















## Genetic diversity, phylogenetic and phylogeographic analyses of *Oncideres impluviata* (Germar, 1823) (Coleoptera: Cerambycidae) in Rio Grande do Sul state, Brazil

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### ABSTRACT

The Cerambycidae *Oncideres impluviata* (Germar, 1823) is an important insect pest for *Acacia mearnsii* De Wild in Southern Brazil. The damage caused by their girdling behavior reduces tree productivity, specially in the early years of plant establishment, when girdling is performed on the main trunk of trees. Here, we used a fragment of the mtDNA COI gene to analyze the genetic diversity, population structure and demography of *O. impluviata* in Southern Brazil, as well as to present the first hypothesis of phylogenetic relationships among species of the genus *Oncideres*. Our results identified five distinct haplotypes among the populations of *O. impluviata*, with the most common haplotype identified as O.imp\_COI\_01. The phylogenetic inferences corroborated the monophyly of *O. impluviata* with maximum statistical support. In addition, the phylogeny recovered three main population strains that are largely congruent with the haplotype network, which includes two lineages that are found in different edaphic regions of Rio Grande do Sul (Serra do Sudeste and Encosta Inferior do Nordeste). This is the first molecular phylogenetic assessment of *O. impluviata*. Our findings provide insights into the evolution of a significant species for the Brazilian forestry sector, as well as new resources for planning of pest management strategies.

### Introduction

The Brazilian forestry sector has significantly contributed to the country's economy, with eucalyptus and pine plantations occupying an estimated area of 7.53 and 1.93 million hectares, respectively (IBÁ, 2022). The forestry sector also includes species from genus *Acacia* Martius, 1829 (Fabaceae), whose contribution has been noted mainly in Southern Brazil, with 51.000 hectares of *Acacia mearnsii* De Wild (2021) in the State of Rio Grande do Sul (AGEFLOR, 2022). The cultivation of *A. mearnsii*, also known as black wattle, plays an important social and

economic role, as it involves more than 35.000 family forest owners who work in the generation of products. These include plant extracts rich in tannins agents and phenols that originate tannins, which are used for leather tanning and animal nutrition; flocculants for water treatment processes (vegetable origin); dispersants used as additives where spraying of liquids is necessary; adhesives, resins and phenolic-based for industries; natural flavoring soluble in water for stimulating food consumption at all stages in the diet of pigs, chickens, sheep, cattle, fish and shrimp (AGEFLOR, 2022).

The increase in acreage and homogeneous plantations of black wattle in Rio Grande do Sul has also promoted an upsurge of insect pests

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damaging the trees, resulting in an environmental imbalance, caused by the insufficient management of forest plantations, and leading to production losses (Silva et al., 2020). The most common insects causing damage in *A. mearnsii* populations are the twig-girdler long horned beetle *Oncideres impluviata* (Germar, 1823) (Coleoptera: Cerambycidae: Lamiinae) and the moth *Adeloneivaia subangulata* (Herrich-Schaeffer, 1855) (Lepidoptera: Saturniidae) (Oliveira and Costa, 2011; Costa et al., 2014; Ono et al., 2014).

There are more than 131 species in the genus *Oncideres* (Monné, 2022). *Oncideres* species are endemic to the Americas and many are classified as pests (Wang, 2017). *Oncideres impluviata* triggers significant damage in *A. mearnsii* plantations in Southern Brazil (Ono, 2015) and has been reported using more than 20 host plants, including native and exotic economically species, such as: *Ilex paraguariensis* Saint-Hilaire (Aquifoliaceae), *Bauhinia forficata* Link, Mimosa scabrella Bentham, *Parapiptadenia rigida* (Bentham) Brenan, *Acacia* spp., (Fabaceae), *Croton floribundus* Sprengel (Euphorbiaceae), *Myrsine coriacea* (Sq.) Roem. & Schult. (Myrsinaceae) (Ricketson and Pipoly, 1997) (Monné, 2022).

Currently, the distribution of *O. impluviata* is restricted to southern South America, being reported in Argentina (Misiones), Brazil (South and Southeast regions), Paraguay, and Uruguay (Monné, 2022). This insect has specific morphological characters for identification (Fig. 1), measuring 13 mm - 20 mm in length and 4 mm - 6 mm in width. Dorsal region has a yellowish-brown color with grayish hairs and yellowish spots on the entire surface of the elytra and glossy black dots near the pronotum (Bondar, 1953; Amante et al., 1976; Pedrozo, 1980). Adults of *O. impluviata* girdle the branches that are going to be used by females to lay eggs (Baucke, 1962). This injury knocks down the branches, which become dry and suitable for feeding and development of larvae (Amante et al., 1976; Pedrozo, 1980). Girdled trees tend to bifurcate their trunk, which drastically reduces wood production (Costa et al., 2014). A high infestation of *O. impluviata* in the early years of forest establishment can result in the death of injured trees (Baucke, 1962). Despite its economic importance, no study has been carried out with the

aim of characterizing the population genetic structure of *O. impluviata* in Southern Brazil. Genetic structure studies of this economically important insect pest can contribute to understand biological and evolutionary aspects that can be applied to pest management.

Mitochondrial DNA (mtDNA) has been used as molecular markers to elucidate genetic diversity in several species (Hebert et al., 2003; Castalanelli et al., 2011; Goldstien et al., 2011; Valmorbidia et al., 2019; Machado et al., 2020a; Perini et al., 2020). The cytochrome c oxidase subunit 1 (COI) gene allows species identification due to its unique genetic signature and can be used in phylogenetic analysis (Wu et al., 2017; Torres-Vila and Bonal, 2018; Wang et al., 2019). The use of COI (DNA barcoding) as a tool for species identification and phylogenetic analysis has demonstrated to be able to discern close relative species (Wilson et al., 2011; Grebennikov and Heiss, 2014; Grebennikov et al., 2017).

In general, mitochondrial genes have an advantage of high rates of evolution, compared to the nuclear DNA, and have relatively conserved regions (Meyer, 1994; Sosa-Gómez et al., 2012); therefore, are remarkably useful for organisms succinctly investigated (Guo et al., 2020). In the present study, we used a fragment of the mtDNA COI gene to analyze the genetic diversity, population structure, and demography of *O. impluviata* in the State of Rio Grande do Sul, Brazil. In addition, the phylogenetic relationships among the populations of *O. impluviata*, as well as among the species of the genus *Oncideres* Lacordaire, 1830 were explored.

## Materials and methods

### *Sampling of adult specimens of O. impluviata*

The sampling procedure was performed in five sites of *A. mearnsii* plantations in Rio Grande do Sul, Brazil. Adults of *O. impluviata* were randomly collected from recently girdled branches which were lying on the ground (Fig. 2a, 2b; Table 1). Five to 15 adults were collected per site. Adult specimens were chosen to be captured instead of larvae because the branches girdled by *O. impluviata* often host other cerambycid opportunistic species, which could lead to inaccurate species identification (Paulino- Neto, 2004).

The samples were placed into 50 mL Falcon type plastic tubes containing 96% ethanol. The species was identified by comparison with the photo of the lectotype (Nearn and Tavakilian, 2015; Nearn and Nascimento, 2019), as well morphological characteristics (Bondar, 1953; Amante et al., 1976; Pedrozo, 1980). After identification, *O. impluviata* adults, both males and females, were stored at -20 °C until extraction of genomic DNA.

### *DNA extraction, amplification and sequencing*

Genomic DNA extraction was performed using parts of adult specimens (head, legs or thoracic muscles). We used the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany), following manufacturer's instructions. Specimens were carefully removed from the Falcon tubes, placed on paper towel and air dried at room temperature. Specimens used for DNA extraction were vouchered and deposited in the Entomological Collection 'Padre Jesus Santiago Moure' (Universidade Federal do Parana, Curitiba, Brazil; DZUP), the Entomological Collection of the National Museum (Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; MNRJ) and Integrated Pest Management Laboratory (Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, Brazil).

Amplification of the partial mtDNA COI gene was performed using primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al., 1994). PCRs



**Figure 1** Adult specimen of *O. impluviata* - Dorsal view.



**Figure 2** Injuries caused by *Oncideres impluviata* to *Acacia mearnsii* in the State of Rio Grande do Sul, Brazil. Girdled fallen branches in a *Acacia* plantation in General Câmara. Red arrows show branches girdled by *O. impluviata* (a). Adults of *O. impluviata* copulating and girdling the main trunk of a young *Acacia* tree in Encruzilhada do Sul (b).

**Table 1**

Sampling sites, dates and the number of *Oncideres impluviata* specimens sequenced from each location.

Locations	Date	Geographic coordinates	Number of sequenced specimens	Haplotype/voucher
Canguçu (CA)	21 Nov. 2019	30°58'43"S - 52°23'57"W	11	O.imp_COI_02 / DNM-0033; DNM-0034; DNM-0035; O.imp_COI_03 / DNM-0036; DNM-0037; DNM-0038; DNM-0039; DNM-0040; DNM-0041; DNM-0042; O.imp_COI_04 / DNM-0043;
Capela de Santana (CS)	16 Nov. 2019	29°43'48"S - 51°17'20"W	10	O.imp_COI_01 / DNM-0001; DNM-0002; DNM-0003; DNM-0004; DNM-0005; DNM-0006; DNM-0007; DNM-0008; DNM-0009; DNM-0010;
Encruzilhada do Sul (ES)	10 Nov. 2019	30°26'45"S - 52°42'45"W	12	O.imp_COI_01 / DNM-0011; DNM-0012; DNM_0013; DNM-0014; DNM-0015; DNM-0016; DNM-0017; DNM-0018; DNM-0019; DNM-0020; DNM-0021; DNM-0022;
General Câmara (GC)	16 Nov. 2019	29°53'35"S - 51°53'54"W	10	O.imp_COI_01 / DNM-0023; DNM-0024; DNM-0025; DNM-0026; DNM-0027; DNM-0028; DNM-0029; DNM-0030; DNM-0031; DNM-0032;
Montenegro (MN)	20 Set. 2017	29°42'6"S - 51°27'58"W	5	O.imp_COI_02 / DSS-0169; DSS-0173; O.imp_COI_03 / DSS-0168; DSS-0174; O.imp_COI_05 / DSS-0170.
<b>Total</b>			<b>48</b>	

were processed with a final volume of 24µL containing 0.25 µL of Taq DNA Polymerase JumpStart™ (5 U / µL); 2.5 µL of JumpStart™ 10x Buffer; 1.25µL of the dNTP mixture (10 mM each); 2µL of each primer (10µM); 1µL of template DNA (70-120 ng / µL); and 15µL of ultra-pure water. PCR amplification comprised the following steps: initial denaturation at 95 °C (5 min), followed by 34 cycles at 94 °C (30 s), 48 °C (30 s) and 72 °C (1, 5 min) and a final extension at 72 °C (5 min). The quality of the PCR products was verified on 1.0% agarose electrophoresis gel stained with Nancy-520 DNA Gel Stain fluorescent (Sigma-Aldrich, St. Louis, MO, USA) using 3 µL of PCR product, and visualized on a gel documentation system. The amplified samples were sequenced by ABI 3500 Genetic Analyzer. All sequences obtained in this study were deposited in GenBank (Accession numbers: from MZ408185 to MZ408227; from MZ427518 to MZ4275528).

#### Sequence editing and alignments

Sequence analysis and editing were performed using Pregap and Gap4 programs within the Staden package (Staden et al., 2000). Partial mtDNA COI sequences were aligned using the software BioEdit version 7.2.6 (Hall, 1999). These sequences were then translated into protein sequences to check for any translation problems and confirm the absence of premature stop codons.

#### Statistical analyses and genetic differentiation

The number of polymorphic sites (S), number of haplotypes (h), haplotype diversity ( $Hd \pm SE$ ), nucleotide diversity ( $\pi \pm SE$ ) and

neutrality tests (Tajima's  $D$  and  $F_S$ ) were calculated individually for each population and for the total populations using the DnaSP v. 5.10.01 (Librado and Rozas, 2009). The nucleotide composition was calculated using MEGA version 7.0.26 (Kumar et al. 2016) and statistical analysis of parsimony was performed using TCS 1.21 (Clement et al., 2000), within the PopART program to generate a haplotype network (Leigh and Bryant, 2015).

Paired population differentiation ( $F_{ST}$ ) and analysis of molecular variance (AMOVA) were performed on Arlequin 3.5.2.2 (Excoffier and Lischer, 2010), and the significant values were estimated with 10,000 permutations.  $F_{ST}$  values were used to estimate the number of female migrants per generation ( $1-F_{ST} / 2F_{ST}$ ) (Wright, 1943). For the AMOVA, the data were grouped according to the physiographic regions of the State of Rio Grande do Sul (<http://coralx.ufsm.br/ifcrs/fisiografia.htm>). The populations of Capela de Santana and Montenegro are in the physiographic region Encosta Inferior do Nordeste; General Câmara in the region known as Depressão Central; Canguçu and Encruzilhada do Sul in the Serra do Sudeste (Fig. 3).

The relationship between genetic distance ( $F_{ST}$ ) and geographic distance (km) was used to test isolation by distance (IBD). The geographical distance between each population pair was estimated using the Google Maps Distance Calculator (<https://www.daftlogic.com/projects-advanced-google-maps-distance-calculator.htm#>), and analysis was performed using IBD version 1.52 with 10,000 randomizations (Bohonak, 2002).

#### Phylogenetic analyses

Phylogenetic analyses were performed to infer the relationships among the populations of *O. impluviata* and included 17 species of *Oncideres* and representatives of five genera of Onciderini (Supplementary material, Table S1), which were used as outgroups. The trees were rooted in *Hypsoma hezia* Dillon & Dillon, 1945 in all analyses. The

data matrix used for the phylogenetic analyses comprised a total of 95 terminals and 612 aligned positions of COI. The alignment included both sequences newly generated for this study and sequences obtained from Barcode of Life Data System (BOLD) (<http://www.boldsystems.org/>). Sequences obtained from BOLD were only reduced to suit them to the length of our alignments. Phylogenetic analyses were performed under Bayesian and Maximum Likelihood criteria.

Prior the phylogenetic analyses, the dataset was partitioned considering codon positions separately and the best-fitting model of nucleotide substitution for each partition was selected under Akaike Information Criterion (AIC) in PartitionFinder 2.1.1 (Lanfear et al., 2017). Bayesian inference (BI) was carried out using MrBayes 3.2.6 (Ronquist et al., 2012), using partitioned data with their corresponding best-fitting evolutionary model (SYM+G, HKY+I, TRN+G for 1st, 2nd and 3rd codon position, respectively) with eight chains of 50 million generations each and sampling trees every 1000 generations. The analysis convergence was assessed in Tracer 1.7.1 (Drummond and Rambaut, 2007) and the maximum clade credibility tree was generated discarding the output of the 10% initial generations in TreeAnnotator 1.8.4 (Heled and Bouckaert, 2013). Posterior probability values (PP) were used to infer branch support in the BI tree. Maximum likelihood (ML) analysis was performed in RAxML 7.2.6 (Stamatakis, 2006) using unlinked GTR + I + G nucleotide substitution models for each partition with 1,000 bootstrap (BS) pseudo-replicates. Both BI and ML analyses were implemented in CIPRES (Miller et al., 2010).

## Results

#### Genetic diversity and phylogenetic analyses

A total of 48 sequences of COI (with 612 bp) from five populations of *O. impluviata* from Rio Grande do Sul, Brazil, were analyzed in the genetic

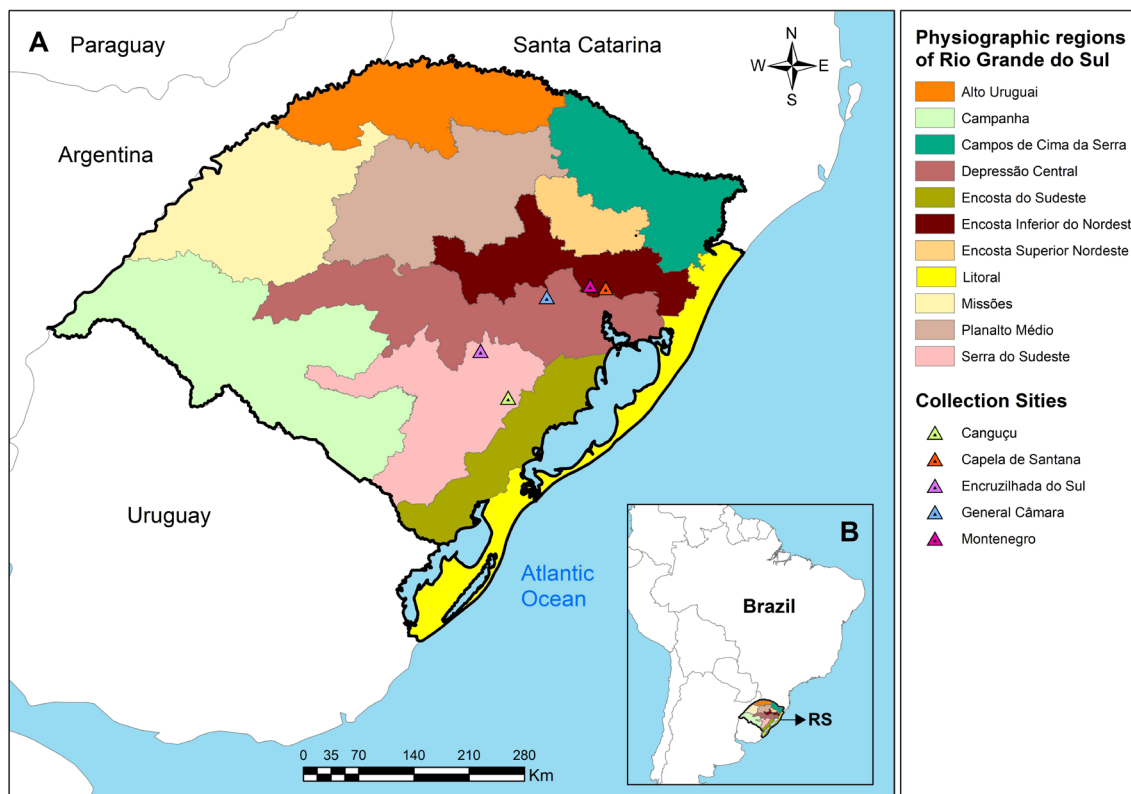


Figure 3 Physiographic regions of Rio Grande do Sul state, Brazil.

diversity study. Twenty-six polymorphic sites (S), haplotype diversity ( $Hd$ ) of  $0.520 \pm 0.073$  and nucleotide diversity ( $\pi$ ) of  $0.01479 \pm 0.00206$  were observed when combining all sequences (Table 2).  $Hd$  in the municipalities of CA and MN varied from 0.564 to 0.800, and  $\pi$  ranged from 0.01014 to 0.01827. In addition, the neutrality tests (Tajima's D:  $-0.34857$ ,  $P = 0.70$ ; and Fu's  $F_s$ :  $2.06$ ,  $P = NA$ ) were not significant, suggesting a pattern of constant size in populations (Table 2). The average nucleotide composition among the 48 sequences of *O. impluviata* was 38.3% thymine (T), 17.2% cytosine (C), 28.5% adenine (A) and 15.9% guanine (G).

Five haplotypes were identified and designated as: O.imp\_COI\_01, O.imp\_COI\_02, O.imp\_COI\_03, O.imp\_COI\_04 and O.imp\_COI\_05). The O.imp\_COI\_01 haplotype was the most common, being present in 32 specimens, and exclusively found in three populations: Capela de Santana (CS = 10), Encruzilhada do Sul (ES = 12) and General Câmara (GC = 10). The populations of Canguçu (CA) and Montenegro (MN) shared two haplotypes, O.imp\_COI\_02 (CA = 02 and MN = 03) and O.imp\_COI\_03 (CA = 07 and MN = 02). In addition, the following haplotypes were represented by only one specimen: O.imp\_COI\_04 (CA = 01) and O.imp\_COI\_05 (MN = 01) (Fig. 4).

Both BI and ML phylogenetic inferences generated congruent results, supporting the monophyly of *O. impluviata* with strong statistical support and recovering three well-supported clades within the species: two closely related including the O.imp\_COI\_02 and O.imp\_COI\_03, both comprising samples from CA and MN; and one containing O.imp\_COI\_01, O.imp\_COI\_04 and O.imp\_COI\_05, comprising all samples from CS, ES, GC and one sample from each CA and MN (Fig. 4). In addition, our analyses recovered a clade containing all samples of a close related species, *Oncideres saga* (Dalman, 1823) (PP = 0.83; BO = 71). *Oncideres* was recovered as paraphyletic in relation to *Psyllotoxus griseocinctus* Thomson, 1868 (PP = 1; BO = 95), which was recovered as sister group of *Oncideres cervina* Thomson, 1868. Except for *Oncideres digna* Bates, 1865 and *Oncideres putator brevifasciata* Dillon & Dillon, 1946 all other *Oncideres* species sampled by more than one specimen in the analyses were strongly supported as monophyletic (Fig. 4).

### Genetic distance between populations

Pairwise genetic distances among the populations of *O. impluviata* evidenced significant distances (Table 3). However, the analysis of

female migrants per generation showed a weak and low evidence for this hypothesis. Furthermore, *O. impluviata* populations showed no statistically significant correlation of genetic distance ( $F_{ST}$ ) vs geographical distance ( $P = 0.572$ ).

### Molecular analysis of variance (AMOVA)

The analysis of molecular variance (AMOVA) was conducted grouping populations according to the physiographic regions of Rio Grande do Sul: Serra do Sudeste, Depressão Central and Encosta Inferior do Nordeste. The results showed significant genetic differentiation among populations within groups ( $\Phi_{SC} = 0.85304$ ;  $P < 0.0001$ ) and within populations ( $\Phi_{ST} = 0.72641$ ;  $P < 0.0001$ ). No statistical significance differences were observed by grouping within physiographic regions ( $\Phi_{CT} = -0.8617$ ;  $P = 1.0000$ ) (Table 4).

### Discussion

#### Genetic diversity, genetic distance, and molecular analysis of variance

Specimens of *O. impluviata*, an important insect pest of *A. mearnsii* in Southern Brazil, were collected in five Acacia plantation sites in Rio Grande do Sul. Analyses of COI resulted in five haplotypes, representing the first molecular characterization and genetic diversity study of *O. impluviata*. In addition, our data are limited to an analysis of a partial mtDNA cytochrome Oxidase I fragment.

Although the specimens of *O. impluviata* were collected in its native range, the genetic diversity among the sampled populations was relatively low, contrary to our expectations of higher genetic diversity which is usually expected from native populations (Puillandre et al., 2008). We also found that O.imp\_COI\_01 was the most common haplotype in the localities with the larger areas of cultivation of *A. mearnsii*, such as General Câmara, Capela de Santana and Encruzilhada do Sul. The two lineages (O.imp\_COI\_04 and O.imp\_COI\_05) found in different edaphic regions, Serra do Sudeste and Encosta Inferior do Nordeste, should be monitored in further landscape adaptation studies. The force of gene flow in each landscape can balance the genetic diversity and form frequency of specific lineages (Dong et al., 2021).

**Table 2**

Results of the polymorphism analysis and neutrality tests based on the mtDNA (COI fragment) of five populations of *Oncideres impluviata* in Rio Grande do Sul, Brazil.

Population	N	S	$\pi$	h	Hd	Tajima's D*	P-value	Fu's FS	P-value
Canguçu (CA)	11	25	0.01014±0.00456	3	0.564±0.134	-1.24665	0.11	6.23	0.99
Capela de Santana (CS)	10	0	-	1	-	0.0000	1.00	0.00	-
Encruzilhada do Sul (ES)	12	0	-	1	-	0.0000	1.00	0.00	-
General Câmara (GC)	10	0	-	1	-	0.0000	1.00	0.00	-
Montenegro (MN)	05	25	0.01827±0.00727	3	0.800±0.164	-0.49618	0.41	4.07	0.96
All sites	48	26	0.01479±0.00206	5	0.520±0.073	-0.34857	0.70	2.06	-

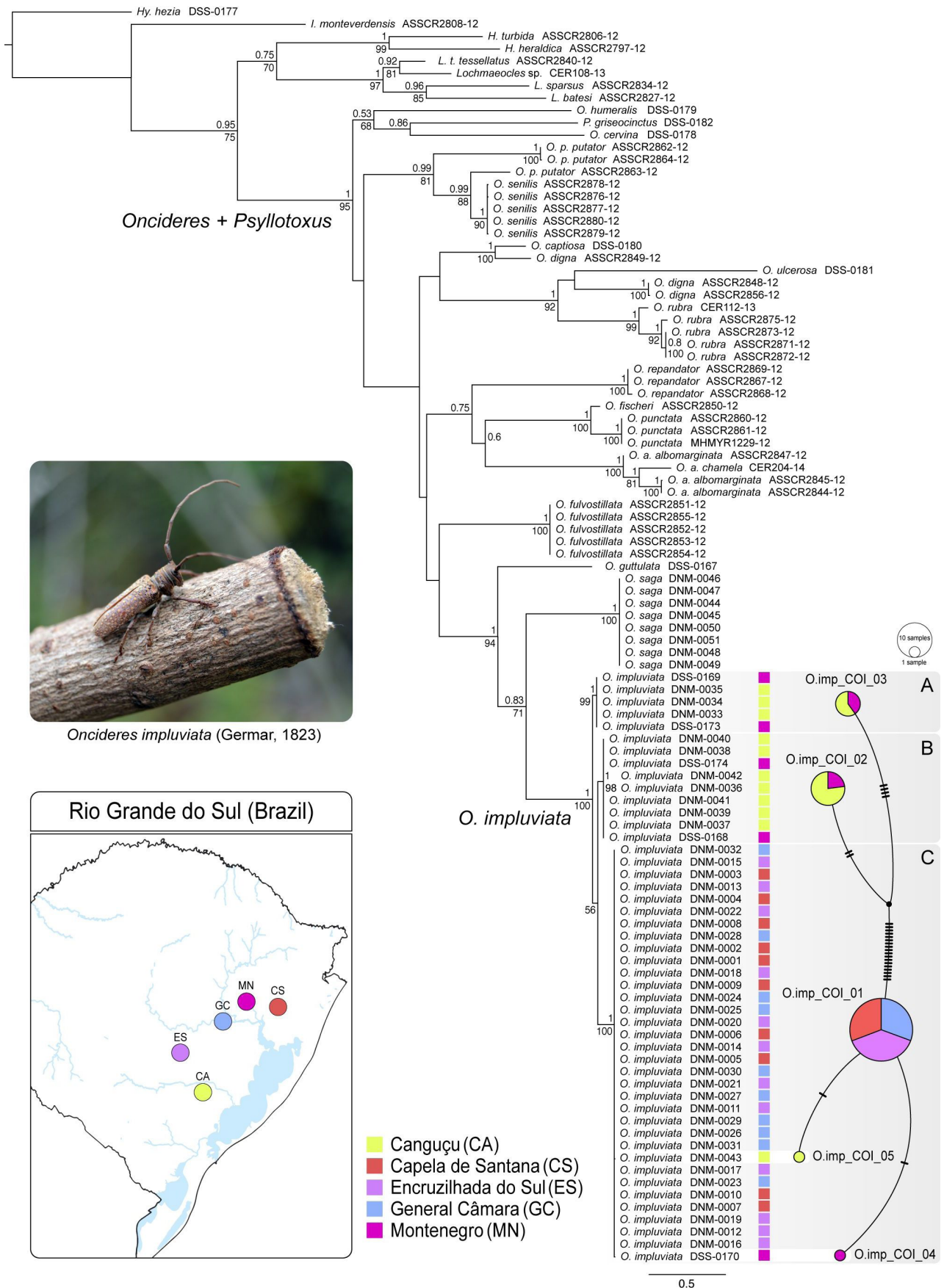
N = number of sequences used, S = number of polymorphic (segregating) sites, h = number of haplotypes, Hd = haplotype diversity,  $\pi$  = nucleotide diversity  $\pm$  standard deviation. Tajima's D and Fu's FS neutrality tests and their respective p values were not included in the table because they have the same haplotype.

**Table 3**

Pairwise FST values (below diagonal) and number of female migrants per generation (above diagonal) among five populations of *Oncideres impluviata* in Rio Grande do Sul, Brazil.

<i>Oncideres impluviata</i>	GC	MN	CS	CA	ES
GC	-	(0.69818)	1.00000	(0.65777)	1.00000
MN	0.77694*	-	(0.69818)	0.99534	(0.67841)
CS	0.00000	0.77694*	-	(0.65777)	1.00000
CA	0.82732*	-0.09650	0.82732*	-	(0.64585)
ES	0.00000	0.80199*	0.00000	0.84161*	-

Populations: GC (General Câmara); MN (Montenegro); CS (Capela de Santana); CA (Canguçu); ES (Encruzilhada do Sul); \*  $P < 0,05$



**Figure 4** Phylogenetic tree summarizing the results of Bayesian inference (BI) and Maximum likelihood (ML). Tree shows the relationships among species of *Oncideres* along with the haplotype network of five populations of *Oncideres impluviata* from Rio Grande do Sul, Brazil. A, B and C depicts clades within *Oncideres impluviata*. The circle areas in the haplotype network are proportional to the frequencies of each haplotype and hatch markers represent the number of differences among haplotypes.

**Table 4**  
Results of the AMOVA analyses of five populations of *Oncideres impluviata*.

Source of variation	Degree of freedom	Sum of squares	Variance components	Percentage of variation	Fixation indices	P-value
Among groups	2	25.997	-3.91811Va	-86.17	$\Phi_{CT}$ -0.8617	1.0000
Among populations within groups	2	133.512	7.22097 Vb	158.81	$\Phi_{SC}$ 0.8530	< 0.0001
Within populations	43	53.491	1.24397 Vc	27.36	$\Phi_{ST}$ 0.7264	< 0.0001
Total	47	213.00	4.54684			

Va, Vb, and Vc are the associated variance components and  $\Phi_{CT}$ ,  $\Phi_{SC}$ , and  $\Phi_{ST}$  are the fixation indices.

The reduction of native areas, the expansion of acaciculture at Rio Grande do Sul, along with the proximity of plantations *A. mearnsii* associated with unappropriated management strategies, have contributed significantly to the increase in population density and dispersion of *O. impluviata*. Older plantations of black wattle can be important bridges of migration of *O. impluviata* to new plantations, especially in areas where legislative measures are not taken for this insect pest management (Costa et al., 2014). This same pattern was observed in old plantations of *Mimosa scabrella* Benth (Baucke, 1958) which is a tree species from the same botanical family as *A. mearnsii*.

The preference of *O. impluviata* for *A. mearnsii* may be associated to the volatile compounds present in this plant species (Berkov et al., 2000). These are fundamental to stimulate feeding, copulation, and oviposition (Paulino-Neto, 2004). Studies should be carried out to know which compounds emitted by *A. mearnsii*, particularly in the initial establishment in the field, are attractive to this insect.

Low diversity of *O. impluviata* can be associated with biotic and biotic factors, in addition to its limited samples. *Oncideres impluviata* is a univoltine insects (Costa et al., 2014; Ono, 2015), as well polyphagous, and reproduces through sexual reproduction, it is worth to note that females of this genus usually oviposit one egg per incision, but there are cases of multiple eggs per branch (Rogers, 1977; Solomon, 1995; Paulino-Neto, 2016). They seek to lay eggs on plants that, due to their characteristics (branch diameter, age, height, vigor, nutritional quality, and others), provides greater fitness to their offspring (Paulino-Neto, 2004). Therefore, it is possible that more than one specimen emerged from the same branch or other branches of the same tree, all of which are maternally related (Machado et al., 2020b), as possibly is the case of insects found in Capela de Santana, Encruzilhada do Sul and General Câmara.

The correlation between genetic distance (FST) and geographical distance didn't show any statistically significance, which suggests absence of distance isolation. However, pairwise genetic distance shows significant population structure when comparing the collection sites (GC x MN, GC x CA, MN x CS, MN x ES, CS x CA, and CA x ES), regardless of its geographic distance. This suggests that most populations are not interbreeding freely, excepting some low FST value (GC x CS, GC x ES, CS x ES, and MN x CA). These pairwise comparisons agree with the AMOVA results, which showed that the populations presented considerable degree of differentiation, within populations lacking haplotype sharing. The number of female migrants of *O. impluviata* between the collection sites was not relevant in this study.

Still, new specimen collections should be carried out in order to expand the possibilities of finding more haplotypes in another areas in Rio Grande do Sul state and Brazil.

#### Phylogenetic analyses

Our results support the monophyly of *O. impluviata*, recognizing three main population strains that are largely congruent with the haplotype network (clades A, B and C), but the statistical support among the clades was negligible to infer with confidence their relationships. *Oncideres impluviata* is morphologically close to *Oncideres guttulata* Thomson, 1868. Both species present rounded yellowish pubescent

spots on the elytra and a white pubescent macula on the metaventrite (Dillon and Dillon, 1946). However, our analyses did not recover an immediate relationship between these species. Instead, *O. saga* was found to be the closest relative of *O. impluviata*, although with moderate statistical support.

This is the first molecular phylogenetic study in the genus *Oncideres*. Our results reveal two important questions about the evolution of Onciderini: (1) the placement of *Psyllotoxus* within *Oncideres*, corroborating the close relationship between these genera, as suggested by some authors in the past (Thomson, 1868; Lacordaire, 1872), and (2) hypotheses about the relationship between the species of *Oncideres*. However, it is still premature to propose taxonomic changes based on our sampling. These findings suggest evidence to redefine the systematic limits of *Oncideres* and must be taken into consideration in future phylogenetic and systematic studies.

#### Implications and insights for pest management

In the agricultural area, insect pests as the Lepidoptera *Chrysodeixis includens* (Walker, 1858) (Lepidoptera: Noctuidae) can get lineages adaptation not only at the local landscapes (Silva et al., 2020b) but also between countries (Perini et al., 2020). Thus, edaphic regions in Rio Grande do Sul can force and create lineages of *O. impluviata* adapted to specific conditions that should be kept under investigation in further years.

Understanding the occurrence and ecology of *O. impluviata* in areas where new *A. mearnsii* tree are being cultivated is essential for the management of this pest species. Main strategies to manage *O. impluviata* populations includes a rotation every 6-7 years, without thinning or pruning and population density of 2-2.5 thousand trees per hectare (AGEFLOR, 2022). Besides our finding of five genetic lineages, pest management strategies at a local-scale management of *O. impluviata* might help to reduce local populations which will not be boosted by long-distance dispersal of adults to another regions.

Furthermore, the destruction of twigs girdled by this species seems not to be efficient, since that *O. impluviata* populations continues to increase in some regions of Rio Grande do Sul state (2006 to 2013), affecting the black wattle plantations (Ono, 2015). This requires, besides legislative tactics, other integrated pest management strategies, including chemical and biological control, plant resistance, behavior control and cultural activities should be performed to manage this pest.

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## Conflicts of interest

The authors declare no conflicts of interest.

## Author contribution statement

DNM and JAA: Conceived, designed the experiments and performed the experiments. ECC and JAA: Supervision. DNM, DSS, RF, GS, LP, MAS, HPG, JO: Methodology, visualization, software, Reviewing and Editing. DNM, CRP, DSS, IV: Validation, Data curation, Writing- Original draft preparation. All authors have read and agreed to the published version of the manuscript.

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**Supplementary material**

The following online material is available for this article:

Table S1 - Taxon sampling *Oncideres impluviata* and other species of genus *Oncideres*.