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PHYLOGENOMICS OF OLMECA, A NEOTROPICAL WOODY BAMBOO, BASED ON NUCLEAR SINGLE NUCLEOTIDE POLYMORPHISM (SNP) DATA

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Abstract

Background: The genus *Olmeca* comprises five species, two of which produce bacoid caryopses, while the rest produce typical caryopsis fruits. *Olmeca* species have a collective geographical distribution in Mexico and Honduras. Previous studies have suggested the potential monophyly of this genus based on plastid markers, but with inconclusive internal phylogenetic resolution.

Questions: Will *O. recta* and *O. reflexa* form a monophyletic group? Will the remaining three *Olmeca* species form a second clade?

Studied species: Species in the genus *Olmeca.*

Study site and dates: Mexico.

Methods: We collected samples from five *Olmeca* species and employed nuclear single nucleotide polymorphisms derived from restriction-site associated DNA sequencing data to construct our phylogenetic hypothesis using maximum likelihood and coalescent methods. We estimated divergence times using the RelTime method and performed ancestral reconstruction of geographic areas using S-DIVA analysis.

Results: The SNP data supports the monophyly of the genus *Olmeca* and the existence of two distinct clades, Fulgor and Reflexa. Our estimate suggests that *Olmeca* originated approximately 7.2 million years ago. The inferred ancestral areas point to Veracruzan + Chiapas Highland provinces or the Veracruzan + Sierra Madre del Sur provinces as the likely origin of the genus. The biogeographic distribution of *Olmeca* can likely be explained by a combination of dispersal and vicariant events.

Conclusions: The analyses recovered *O. recta* and *O. reflexa* as a monophyletic clade. Additionally, the remaining three *Olmeca* species formed a second clade, with *O. fulgor* as the sister species to *O. zapotecorum*, consistent with their morphological similarities.

Keywords: bacoid caryopsis, Guaduinae, montane cloud forest, Neotropical woody bamboos, RADSeq, tropical perennial forest, typical caryopsis.

Resumen

Antecedentes: *Olmeca* comprende cinco especies, dos producen cariópsides bacoides, mientras que el resto produce cariópsides típicas. Las especies de *Olmeca* se distribuyen en México y Honduras. Estudios previos sugeieren la monofilia del género basándose en marcadores de plástidos, pero con una resolución filogenética interna no concluyente.

Preguntas: ¿Formarán *O. recta* y *O. reflexa* un grupo monofilético? ¿Formarán las otras tres especies de *Olmeca* un segundo clado?

Especies estudiadas: Especies del género *Olmeca.*

Sitio de estudio y fechas: México.

Métodos: Recolectamos muestras de cinco especies de *Olmeca* y empleamos polimorfismos de nucleótido único (SNP) derivados de datos de secuenciación RAD (Restriction-site Associated DNA) para construir nuestra hipótesis filogenética utilizando métodos de máxima verosimilitud y coalescencia. Estimamos los tiempos de divergencia utilizando el método RelTime y realizamos una reconstrucción ancestral de áreas geográficas mediante análisis S-DIVA.

Resultados: Los datos de SNP respaldan la monofilia del género *Olmeca* y la existencia de dos clados, Fulgor y Reflexa. Nuestra estimación sugiere que *Olmeca* se originó hace aproximadamente 7.2 millones de años. Las áreas ancestrales inferidas apuntan a las provincias de Veracruz + Chiapas Highland o Veracruz + Sierra Madre del Sur como el probable origen del género. La distribución biogeográfica de *Olmeca* probablemente puede explicarse por una combinación de eventos de dispersión y vicarianza.

Conclusiones: Los análisis recuperaron a *O. recta* y *O. reflexa* en un clado. Además, las otras tres especies de *Olmeca* formaron un clado, *O. fulgor* es la especie hermana de *O. zapotecorum*, lo que es consistente con sus similitudes morfológicas.

Palabras clave: bambúes leñosos Neotropicales, bosque mesófilo de montaña, bosque tropical perennifolio, cariópside bacoide, cariópside típica, Guaduinae, RADSeq.

The Neotropical woody bamboo clade is a lineage comprising three subtribes (Arthrostylidiinae, Chusqueinae, and Guaduinae) within the Bambuseae tribe, which belongs to the Bambusoideae subfamily of the Poaceae family (Sungkaew *et al*. 2009, BPG 2012, Kelchner *et al*. 2013, Clark *et al*. 2015). *Olmeca* Soderstr. is one of the six genera belonging to the Guaduinae subtribe, alongside *Apoclada* McClure, *Eremocaulon* Soderstr. & Londoño, *Guadua* Kunth, *Otatea* (McClure & E.W. Sm.) C.E. Calderón & Soderstr., and *Tibisia* C.D. Tyrrell, Londoño & L.G. Clark. It includes five described species (Ruiz-Sanchez *et al*. 2011, Tyrrell *et al*. 2018, Ruiz-Sanchez *et al*. 2021).

The geographical distribution of *Olmeca* ranges from east-central Mexico (Veracruz) to northern Honduras (Ruiz-Sanchez *et al*. 2011, 2020). Four of the five *Olmeca* species are endemic to Mexico (Ruiz-Sanchez *et al*. 2020). Two species, *O. recta* Soderstr. and *O. reflexa* Soderstr., are distributed in southern Veracruz, Oaxaca, and Chiapas. They inhabit tropical perennial forests and produce bacoid caryopses (Figure 1; Rzedowski 1978, Soderstrom 1981, Ruiz-Sanchez *et al*. 2011, Ruiz-Sanchez & Sosa 2015, Ruiz-Sanchez *et al*. 2017, 2020). Both species have been recorded in sympatry (Ruiz-Sanchez *et al*. 2011), in the region known as Uxpanapa in southern Veracruz. The other three *Olmeca* species— *O. clarkiae* (Davidse & R.W. Pohl) Ruiz-Sanchez, Sosa & Mejía-Saulés, *O. fulgor* (Soderstr.) Ruiz-Sanchez, Sosa & Mejía-Saulés, and *O. zapotecorum* Ruiz-Sanchez, Sosa & Mejía-Saulés—are distributed in Veracruz, Oaxaca, and Chiapas in Mexico, with *O. clarkiae* also found in Chiapas and Lempira, Honduras. They inhabit montane cloud forests and produce typical caryopsis fruits (Figure 2; Rzedowski 1978, Ruiz-Sanchez *et al*. 2011).

Previous molecular phylogenetic hypotheses based on plastid markers have shown that the two *Olmeca* species (*O. recta* and *O. reflexa*) form a clade, albeit without strong nodal support. This clade was closely related to *Aulonemia clarkiae* Davidse & R.W. Pohl, *A. fulgor* Soderstr., and an undescribed species of *Aulonemia* (Ruiz-Sanchez *et al*. 2011). Based on those phylogenetic results and morphological characters, Ruiz-Sanchez *et al*. (2011) proposed the inclusion of the three *Aulonemia* species into the genus *Olmeca*. However, it is important to note that Ruiz-Sanchez *et al*. (2011) did not achieve good phylogenetic resolution among the branches of the three former *Aulonemia* species. The relationships between *O. clarkiae*, *O. fulgor*, and *O. zapotecorum* remain unresolved, likely due to the limited resolution provided by the five plastid markers used (*matK*, *rbcL*, *psbK-psbI*, *rpl16*, and *psbA-trnH*). The sister relationship between *Olmeca* and *Otatea* (McClure & E.W. Sm.) C.E. Calderón & Soderstr. has been consistently supported by studies using a few chloroplast markers or complete plastomes (Ruiz-Sanchez *et al*. 2011, Ruiz-Sanchez & Sosa 2015, Saarela *et al*. 2018, Gallaher *et al*. 2022).

Ruiz-Sanchez & Sosa (2015) expanded the sampling of taxa and molecular markers (including *atpB-rbcL*, *matK*, *rps16*, *rps16-trnQ*, *trnC-rpoB*, *trnD-trnT*, *trnL-trnF*, t*rnT-trnL*, GBSSI, and ITS) to investigate the origin and evolution of fleshy fruits in woody bamboos. Their analysis included all five known *Olmeca* species and confirmed that *Olmeca* is monophyletic. They found that *O. reflexa* is sister to the rest of the genus, followed by *O. recta*, and then a clade comprising *O. fulgor* as sister to the clade formed by *O. clarkiae* and *O. zapotecorum*. This finding suggests that fleshy fruits (bacoid caryopses) evolved once within *Olmeca*, as both *O. recta* and *O. reflexa* produce bacoid caryopses and then reverted to a typical caryopsis in the ancestor of the clade with *O. fulgor*, *O clarkiae*, and *O. zapotecorum*. Additionally, *O. fulgor* is not closely related to *O. zapotecorum*, despite their morphological similarities. Ruiz-Sanchez & Sosa (2015) estimated that the divergence between *Olmeca* and *Otatea* occurred approximately 7.2 million years ago (Ma). Similarly, Gallagher *et al*. (2022) estimated this divergence to be around 8 Ma.

Approximately 67 species across 12 genera within two tribes (Arundinarieae and Bambuseae) produce bacoid caryopses (Ruiz-Sanchez *et al*. 2017). Bacoid caryopses have evolved independently at least seven times within the Bambusoideae subfamily since the late Miocene (Ruiz-Sanchez & Sosa 2015). Ruiz-Sanchez & Sosa (2015) found that current climatic variables and soil parameters were not significantly correlated with the appearance of this type of fruit. However, Liu *et al*. (2024) demonstrated that topography and climate are correlated with the presence of bacoid caryopses in Paleotropical woody bamboos. Van der Pijl (1982) hypothesized that bacoid caryopses in Bambusoideae are related to the retention of water and nutrients, favoring rapid germination and growth, thereby producing vigorous seedlings. De la Cruz Aparicio (1985) and Ruiz-Sanchez *et al*. (2017) reported seedlings of *Olmeca recta* and *O. reflexa*, respectively, growing up to 1 meter tall within two months. These findings align with van der Pijl's (1982) hypothesis.

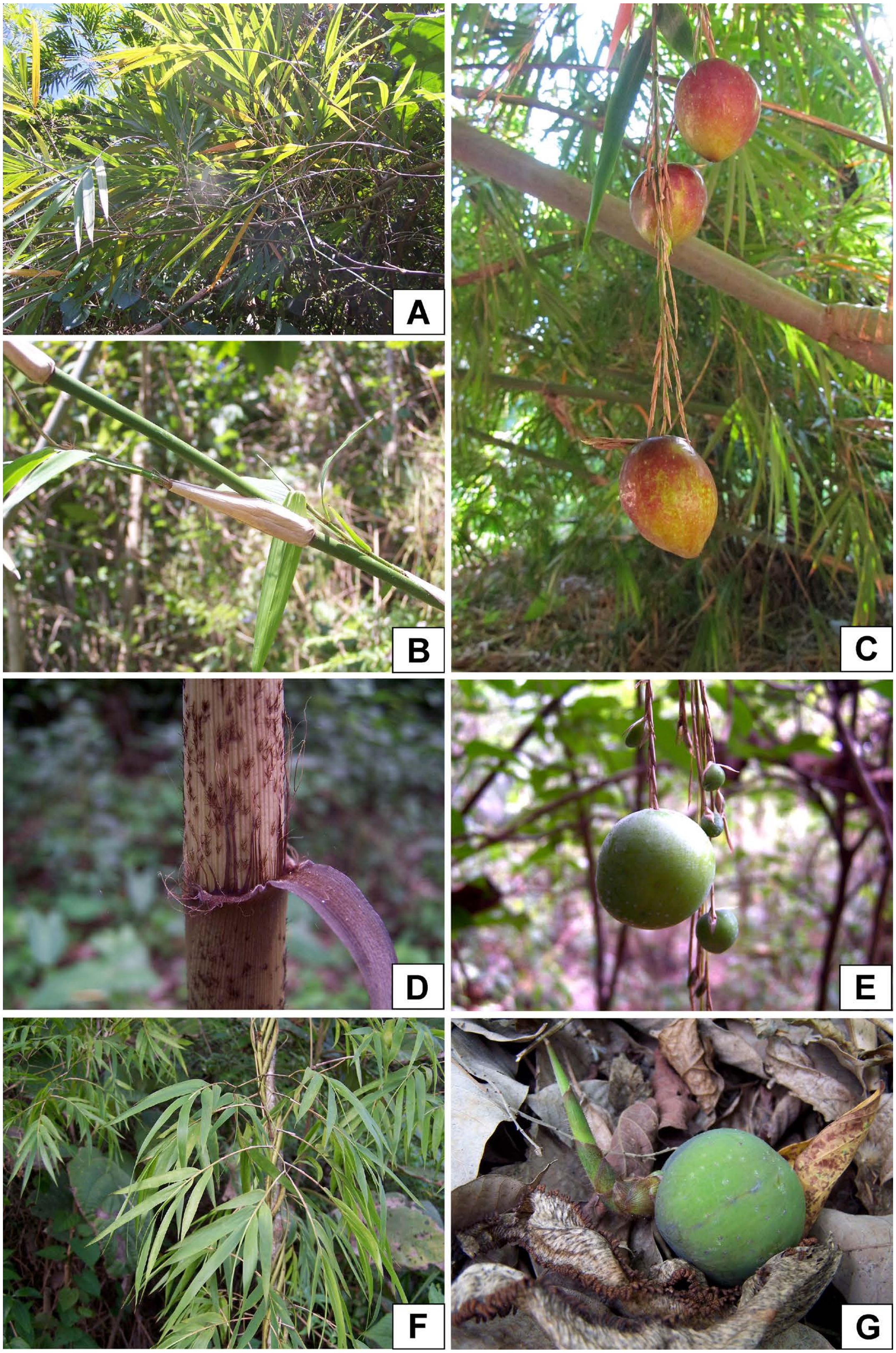

Figure 1. A-C. *Olmeca recta*. A. Foliage leaves. B. Young culm showing branching. C. Bacoid fruits. D-G. *Olmeca reflexa*. D. Culm leaf, showing reflexed blade. E. Bacoid fruits in distinct maturation periods. F. Foliage leaves. G. Germinating bacoid fruit.

Figure 2. A-B. *Olmeca clarkiae*. A, Arching culm showing branches and foliage leaves. B. Foliage leaves. C-D. *Olmeca fulgor*. Main culm branching, showing culm leaves and blades. D. Foliage leaves. E-F. *Olmeca zapotecorum*. E. Foliage leaves. F. New shoot showing culm leaves and blades.

In recent years, genome subsampling techniques, such as restriction site-associated DNA sequencing (RADseq; Baird *et al*. 2008), have proven effective for resolving phylogenetic issues in woody bamboos. These methods have successfully clarified phylogenetic relationships at various taxonomic levels, from genus to more complex groups. Specifically, these techniques have been applied to resolve phylogenetic relationships at the genus and subtribe levels in temperate woody bamboos (Wang *et al*. 2017, Ye *et al*. 2019), paleotropical woody bamboo species (PWB) (Liu *et al*. 2020, 2024), and the Neotropical genus *Guadua* (Ruiz-Sanchez *et al*. 2025). Notably, Liu *et al*. (2024) reconstructed the relationships at the subtribe and generic levels among PWB species. They confirmed the monophyly of the four major subtribes of PWB, contrasting with the eight subtribes proposed by Wong *et al*. (2016).

Phylogenetic hypotheses based on a few plastid markers (Ruiz-Sanchez *et al*. 2011, Ruiz-Sanchez & Sosa 2015) have shown poor phylogenetic resolution (Ruiz-Sanchez *et al*. 2011), weak nodal support among *Olmeca* species (Ruiz-Sanchez & Sosa 2015), or non-monophyly (Tyrrell *et al*. 2018). This raises the compelling questions: could RADseq data better resolve the internal relationships within *Olmeca*? Will *O. recta* and *O. reflexa* form a clade, given that both species share the ability to produce bacoid caryopsis? Will the remaining three *Olmeca* species form a second clade, with *O. fulgor* as the sister species to *O. zapotecorum* due to their morphological similarities?

Material and methods

Sample collection. In this study, we sampled all five *Olmeca* species (Table 1). Leaf tissue from living specimens was collected from wild populations. Voucher specimens were deposited at the University of Guadalajara Herbarium (IBUG) and the Instituto de Ecología, A.C. Herbarium (XAL) (Thiers 2024).

DNA extraction and sequencing. Total DNA was extracted from leaf tissues using the CTAB procedure (Doyle & Doyle 1987, Cota-Sánchez *et al*. 2006). DNA quality was assessed by visualization on 1 % agarose gels, and quantification was performed using a Nanodrop (Thermo Scientific, USA) and a Qubit 2.0 fluorometer (Life Technologies, Carlsbad, CA, USA).

Library preparation was based on a modified double-digest restriction site-associated DNA approach (3RAD; Bayona-Vásquez *et al*. 2019). Genomic DNA was digested using the EcoRI-HF and NheI restriction enzymes. Sequencing was conducted on an Illumina HiSeq 2500 platform (Illumina, San Diego, CA, USA), producing paired-end reads of 150 bp. Library preparation and sequencing were carried out in the Department of Environmental Health Science at the University of Georgia, USA.

Table 1. Sample species, geographical distribution and voucher records.

Species	Distribution	Collection
Olmeca. clarkiae (Davidse & R.W. Pohl) Ruiz-Sanchez, Sosa & Mejía-Saulés	D	*A. Vázquez 504* (XAL)
O. fulgor (Soderstr.)Ruiz-Sanchez, Mejía-Salues & Sosa	A, C	*E. Ruiz 519* (IBUG)
O. recta Soderstr.	B	*E. Ruiz 132* (XAL)
O. reflexa Soderstr.	B	*T. Mejía-Saulés 2045* (XAL)
O. zapotecorum Ruiz-Sanchez, Sosa & Mejía-Saulés	C	E. Ruiz 864 (IBUG)
Otatea acuminata (Munro) C.E. Claderón & Soderstr.	A	*E. Ruiz 644* (IBUG)

A = Transmexican Volcanic Belt province, B = Veracruzan province, C = Sierra Madre del Sur Province, D = Chiapas Highland province.

Samples were assembled into orthologous loci using ipyrad v.0.9.85 (Eaton & Overcast 2020) with a reference-guided approach. Specifically, the reference genome of *Dendrocalamus latiflorus* (GCA_017311315.1, Zheng *et al*. 2022) was used for this purpose. On average, we recovered 1.54 million (±570,922 SD) raw reads per sample. The proportion of mapped reads varied across samples, with a mean of 39.7 % (SD = 4.78 %). Notably, coverage was relatively uniform among clusters of mapped reads, with a mean of 79,434 clusters (SD = 15,156). Ultimately, the assembly yielded a final number of 54,614 non-overlapping loci, averaging 9,102 loci per sample (SD = 3,533). Further detailed information is available in the Supplementary material. Sequences were uploaded to GenBank PRJNA1267969.

Phylogenetic Analysis. We employed maximum likelihood (ML) to infer phylogenetic trees using five ingroup accessions. To root the trees, we included a specimen of *Otatea acuminata* (Munro) C.E. Calderón & Soderstr. Initially, we filtered all assembled loci to retain those with a minimum coverage of at least 25 % of all samples, subsequently removing any samples that contained data for less than 10 % of all sites. This resulted in a filtered dataset comprising 411 loci, which we concatenated using the *window extracter* module in the *ipyrad-analysis toolkit* to generate a final alignment. For the main reconstruction, we utilized RAxML v. 8.2.12 (Stamatakis 2014), employing the GTRCAT evolutionary model and executing 100 repetitions for bootstrap support. Additionally, we conducted a cross-validation reconstruction using I-QTREE v. 2.2.0.3 (Minh *et al*. 2020) with the TVM+F+G4 model, as suggested by ModelFinder (Kalyaanamoorthy *et al*. 2017). This involved running 1,000 replicates to obtain ultrafast bootstrap (BS) support values (Hoang *et al*. 2018) and 1,000 replicates to calculate Shimodaira-Hasegawa-like approximate likelihood ratio test (SH-aLRT) support (SH-aLRT, Anisimova *et al*. 2011).

With the aim of exploring possible incomplete lineage sorting or reticulate relationships among *Olmeca* species, we inferred a species tree based on a multispecies coalescent approach using ASTRAL v. 5.7.1 (Zhang *et al*. 2018). To acquire the required data for this analysis, we first determined how many informative gene trees could be obtained by splitting scaffolds of the reference genome into non-overlapping 2 Mb windows and concatenating assembled RAD loci within each window. This was performed using the *treeslider* module in the *ipyrad-analysis* toolkit, which also applied a filter to each alignment to remove sites with missing data across the ingroup samples. This yielded a total of 1,336 windows. We further refined this set by requiring more than 10 SNPs per window. RAxML was then used to infer a maximum likelihood tree for each of the remaining 102 alignments, which were subsequently used in our ASTRAL reconstruction.

Time tree and historical biogeography. Conventional Bayesian methods for estimating divergence times face statistical and computational challenges when applied to RADseq datasets. These challenges primarily arise from the abundance of short, unidentified RAD loci, which complicate their integration into phylogenetic partitioning strategies. To address this, we adopted the strategy of Donoghue *et al*. (2022) to calibrate our tree using RelTime (Tao *et al*. 2020) in MEGA 11 (Tamura *et al*. 2021). RelTime redistributes molecular branch lengths into branch-specific segments of both geological time and evolutionary rates while adhering to user-specified constraints. We used *Otatea acuminata* as an outgroup and calibrated the divergence between *Olmeca* and *Otatea* using the Timetree of Life tool (Kumar *et al*. 2022), which provides a median estimate of 7.4 Ma (95 % confidence interval [CI]: 2.9-15.7 Ma) using a normal prior to incorporate the confidence interval.

For the historical biogeography analysis, we reconstructed the ancestral species ranges for *Olmeca* species using the Statistical Dispersal-Vicariance Analysis (S-DIVA) approach (Yu *et al*. 2015). S-DIVA statistically evaluates alternative ancestral ranges at each node in a tree, accounting for phylogenetic uncertainty and uncertainty in DIVA optimization. S-DIVA was executed using RASP v. 4 (Yu *et al*. 2020). This method requires a phylogeny and discrete range data as input. For the phylogeny, we used the RAxML tree. For discrete range data, we coded the *Olmeca* species according to their geographical distribution (Ruiz-Sanchez *et al*. 2020), by dividing it into four areas based on the biogeographical regionalization of the Neotropical Region proposed by Morrone (2014): (A) Transmexican Volcanic Belt province; (B) Veracruzan province; (C) Sierra Madre del Sur province; and (D) Chiapas Highland province (Table 1).

Results

Phylogenetic Analysis. The final alignment consisted of 4,292,771 base pairs (bp) and included 9,592 single nucleotide polymorphisms (SNPs) with 66.2 % missing data. Maximum likelihood analyses (RAxML and IQ-TREE) and coalescent-based analyses produced identical topologies (Figure 3A). *Olmeca* was confirmed to be monophyletic with strong support across all metrics: 100 % bootstrap support (BS), SH-aLRT of 100, and posterior probabilities (PP) of 1.0. Two well-supported clades were identified (100 % BS, 100 SH-aLRT, 1.0 PP). The first, informally named the Reflexa clade, includes *O. recta* and *O. reflexa* (Figure 3A). The second, informally named the Fulgor clade, consists of *O. fulgor* and *O. zapotecorum* as sister species (100 % BS, 100 SH-aLRT, 1.0 PP), with *O. clarkiae* sister to this pair (Figure 3A).

Time tree and historical biogeography. The time-tree estimation analysis indicates that the divergence between *Olmeca* and *Otatea* occurred at a mean age of 7.2 million years ago (Ma) (95 % confidence interval [CI] = 6-13.4 Ma). The crown age of *Olmeca* was estimated at 5.89 Ma (95 % CI = 4.69-7.4 Ma). The Reflexa clade was dated to 2.68 Ma (95 % CI = 1.48-4.84 Ma), while the Fulgor clade was estimated to be 4.8 Ma (95 % CI = 3.98-5.77 Ma). The clade formed by *O. fulgor* and *O. zapotecorum* had an estimated age of 3.01 Ma (95 % CI = 2.03-4.48 Ma) (Figure 3B).

The biogeographic analysis using the S-DIVA model identified two possible combined ancestral areas for *Olmeca*: Veracruzan + Chiapas Highland provinces (50 %) or Veracruzan + Sierra Madre del Sur provinces (50 %). For the Reflexa clade, the ancestral area was identified as the Veracruzan province. In the Fulgor clade, a combined ancestral area of Chiapas Highland + Sierra Madre del Sur provinces was determined (100 %). Finally, the clade formed by *O. fulgor* and *O. zapotecorum* was found to have the Sierra Madre del Sur province as its ancestral area (100 %). Additionally, the analysis supports a series of events throughout the evolutionary history of *Olmeca*, including three dispersal and three vicariance events (Figure 3B, C).

Discussion

Our phylogenetic hypothesis supports that *Olmeca* is a monophyletic group comprising two well-supported clades: the Reflexa and Fulgor clades (Figure 3A). Previous phylogenetic studies using a limited number of chloroplast markers suggested that *Olmeca* was non-monophyletic (Ruiz-Sanchez *et al*. 2011, Tyrrell *et al*. 2018). In contrast, Ruiz-Sanchez & Sosa (2015), using a combined nuclear and chloroplast dataset, recovered *Olmeca* as monophyletic. However, their results showed that *O. recta* and *O. reflexa* did not form a clade. Despite containing 66.2 % missing data, our analysis successfully resolved the phylogenetic relationships within *Olmeca*. This aligns with findings by Tripp *et al*. (2017), who reconstructed *Petalidium* Nees (Acanthaceae) phylogeny with 90 % missing data. The use of SNPs from RADseq provided significantly more molecular characters than previous phylogenetic studies where Ruiz-Sanchez *et al*. (2011) had only 97 and Tyrrell *et al*. (2018) had only 265 parsimony informative characters (*vs*. ~9,000 here), resulting in a more resolved phylogenetic tree with strong nodal support. The Reflexa clade comprises two sister species, *Olmeca recta* and *O. reflexa*, both strongly supported by high nodal values (Figure 3A). Both *O. recta* and *O. reflexa* inhabit tropical perennial forests. According to Ruiz-Sanchez *et al*. (2020), few Mexican woody bamboo species occur in this vegetation type, including *Rhipidocladum bartettlii* (McClure) McClure, *Chusquea simpliciflora* Munro, *Guadua tuxtlensis* Londoño & Ruiz-Sanchez, and *G. longifolia* (E. Fourn.) R.W. Pohl.

The Fulgor clade consists of *O. fulgor* as sister to *O. zapotecorum*, with *O. clarkiae* sister to that pair (Figure 3A). Ruiz-Sanchez *et al*. (2011) recovered the same clade but noted a lack of internal resolution (*i.e.,* a polytomy). Ruiz-Sanchez & Sosa (2015) also identified this clade but with a conflicting topology, placing *O. fulgor* as sister to *O. clarkiae* under weak support. Our new phylogeny, based on nuclear SNPs, resolves these relationships with strong support and well-resolved clades.

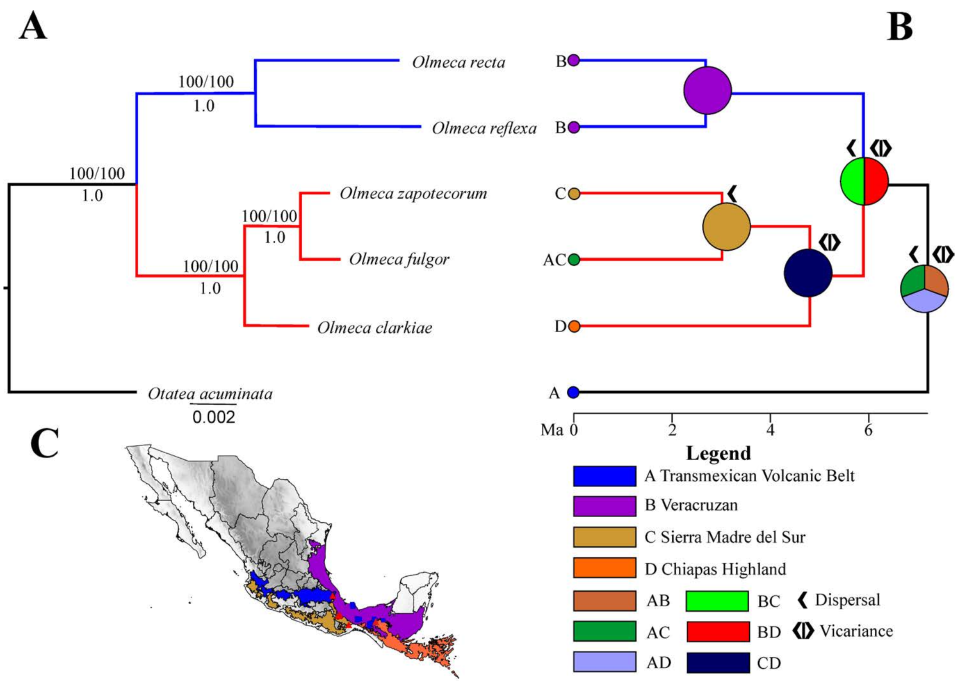

Figure 3. A. Maximum likelihood tree inferred using RAxML, numbers above the branches are bootstrap support values (left) and SH-aRLT support values (right); numbers below the branches are posterior probabilities. Blue branches indicate the informal Reflexa clade and red branches the informal Fulgor clade. B. RelTime (MEGA 11) calibrated chronograph derived RAxML tree based on SNPs and ancestral range inferences. Circles at nodes represent ancestral area optimizations. C. Map showing biogeographical regions according to Morrone (2014) and legend of the biogeographical areas used.

We found that the crown age of *Olmeca* was 5.89 Ma, while Ruiz-Sanchez & Sosa (2015) estimated it at 6.05 Ma. Both time estimates are very similar and fall within the Late Miocene period. However, the topologies of the two phylogenetic trees differ significantly. The estimated crown age for Reflexa clade, the clade that includes two species with bacoid fruits, was 2.68 Ma (Figure 3B), which corresponds to the Late Pliocene period. In contrast, Ruiz-Sanchez & Sosa (2015) found a paraphyletic relationship between these two species and reported an older divergence time. Our time estimate for these bacoid fruit-producing species is similar to those of *Dinochloa* Buse and *Melocalanna* Trin., two Asian genera that also produce bacoid fruits (Liu *et al*. 2024). However, the estimated age is younger than that of *Melocalamus* Link & Otto (7.66 Ma), another Asian genus producing bacoid fruits (Liu *et al*. 2024). Additionally, *Alvimia* C.E. Calderón ex Soderstr. & Londoño, a Neotropical woody bamboo genus that produces bacoid fruits, shows a divergence time similar to that of *Olmeca* (Ruiz-Sanchez & Sosa 2015). According to Liu *et al*. (2024), bacoid fruits may have evolved independently in the Arundinarieae and Bambuseae tribes, following similar evolutionary patterns as adaptations to comparable environments.

The Fulgor clade has an estimated crown age of 4.89 Ma (Figure 3B), corresponding to the middle Pliocene. This clade is older than the Reflexa clade. The three species that compose this clade inhabit montane cloud forests (Ruiz-Sanchez *et al.* 2011, 2020). Montane cloud forests are among the richest ecosystems in Mexico, accounting for 10 % of the entire Mexican flora (Ornelas *et al.* 2013). This ecosystem harbors nearly 50 % of Mexico's total woody bamboo diversity (Ruiz-Sanchez *et al*. 2015, 2020), and most of its woody bamboo species fall into some risk category (Ruiz-Sanchez *et al.* 2020). Notably, the montane cloud forest is one of the most threatened ecosystems in Mexico (Ornelas *et al.* 2013). *Olmeca clarkiae* and *O. zapotecorum* have been classified as Critically Endangered, while *O. fulgor* is listed as Endangered. In contrast, *O. recta* is categorized as Near Threatened, and *O. reflexa* as Vulnerable (Ruiz-Sanchez *et al.* 2020).

As mentioned earlier, the Reflexa clade includes two species that inhabit tropical perennial forests, while the Fulgor clade comprises three species that inhabit montane cloud forests. A similar pattern of two clades was found in the phylogeny of *Tridimeris* Baill., a perennial tree from the Annonaceae family (Ortiz-Rodriguez *et al.* 2024). *Tridimeris* is now taxonomically classified into two sections: *Tridimeris* sect. *Tridimeris* and *T*. sect. *Zoque* Ortiz-Rodr. & Couvreur. Species in sect. *Tridimeris* inhabit montane cloud forests, with some occurring in sympatry with species from the Fulgor clade. Conversely, species in sect. *Zoque* inhabit tropical perennial forests, with some coexisting with *Olmeca recta* and *O. reflexa* in Los Tuxtlas, Uxpanapa, and La Pera (Ortiz-Rodriguez *et al*. 2024). Unfortunately, the phylogeny of *Tridimeris* has not been dated, but it would be interesting to determine whether both genera share similar diversification ages.

Our ancestral area reconstruction identified two possible ancestral regions for *Olmeca*, each with equal probability (50 %): Veracruzan + Chiapas Highland provinces or Veracruzan + Sierra Madre del Sur provinces. The reconstruction suggests that, in the first scenario, a dispersal event occurred from Veracruzan to Sierra Madre del Sur, whereas in the second scenario, dispersal occurred from Veracruzan to Chiapas Highland. However, in both cases, a vicariance event took place, leading to the separation of the ancestral Veracruzan province from the other two areas. This implies a division between montane cloud forests and tropical lowland ecosystems.

A distinctive feature of montane cloud forests is their location at elevations higher than tropical lowland ecosystems (such as tall or medium evergreen and semi-evergreen forests) but lower than the temperate forests found in mountainous regions (*e.g.*, Pine, Pine-Oak, or Oak forests). In addition to species exclusive to these forests, montane cloud forests also host species typical of both lowland humid forests and temperate forests from colder mountain regions, where the combination of altitude, humidity, and temperature fosters their coexistence (Villaseñor 2010).

The crown age of the Fulgor clade was estimated at 4.89 Ma, while the Reflexa clade dates to 2.68 Ma - representing a 2.21 Myr divergence. This extended temporal gap may reflect the time required for environmental stabilization, particularly the re-establishment of tropical perennial forest conditions that potentially facilitated the evolution of bacoid-type fruits in both species. These findings align with Ruiz-Sanchez & Sosa (2015) and Liu *et al.* (2024), who reported that bacoid fruits have independently evolved in different bamboo lineages within tropical forests. Moreover, they highlighted that this type of caryopsis represents an evolutionary novelty not only in bamboo species but also among grasses in general.

The crown age of *Olmeca* was estimated at 5.89 Ma, corresponding to the Late Miocene when the divergence between the Fulgor and Reflexa clades occurred. According to Graham (1975), tropical perennial forests were replaced by warm-temperate oak forests during this period. This finding was further supported by Palacios-Chávez & Rzedowski (1993), who documented the replacement of tropical perennial forests with montane cloud forests in northwestern Chiapas. These paleoenvironmental changes suggest that periods of environmental instability followed by stabilization may have driven the divergence of these *Olmeca* clades in southern Mexico. It is important to note that *Olmeca clarkiae* is geographically isolated from other Fulgor clade species by the Isthmus of Tehuantepec. This lowland valley, which separates the Sierra Madre del Sur from the Chiapas Highlands, formed approximately 6 Ma (Barrier *et al*. 1998). The Isthmus serves as a classic example of vicariance through habitat fragmentation (Ornelas *et al*. 2010, 2013, Gutiérrez-Ortega *et al*. 2020, 2024).

Our results suggest that RADseq data can effectively resolve the internal relationships within *Olmeca*, providing a well-supported phylogenetic framework for the genus. The analyses recovered *O. recta* and *O. reflexa* as a monophyletic group, supporting the hypothesis that the ability to produce a bacoid caryopsis is a shared, derived trait. Additionally, the remaining three *Olmeca* species formed a second clade, with *O. fulgor* as the sister species to *O. zapotecorum*, consistent with their morphological similarities. These findings highlight the power of RADseq data in resolving complex phylogenetic relationships and contribute to a better understanding of the evolutionary history of *Olmeca*.

Supplementary material

Supplemental data for this article can be accessed here: https://doi.org/10.17129/botsci.3676

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