L. chinensis* | 16.9–20.1 (mean = 18.6 ± 0.7) | 14.5–21.1 (mean = 17.9 ± 1.3) |
| *L. eupatorii* | 13.4–13.7 (mean = 13.5 ± 0.1) | 11.2–14.2 (mean = 13.8 ± 0.5) |
| *L. cicerina* | 13.4–17.0 (mean = 14.9 ± 0.7) | 10.6–15.5 (mean = 13.9 ± 1.0) |
| *L. fricki* | 14.7–18.7 (mean = 16.7 ± 0.4) | 10.1–16.4 (mean = 12.8 ± 0.7) |
| *L. helianthi* | 14.1–16.2 (mean = 15.2 ± 0.4) | 9.9–15.4 (mean = 13.2 ± 0.8) |
| *L. huidobrensis* | – | 9.9–13.7 (mean = 12.4 ± 0.6) |
| *L. kenti* | 15.5–18.7 (mean = 16.7 ± 0.8) | 12.5–17.0 (mean = 15.1 ± 0.7) |
| *L. langei* | 6.3–7.9 (mean = 7.1 ± 0.2) | 11.1–15.6 (mean = 13.5 ± 0.8) |
| *L. lima* | 15.0–16.5 (mean = 16.3 ± 0.3) | 14.1–16.9 (mean = 15.4 ± 0.4) |
| *L. ptarmicae* | 20.5–21.1 (mean = 21.0 ± 0.1) | 15.6–19.1 (mean = 17.7 ± 0.7) |
| *L. ranunculoides* | 16.5–17.6 (mean = 17.0 ± 0.4) | 10.6–13.1 (mean = 11.7 ± 0.4) |
| *L. philadelphivora* | 20.3–22.1 (mean = 21.5 ± 0.4) | 16.4–19.9 (mean = 18.0 ± 0.4) |
| *L. sativae* | 9.9–13.7 (mean = 12.4 ± 0.6) | 12.7–17.1 (mean = 14.4 ± 0.6) |
| *L. septentrionalis* | 14.6–15.9 (mean = 15.5 ± 0.2) | 11.0–15.1 (mean = 13.3 ± 0.8) |
| *L. sylvatica* | 11.2–14.2 (mean = 13.2 ± 0.9) | 6.1–12.2 (mean = 10.1 ± 1.3) |
| *L. trifolii* | 10.8–14.8 (mean = 14.0 ± 0.6) | – |
| *L. trifoliearum* | 11.7–15.6 (mean = 12.3 ± 0.4) | 7.3–13.2 (mean = 9.8 ± 0.7) |

Table 5. Range, mean, and standard deviation of COI-3′ interspecific sequence divergences (uncorrected) expressed as percentages for *L. huidobrensis* and *L. sativae*

| Species       | *L. huidobrensis* | *L. sativae* |
|---------------|-------------------|-------------|
| *L. baptisiae* | 15.1–17.2 (mean = 16.2 ± 0.2) | 13.1–17.2 (mean = 14.1 ± 0.6) |
| *L. brassicae* | 12.5–14.8 (mean = 13.2 ± 0.3) | 11.2–14.7 (mean = 13.1 ± 0.4) |
| *L. bryoniae* | 8.7–11.6 (mean = 9.3 ± 0.3) | 10.2–14.5 (mean = 11.8 ± 0.8) |
| *L. chenopodii* | 13.7–14.8 (mean = 14.2 ± 0.1) | 10.5–14.6 (mean = 12.2 ± 0.7) |
| *L. chinensis* | 13.5–15.7 (mean = 14.1 ± 0.3) | 9.7–15.3 (mean = 12.1 ± 0.7) |
| *L. cicerina* | 13.3–14.7 (mean = 14.0 ± 0.2) | 12.2–18.7 (mean = 14.4 ± 0.9) |
| *L. fricki* | 11.8–14.2 (mean = 11.9 ± 0.2) | 10.7–16.7 (mean = 12.5 ± 1.0) |
| *L. huidobrensis* | – | 10.0–14.6 (mean = 12.4 ± 0.4) |
| *L. langei* | 4.6–9.1 (mean = 5.8 ± 0.4) | 10.0–14.4 (mean = 12.0 ± 0.6) |
| *L. philadelphivora* | 16.4–20.8 (mean = 16.7 ± 0.4) | 12.6–16.7 (mean = 14.2 ± 0.6) |
| *L. sativae* | 10.0–14.6 (mean = 12.4 ± 0.4) | – |
| *L. trifolii* | 8.7–14.2 (mean = 12.1 ± 0.3) | 7.5–12.5 (mean = 9.4 ± 0.8) |
| *L. trifoliearum* | 15.5–20.8 (mean = 16.4 ± 0.5) | 11.8–17.3 (mean = 13.2 ± 0.9) |

was previously only supported based on COI-3′ sequences (Parish et al. 2017). The Brazilian haplogroup *L. sativae*-B is separated by *L. sativae*-A by five substitutions in COI-5′ and three substitutions in COI-3′. Interestingly, most of these major clades can be distinguished by aminoacid changes in COI alignments. In the COI-5′ alignment, aminoacids at nucleotide positions 334–336 coded M for *L. sativae*-A, I for *L. sativae*-B, and V for *L. sativae*-W (as in *L. trifolii*). In the COI-3′ alignment, there is more aminoacid variation among groups, aminoacids at nucleotide positions 340–342 coded I for all *L. sativae* clades, except V for *L. sativae*-W (as in *L. trifolii*); positions 514–516 coded M for *L. sativae*-A and *L. sativae*-B and V for *L. sativae*-L and *L. sativae*-W (as in most *L. trifolii*); and positions 670–672 coded Y for *L. sativae*-A and *L. sativae*-B and F for *L. sativae*-L and *L. sativae*-W (as in most *L. trifolii*).

Taxonomy

*Liriomyza huidobrensis* (Blanchard)  
(Figures 7a–k and 8a–e)  
*Liriomyza strigosa* Spencer 1963. Syn. Nov.

* Diagnosis. Wing length 1.75–2.6 mm (♂), 1.9–2.7 (♀); two ors directed inwardly or dorsally (anterior ori sometimes shorter; one in some females); two ors; head light yellow (some males, pale yellow), with back of head brown to base of outer vertical setae; antenna yellow (some specimens with pedicel and postpedicel brown); calypter grey, margin and fringe brown; halters yellow; legs with coxae and femora mostly yellow with light brown maculae, tibiae and tarsi dark brown; abdomen dark brown, sometimes yellowish medially on second tergite; male terminalia black (Fig. 7e); oviscape dark brown (Fig. 7k); epandrium...
Table 6. Range, mean, and standard deviation of COII interspecific sequence divergences (uncorrected) expressed as percentages for *L. huidobrensis* and *L. sativae*

| Species      | L. huidobrensis | L. sativae  |
|--------------|-----------------|-------------|
| *L. huidobrensis* | 6.8–7.8 (mean = 7.3 ± 0.2) | 14.1–16.3 (mean = 15.1 ± 0.4) |
| *L. chinensis* | 15.2–16.2 (mean = 15.8 ± 0.1) | 14.3–15.0 (mean = 14.6 ± 0.1) |
| *L. langei*   | 4.7–6.0 (mean = 5.1 ± 0.1) | 13.6–15.6 (mean = 14.4 ± 0.3) |
| *L. sativae*  | 14.8–16.8 (mean = 16.6 ± 0.3) | – |
| *L. trifoli*  | 14.8–16.6 (mean = 15.3 ± 0.2) | 7.4–10.2 (mean = 8.2 ± 0.4) |
| *L. huidobrensis* | 8.4–9.4 (mean = 8.8 ± 0.2) | 14.8–16.8 (mean = 16.6 ± 0.3) |

with one apical spine; surstylus with single subapical spine (Fig. 7g); basiphallus with a long gap before mesophallus; hypophallus well-developed; mesophallus sclerotized laterally; distiphallus cylindrical, with apex less sclerotized and basal margin angled, sclerotized and upcurved in lateral view (Fig. 7h–i); ejaculatory apodeme short and narrow, with sperm pump rounded (Fig. 7j).

**Early Stages.** Pupa orange (Fig. 8a); larva white (Fig. 8b); anterior spiracle (Fig. 8c); cephalopharyngeal skeleton, lateral view (Fig. 8d); posterior spiracle (Fig. 8e).

**Host Plants.** Linear mine in *Gypsophila paniculata* L. (Fig. 9a) and linear mine in *Chrysanthemum morifolium* Ramat. (Fig. 9b).

**Material Examined.** Brazil. Ceará, São Benedito: 2 ♂, 3 ♀ (27.ix.2016) – ex: *Gypsophila paniculata* L., Sousa, V.R. col.; 10 ♂, 28 ♀ (26.i.2017) – ex: *G. paniculata*, Sousa & Braga cols.; 2 ♂, 2 ♀ (27.i.2016) – ex: *Chrysanthemum morifolium* Ramat., Sousa, V.R. col.

**Santa Catarina, Seara [Nova Teutonia]:** 1 ♂ holotype of *L. strigosa* Spencer 1963, 24.09.1937, 27°11′S, 50°23′L, Fritz Plaumann (BMNH).

**Comments.** Lonsdale (2011) observed paler specimens found mostly among males and a few females from South American countries, including Brazil, congruent with our specimens. He mentioned that these paler specimens have a wing length 1.9–2.6 mm in males, a dark brown abdomen, and sometimes a yellow lateral stripe on scutum connected along the posterior margin of tergite 6 (Lonsdale 2011). Although the yellow lateral line on the abdomen is found in specimens herein studied, the anteromedial spot on tergite 6 was not observed.

Spencer (1963) described *Liriomyza strigosa* from unreared specimens collected in Santa Catarina, Brazil, and mentioned that it could be easily recognized by the matt scutum and two rows of acrostichals. We analyzed the holotype of *L. strigosa* (Fig. 10a–e) deposited at the BMNH and observed that there are no differences between the male terminalia of *L. huidobrensis* (Fig. 7h–i) and *L. strigosa* (Fig. 10d). Both species have one strong spine at the epandrium and one on surstylus, a long membranous gap between basiphallus and distiphallus, as well as a membranous gap before the distiphallus. The opaque scutum and presence of two rows of acrostichals as in the original description are also found among populations of *L. huidobrensis*. Based on these observations, we propose *L. strigosa* as a junior synonym of *L. huidobrensis*.

**Liriomyza sativae** Blanchard (Figures 11a–k and 12a–e)

**Diagnosis.** Wing length 1.27–1.6 mm (♂), 1.4–1.8 mm (♀); two or three (♂) or sometimes short or absent (♀), sometimes three; two ors upcurved; acrostichal setulae in four irregular rows; scutum dark brown, with hind corners and a line before scutellum yellow; calyptr greyish, margin and fringe greyish to brownish yellow; haltere yellow; legs entirely yellow, some specimens with tibiae, tarsi, and base of coxae brown; abdomen brown, yellow laterally, most specimens have a straight yellow band at posterior margin of tergites; male terminalia brown (Fig. 11g–i); surstylus with single subapical spine (Fig. 11g); paraphallus sclerotized; hypophallus well-developed; mesophallus long and fused to distiphallus; distiphallus short and C-shaped in profile (Fig. 11h–i); ejaculatory apodeme large, with sperm pump sclerotized (Fig. 11j).

**Early Stages.** Pupa orange (Fig. 12a); larva yellow (Fig. 12b); anterior spiracle (Fig. 12c); cephalopharyngeal skeleton, lateral view (Fig. 12d); posterior spiracle (Fig. 12e).

**Host Plants.** *Cucumis melo* L. (Fig. 9c), *Solanum lycopersicum* L. (Fig. 9e), and *Allium cepa* L. (Fig. 9f).

**Material Examined.** Brazil. Rio Grande do Norte, Mossoró. 10 ♂, 15 ♀ (23.i.2017) – ex: *Cucumis melo* L., Sousa & Braga cols; 6 ♂, 5 ♀ (22.ix.2016), Sousa, V.R. col.; 2♂, 2♀ (23.i.2017) – ex: *Citrullus lanatus* (Thunb.) Matsum. & Nakai, Sousa & Braga cols. Ceará, Guraciaba do Norte. 79 ♀, 100 ♂ (26.ix.2016) – ex: *Solanum lycopersicum* L., Sousa, V.R. col. Juazeiro, Bahia. 3 ♂, 13 ♀ (07.iv.2017) – ex: *Allium cepa* L., Damascoeno, G.C.C. col.

**Comments.** Color variation was found among studied specimens with hind tibiae and tarsi brown, calyptr greyish and fringe brownish yellow, anepisternum with only 1/3 or less brown. Lonsdale (2011) mentioned some color variations that he observed in his specimens, including variable brown markings on the anepisteron and the anterocentral corner of the anepisternum, and the anepisternum is sometimes predominantly brown along the ventral margin. He also observed that specimens from western North America are sometimes darker with only the dorsal 1/4 of the anepisternum (similar to that seen in our analyzed specimens from Brazil), meron, and katepisternum yellow. Specimens reared from *Jacaranda* Juss. have also a total lack of pigment, and other specimens rarely have the lateral yellow stripes on scutum connected along the posterior
Fig. 2. Bayesian consensus of COI-5′ sequences (470 bp) from 926 individuals of Liriomyza. Liriomyza sativae clade names follow Scheffer and Lewis (2005) and Parish et al. (2017). Number above clades refer to posterior probabilities and below to NJ bootstrap support.
margin of the scutum (Lonsdale 2011). Slightly larger wings length (1.3–1.6 in males and 1.4–1.8 in females) were observed in specimens from North American countries by Lonsdale (2017).

**Discussion**

**Among-Crop Genetic Structure**

For both species, no genetic structuring was found among crops sampled. For Brazilian populations of *L. huidobrensis* from *Gypsophila* and *Chrysanthemum*, a single haplotype was found for each molecular marker. The Brazilian haplotype of COI-3′ sampled herein from *L. huidobrensis* was the same found previously by Parish et al. (2017) sampled from six individuals from lettuce, cucumber, and bean from Minas Gerais and São Paulo states. Similarly, based on other molecular markers of *L. huidobrensis*, no genetic structuring among different crops has previously been found by Scheffer (2000) based on COII, where a single haplotype from W. Java, Sri Lanka, Israel, and Guatemala, was found from celery, cabbage, potato, fava bean, mustard, snow pea, leek, and *Chrysanthemum*. Additionally, a
single haplotype of COII from *L. huidobrensis* was sampled from nine different host plants throughout Yunnan Province in China (He et al. 2002).

Likewise, although more genetic variation was found among samples of Brazilian *L. sativae* in both markers as compared to *L. huidobrensis*, no evidence of genetic structuring was found among crops. As an example, the most sampled haplotype of *L. sativae* in Brazil of COI-5′ (Fig. 13, SA.1) was collected from all four crops studied herein (tomato, melon, watermelon, and onion) in addition to bean (Ferreira et al. 2017) from Espírito

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**Fig. 4.** Bayesian consensus of COII sequences (629 bp) from 184 individuals of *Liriomyza*. Number above clades refer to posterior probabilities and below to NJ bootstrap support.
Fig. 5. Haplotype networks based on COI and COII from individuals of *Liriomyza huidobrensis*: (a) COI-5′ (*n* = 26); (b) COI-3′ (*n* = 93); and (c) COII (*n* = 52). Each circle represents a unique haplotype and its size the number of individuals sampled, transverse lines along connecting haplotype lines indicate mutational steps, and colors refer to the geographic origin of individual sampled.
Similarly, no genetic structuring of \textit{L. sativae} populations among crops was found in previous studies in Mexico (between bean and tomato, Pérez-Alquicilla et al. 2018) and within lineages of \textit{L. sativae}-A and \textit{L. sativae}-W around the world (from bean, tomato, and cucurbits, Scheffer and Lewis 2005). In contrast, members of the \textit{L. sativae-L} clade were exclusively swept from lupines (Fabaceae) in Colorado, which seems to represent an example of a host-associated group of this species complex (Scheffer and Lewis 2005).
Genetically Homogeneous *Liriomyza huidobrensis*

Different haplotypes found for *L. huidobrensis* from around the world show very few differences, with pairwise intraspecific distances up to 1.1% (COI-5'), 2.0% (COI-3'), and 0.9% (COII). Considering the COII marker, there is more genetic structuring within South America (three Brazil and Ecuador haplotypes separated by 5 substitutions) when compared to the other genetically homogeneous regions of the world (three haplotypes separated by two substitutions). Previous
authors have suggested that the European and recently introduced populations (e.g., Middle East and Asian) of *L. huidobrensis* originated from somewhere in South America (de Goffau 1991, van der Linden 1993, Scheffer 2000), which could be true given the higher genetic variability seen among COII South American populations studied herein. Nevertheless, a larger sampling of South American populations is needed to corroborate this hypothesis.

In disagreement with our findings, Scheffer (2000) reported very high pairwise intraspecific distances up to 5.33% for COI-3′+COII, distances calculated from individuals belonging to two monophyletic clades, one consisting of samples from California and Hawaii and the other from Central and South America. However, Scheffer et al. (2014) elaborated a new multiplex PCR method and found that those *L. huidobrensis* reported from California and Hawaii are truly *L. langei* and that probably *L. huidobrensis* does not occur in California and Hawaii (Scheffer 2000, Lonsdale 2011 and S. Scheffer personal communication). In the present analysis, these mistaken sequence identifications were corrected to *L. langei*.

**Liriomyza sativae**: Adding Evidence for a Species Complex

Within *L. sativae*, high intraspecific pairwise genetic divergences for both fragments of COI, with 0–6.9% (Table 3) for COI-3′, and 0–9.6% (Table 3) for COI-3′ were observed. These findings are in agreement with previous results, which demonstrated high distances between COI clades within *L. sativae* ranging from 2.5–8.8%. High divergence values were also observed in another related species, *L. trifoli.* with uncorrected pairwise distances between major clades ranging from 4.7–5.7% (Scheffer and Lewis 2006). Nevertheless, according to Scheffer and Lewis (2005), the mitochondrial variation found within *L. sativae* exhibits some geographic structure. The clade ‘*sativae*-A’ was found only in Florida, Guatemala, Honduras, and Mexico (recently found by Pérez-Alquicira et al. 2018); clade ‘*sativae*-L’ was found only in Colorado on wild lupines; and clade ‘*sativae*-W’ was more widespread, being found in Florida, California, Arizona, Mexico (recently found by Pérez-Alquicira et al. 2018), and all invasive Old World populations. Our present phylogenetic results from COI-3′ (Fig. 2) recovered three clades with maximum posterior probability values: *L. sativae*-W (formed by Australia, Bangladesh, China, French Polynesia, India, Japan, Pakistan, Papua New Guinea, and Sri Lanka populations) and sister to a clade composed of *L. sativae*-A (Mexico) + *L. sativae*-B (Brazil), while the COI-3′ analysis (Fig. 3) recovered four clades also with maximum posterior probability values: *L. sativae*-A (also including Guatemala, USA: Florida and Honduras) + *L. sativae*-B (Brazil) as sister to the clade composed by *L. sativae*-L (USA: Colorado) + *L. sativae*-W (formed by Israel, Japan, Malaysia, Papua New Guinea, Philippines, Saudi Arabia, Sri Lanka, USA, Venezuela, and Vietnam); These clades had previously been recovered by Scheffer and Lewis (2005) and Parish et al. (2017), while the confirmation of the Brazilian clade with the COI-3′ marker is unprecedented.

Scheffer and Lewis (2005) suggested that more genetic diversity may be found in the Americas. Two studies on *L. sativae* populations from Brazil have been previously conducted (Parish et al. 2017, Ferreira et al. 2017) and although both used the COI gene, each sequenced a different region of it. Thus, we have decided to work with both fragments herein, to be able to contrast our results.
Parish et al. (2017) studied *L. sativae* populations in Southeastern (Espírito Santo, Minas Gerais, Rio de Janeiro, and São Paulo states) and Northeastern (Bahia, Ceará, and Pernambuco states) Brazil and showed that all Brazilian haplotypes were part of a fourth clade ‘sativae-B’ quite divergent from other available *L. sativae* sequences (11 and 36 mutational steps). All sequences of *L. sativae* COI-3′ generated herein group with Parish et al.’s (2017) sequences found in this ‘sativae-B’ clade (Fig. 3). On the other hand, Ferreira et al.’s (2017) study found 14 haplotypes of COI-5′ from Northeastern (Bahia, Ceará, Pernambuco, and Rio Grande do Norte states) and

Fig. 9. Damage by leafminers in crops investigated. (a–b) *Liriomyza huidobrensis* (Blanchard): (a) linear mine on *Gypsophila paniculata* L.; (b) linear mine on *Chrysanthemum morifolium* Ramat. (c–f) *Liriomyza sativae* Blanchard: (c) linear mine on *Cucumis melo* L.; (d) linear mine on *Citrus lanatus* (Thunb.) Matsum. & Nakai; (e) linear mine on *Solanum lycopersicum* L.; (f) linear mine on *Allium cepa* L. (Photo credit [f]: Costa-Lima, T.C. [Embrapa Semiárido]).
Southeastern Brazil (Espírito Santo State), but they did not compare their sequences to other available *L. sativae* sequences. They do show a maximum likelihood tree, including their sequences and a single other *L. sativae* sequence (KF962593) from Bangladesh, that shows a very long branch separating them, but unfortunately, the tree has no scale bar and no sequence divergence is reported between these. In any case, all of *L. sativae* COI-5′ sequences generated herein group with Ferreira et al.’s sequences and also newly corroborate the ‘sativae-B’ clade with this marker (Fig. 2). Thus, both markers indicate a single unique lineage of *L. sativae* in Brazil, sister to the ‘sativae-A’ clade, which shows sequence divergences of 2.4–4.6% in COI-5′ and 1.8–2.6% in COI-3′ to members of the ‘sativae-A’ clade.

Deep mitochondrial divergences and phylogenetic clade structure found within species of *L. cicerina*, *L. sativae*, and *L. trifolii* made previous authors suggest the presence of cryptic species (Scheffer and Lewis 2005, 2006; Carapelli et al. 2018). Considering this, we are following Scheffer and Lewis (2005) in the assumption that the Brazilian endemic population also represents another possible cryptic species in this complex. Apparently, these cryptic species may show particular geographic restrictions and/or host preferences, such as the case of lupin-associated *L. sativae* in Colorado (Scheffer and Lewis 2005) and host-associated cryptic species of *L. cicerina* in Tunisia (Carapelli et al. 2018). Although the Brazilian endemic populations reported herein do not have any particular host preference, these haplotypes have not yet been found outside of Brazil. Thus, more sampling of *L. sativae* in other areas of South America is still needed to corroborate or refute the geographical restriction of this supposedly cryptic species. For example, Scheffer and Lewis (2006) suggested that within the *L. trifolii*-W clade, a shallowly diverged, but phylogenetically distinct subclade restricted to peppers (from Mexico, Hoduras, Florida, and California) provided substantial evidence for another separate species in this complex that seemed to be host-associated. However, the distinction of this supposedly pepper-associated clade was refuted by Pérez-Alquicilla et al. (2018) in a study involving Mexican *L. trifolii* populations of peppers, tomatillos, and onions.

**Conclusions**

Brazilian populations of both *Liriomyza* leafminer species studied have shown no or little intraspecific genetic divergences in all mtDNA markers studied, even though specimens were collected in different crops and geographic regions of the country. In the case of *L. huidobrensis*, only a single haplotype was found in each of the three markers, and in the case of COI (HA.1 and HB.1) haplotypes, they were also shared with Old World populations. However, in the case of the COII the Brazilian haplotype found was restricted to
Brazil, and was slightly more divergent from Ecuadorian populations than other Old World haplotypes. This apparent higher genetic variability suggests that future mtDNA studies of *L. huidobrensis* populations in South America should perhaps focus on this genetic region. For *L. sativae*, our study added support to the previous suggestion that this species may be in fact a complex of cryptic species, and that further sampling of American populations is needed to test this hypothesis and to investigate the origin of introduced Old World populations. The present study also confirmed the presence of a single divergent *L. sativae* lineage in Brazil based on two
genetic markers. More sampling is needed to check for the presence of other *L. sativae* lineages in other Brazilian regions and South American countries occurring in crops and noncrop relatives.

Further investigations on leafminers focusing on morphology, biology, host plants, and integrated molecular data for different countries are strongly recommended. This will provide more information to establish the pest status, invasion processes, gene flow, and speciation.

**Supplementary Data**

Supplementary data are available at *Annals of the Entomological Society of America* online.

**Acknowledgments**

We thank Reijeres, Estufa Timbauba, and Norfruit companies for allowing the collection of specimens on their crops; BMNH curator, Daniel Whitmore for images provided; and Tiago Cardoso da Costa Lima from ‘Embrapa Semiárido’ for donating part of specimens and reagents used in this study. MSC thanks to CNPq (Proc. 303414/2018-9) and FAPERJ (Proc. E-26/202.875/2017) Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) is acknowledged for research funding to NSDP. VRS acknowledges a CNPq doctoral fellowship received during this study. DMT is a research productivity fellow from CNPq (Proc. 313677/2017-4) and a Cientista do Nosso Estado fellow from Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ, Proc. E-26/202.672/2019). To Dr. Owen Lonsdale (Canadian National Collection) for suggested revisions on a preliminary version of this manuscript.

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**Fig. 12. *Liriomyza sativae* Blanchard: (a) pupa; (b) larvae; (c) anterior spiracle; (d) cephalopharyngeal skeleton, lateral view; (e) posterior spiracle.**
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