Morphoanatomical study and genomic DNA extraction for molecular characterization of *Sinningia warmingii* (Hiern) Chautems “papa madre”

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**Abstract:**

**Introduction:** *Sinningia warmingii* (Hiern) Chautems (Gesneriaceae) is used in north eastern from Peru to relieve postpartum diseases and vaginal descents. The objective of the present study: to carry out morpho-anatomic analysis and extraction of the genomic DNA of the *Sinningia warmingii* "mother potato" species to identify the species. The sample was collected in Utcubamba-Amazonas. The morpho-anatomic study applied histological cutting techniques and genetic characterization with rbc-L, matK, ITS and trnH-psbA markers.

**Method:** Morphologically, it is a herbaceous species, with opposite leaves, shortly petiolated, the apical: sessile, oval, symmetrical, regularly closed edges, obtuse base, and acute apex. Flowers with greenish bell-shaped calyx, lobes with acute apex; tubular corolla, pink-fuchsia and yellow inside. Pubescent capsule fruit. Anatomically, abundant filiform and glandular multicellular trichomes, anomocytic stomata and vacuoles of phenolic substances (anthocyanins and tannins) were observed in the stem and leaves. open vascular bundle the stem has woody vessels (helicollen tracheids) and open lateral vascular bundles. In the root ("papa madre"), ovoid amyloplasts and brachisclereids were recognized.

**Results:** For the genetic characterization, genomic DNA was obtained, which was used to amplify by PCR: segments of the genes used for the bar code have been amplified with chloroplastidial markers (rbc-L, matK and ITS2) whose amplifications were positive. Sequencing has been performed with other species of the Sinningia genus that have confirmed the molecular characteristics of the genus.

**Conclusión:** the morpho-anatomic characteristics of the studied species correspond to the *Sinningia warmingii* species, which with this biomolecular study confirmed its correspondence to the *Sinningia genus*.

**Keywords:** *Sinningia warmingii*, morphology, histology, genomic DNA, molecular characteristics.

**Introduction:**

The species mother potato or pumachilca corresponds to the scientific name *Sinningia warmingii* (Hiern) Chautems, it is characterized as a herbaceous plant with tuberous roots, short trunk, velvety dark green leaves, oval, crenulate, petiolate and fleshy edges, with bilabiadas flowers.
They are found in Peru in Cajamarca and Huancavelica, between 2000-2500 m.a.s.l., they usually grow in areas with a dry season or exposed to the sun. A species of great importance to relieve female diseases, such as: postpartum inflammations and vaginal discharge (Mostacero, 2009; Sagástegui, 1995; Klouman, 2005).

The objective of the study is to evaluate by molecular analysis, using the model according to Robert (1979) that will generate the DNA barcode corresponding to the species, using the matK, rbcL and ncpGS genes, sequences that will be deposited in the databases of the international barcode project of life (iBOL). The purpose of this work is to determine the morpho-anatomic and molecular characterization of Sinningia warmingii. The tuberous roots of Sinningia warmingii were collected in the province of Utcubamba department from Amazonas; located at 400 m.a.s.l. The taxonomic identification was carried out in the Natural History Museum –Universidad Nacional Mayor de San Marcos.

Material and Method:

Botanical Analysis

It involves the morpho-anatomic and histochemical study; making histological cuts of root, stem and leaf to determine the presence of structures and tissues of the species.

Molecular Biological Analysis

Genomic DNA extraction

The plant material was used to extract genomic DNA (tuberous root), according to the Doyle extraction method & Doyle, 1990 in 100 mg of plant material.

DNA Spectrophotometric Measurements.

For each sample, the absorbance reading was taken at 280nm and 260 nm, using Nanodrop equipment. The DNA concentrations were made with the reading at 260 nm and its degree of contamination with the A260/A280 ratio (between 1.8 -2.0, good degree of DNA purity).

The measurements obtained were: concentrations between 34ng/ul - 50.9ng/ul and purity A260/A280 between 1.7-1.82. The purity ratios are between the expected values of 1.8-2.0. All samples could be amplified by PCR.

Results:

Botanical Analysis

Abundant multicellular and glandular trichomes were found in the stem and leaf sections. The presence of anthocyanins and tannins was observed in the epidermal layer and cortical parenchyma. Likewise, the histochemistry that tested positive for the lugol reaction was performed, observing abundant presence of starch granules in relation to the tuberous root; The reaction of iron trichloride was also positive, observing the presence of phenolic compounds also in stem and leaf.
Figure 1: (a) Flower, (b) flower and leaf, (c) root and stem, *Sinningia warmingii* "papa madre"

Figure 2: Photomicrograph of histological sections of *Sinningia warmingii*. A and B (Stem), C (Root), D (Leaf), E (Yema) and F (Root tuberosa).

### Molecular Characterization

**DNA Spectrophotometric measurements**

For each sample, the absorbance reading was taken at 280 nm and 260 nm, using Nanodrop equipment. DNA concentrations were performed with the reading at 260 nm and the degree of contamination with the A260/A280 ratio (between 1.8-2.0), good degree of DNA purity.

The measurements obtained were: at a concentration between 34 ng/uL - 50.9 ng/uL, purity A260/A280 between 1.7-1.82. The purity ratios are between the expected values of 1.8 - 2.0. All samples could be amplified by PCR.
Genomic DNA Visualization

Once the genomic DNAs were quantified, they were observed in 1% agarose gels using TBE1X electrophoresis buffer, loading for each well an approximate amount of 200ng of DNA. Electrophoresis in 1 hour at 100V. Staining was performed in 0.05% ethidium bromide solution to be visualized in a UV transilluminator. Photographic record of the observations and their labeling was taken.

![Visualization of genomic DNA of 4 samples collected (Sin1, Sin2, Sin3, Sin4), “maca” (positive control), C(+) positive control](image)

**Figure 3:** Visualization of genomic DNA of 4 samples collected (Sin1, Sin2, Sin3, Sin4), “maca” (positive control), C(+) positive control

a) **Evaluating DNA quality for PCR amplification: Amplifying the rbc-L chloroplastidial marker** (coding sequence)

The rbc-L chloroplastidial marker was used, whose primers have amplification efficiency close to 100% in different plant taxa and do not require a protocol with special PCR conditions for amplification. The primers used amplify the 5’ region of the coding region of the gene comprising position 1 to 599.

The PCR reaction was performed in a volume of 25uL with 1X of PCR buffer, 2.5mM of magnesium chloride, 0.05mM of dNTPs, 0.1uM of each first (rbcLa-F and rbcLa-R), 0.625U of Taq polymerase and 60-150 ng of DNA. With the 94 °C amplification program for 4 minutes followed by 35 cycles of 94 °C for 30 seconds, 55 °C for 30 seconds, 72 °C for 1 minute, and a final extension of 72 °C for 10 minutes.

5uL of the amplified product was visualized on a 2% agarose gel, in 1X TBE electrophoresis buffer for 100V for 45 minutes; for subsequent staining with 0.05% ethidium bromide and visualization in a UV transilluminator and photographic record of the observations.
Amplification of the rbc-L gene in the Sinningia warmingii replicas provided (1.2: leaf; 3.4: tuber) with two positive controls (plant samples that amplify the marker)

b) Nuclear marker amplification: ITS2 marker (Intragenic Sequence)

This marker is being proposed as a DNA barcode for plants with efficiency and similar in the identification of species reached by the IOC gene in animals allowing itself to correctly discriminate between 92.7% at the species level and 99.8% at the genus level in medicinal plants. It comprises a short region between 160pb and 320pb and is easily amplifiable with universal primers whose conditions are given below:

PCR reaction performed in 50uL volume with 1X PCR buffer, 2.5mM magnesium chloride, 0.05mM dNTPs, 0.1uM of each first (ITS-S2F and ITS4), 0.625U of Taq polymerase and 60- 150ng of DNA With the following amplification program 94 ° C for 5 minutes followed by 35 cycles of 94 ° C for 30 seconds, 56 ° C for 30 seconds 72 ° C for 45 seconds, and final extension of 72 ° C for 10 minutes, 5uL of the amplified product was visualized in 2% agarose gel, in 1X TBE electrophoresis buffer for 100V for 45 minutes. For its subsequent staining with 0.05% ethidium bromide and its visualization in a UV transluminator and photographic record of the observations.

<table>
<thead>
<tr>
<th>Región</th>
<th>Primer</th>
<th>Sequence (5’ 3’)</th>
<th>pb</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITS2</td>
<td>F</td>
<td>ATGCCGATACGTGTGGAAT</td>
<td>160</td>
<td>Chen et. al</td>
</tr>
<tr>
<td>R</td>
<td>ITS4</td>
<td>TCCTCCGCTTATTGATATGC</td>
<td>320</td>
<td>White et. al</td>
</tr>
</tbody>
</table>

b) Amplification of the chloroplastidial markers: mat-K marker (coding sequence)

The chloroplastidial mat-K marker was used, whose primers do not offer the universality characteristic of those used for the rcb-L gene. The primers used, belong to the one reported by Ki-Joong and are the ones that have presented the least problems in studies in other plant taxa. These amplify the internal coding region of the gene that comprises position 173 through 1046. The PCR reaction was performed in a volume of 50uL with 1X of PCR buffer, 1.5mM of magnesium chloride, 0.2mM of dNTPs, 1uM of each first (matK-KIM3F and matK-KIM1R), 2U of Taq polymerase and 10 - 25ng of DNA. With the 94 ° C amplification program for 1 minute followed by 35 cycles of 94 ° C for 30 seconds, 52 ° C for 20 seconds, 72 ° C for 50 seconds, and a final extension of 72 ° C for 5 minutes.
5μL of the amplified product was visualized on a 2% agarose gel, in 1X TBE electrophoresis buffer for 100V for 45 minutes. For its subsequent staining with 0.05% ethidium bromide and its visualization in a UV transluminator and photographic record of the observations.

**Figure 4:** Amplification of the mat-K gene in the *Sinningia warmingii* replicas provided (SINING1,2: leaf; SINING 3,4: tuber) with two positive controls (plant samples that amplify the marker).
Figure 5: Amplification of the ITS2 gene in the *Sinningia warmingii* replicas provided (SIN1,2: leaf; SIN3,4: tuber) with two positive controls (plant samples that amplify the marker).

![Image of ITS2 amplification](image)

Figure 6: Amplification of the rbc-L gene in the *Sinningia warmingii* replicas provided (SIN1,2: leaf; SIN3,4: tuber) with two positive controls (plant samples that amplify the marker).

It has been possible to isolate genomic DNA from the foliar material and the plant tuber, this material has been amplified with three chloroplastidial markers (rbc-L, matK and ITS2) whose amplifications were positive. The amplified products have been sent to an external sequencing service to obtain the DNA sequences that will allow them to be compared with those of other *Sinningia* species.

**Discussion:**

The "mother potato" *Sinningia warmingii* species of great importance to relieve female diseases: treatments of postpartum inflammations and vaginal discharge (Mostacero 2009; Sagástegui 1995; Klouman, 2005), has demonstrated its high antioxidant power, which would favor its pharmacological effect.

*Sinningia warmingii* is a scientific synonym for *Reschsreineria peruviana* called pumachilca that mentions that crushed leaves are applied as a poultice for pain, bumps and fractures. (Vicuña 2011). Molecular analyzes of the nuclear glutamine synthase (ncpGS) gene indicate that the root of the Sinningieae tribe probably corresponds to the rainforests of the Atlantic (San Francisco Region-Brazil), where approximately 57% of the events occur, speciation. The ancestral area of the Dircaea clade was limited to that of the southern Atlantic rainforest, while the northern Atlantic region was probably part of the ancestral distribution of the Corytholoma and *Sinningia clades* (Perret 2006).

The *Sinningia* genus is a large and variable genus, with approximately 75 species; distributed from Mexico to Argentina but focused on Brazil. *Sinningia elatior* and *Sinningia warmingii* are found in Peru, with bilabiated flowers; They grow normally in areas with dry season or exposed to the sun. Paul Hebert, a researcher at the University of Guelph in Ontario, Canada, proposed in 2003 the "DNA barcode" as a way to identify species. The barcode is the use of one or several short DNA sequences, of standardized locus (or loci), as a tool to identify species of living organisms (Perret 2007). In the present study the morpho-anatomic characteristics of the studied species...
correspond to the Sinningia warmingii species and thanks to the biomolecular study its correspondence to this genus could be corroborated. The generation of the DNA barcode will be a new taxonomic tool that is easy and fast to identify, will allow monitoring of cases of adulteration of commercial pharmacological preparations, as well as guarantee the authenticity and quality of the products made by manufacturers and the defense of their patent. It will also contribute to the international project of barcodes of life, in its eagerness to inventory the living diversity of the planet.

DNA was extracted from plant samples of Sinningia warmingii using the method described by Doyle & Doyle. The final lyophilisate of DNA was resuspended in 100uL of Tris: 20mM EDTA: 1mM and the spectrophotometric measurements resulted A260 / A280 of 1.8 concentration of 50ng / uL. The electrophoresis of the genomic DNA obtained revealed the integrity of the DNA for subsequent PCR tests. The PCR primers or primers proposed by the "Consortium for the Barcode of Life" were selected with which PCR amplification of the rbc-L, mat-K, ITS and trnH-PsbA markers was achieved. These PCR products were sequenced by the Macrogen USA sequencing service.

Conclusion:

The morphoanatomic characteristics of the studied species correspond to the Sinningia warmingii species and the biomolecular study corroborated its correspondence to the Sinningia genus.

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Reference


