

## Project Update: February 2022

Period: September 27th, 2021 - February 13th, 2022

Progress of activities of the first main objective:

### 1. Development of protocols for the generation of seedlings of *Myrica parvifolia* and other forest species in vitro and in nursery

Related projects:

Title	Reforestation of areas degraded by forest fires in the Tambillo Community Protected Area
Institution:	Universidad de Cuenca
Modality:	Social Outreach Hours
Principal Researcher:	Ph.D. Juan Pablo Ñamagua – Researcher and lecturer

Title	Establishment of a protocol for in vitro propagation of <i>Myrica</i> sp. ecosystem restoration and conservation purposes in the Tambillo Community Protected Area
Institution:	Universidad Politécnica Salesiana
Modality:	Pre-professional internships
Principal Researcher:	Ph.D. Inés Malo – Researcher and Lecturer M. Sc. Mateo León - External Researcher

Tentative Title	<i>In vitro</i> multiplication of woody species for reforestation purposes in the Tambillo Community Protected Area
Institution:	Universidad Técnica de Manabí
Modality:	Undergraduate thesis
Principal Researcher:	Ph.D. Liliana Corozo - Researcher and Lecturer Ph.D. (c) Miryan Pinoargote - Researcher and Lecturer

#### 1.1. Collection of biological material

Seeds and vegetative parts (apical shoots and nodal parts) of *M. parvifolia* collected in the montane cloud forest (middle zone) of the Tambillo Community Protected Area were randomly. In May 2021, the fruits of *M. parvifolia* were immature (Image 1); between September and October 2021, the fruits reached maturity (Image 2). Specimens approximately 2 years old were selected.

#### 1.2. Propagation in nursery

##### 1.2.1. Seed pretreatment

To break dormancy and stimulate germination of *M. parvifolia* seeds, two pretreatments were carried out. 1) Seeds were placed in boiling water, and by the action of detergent, the waxy layer was removed manually; 2) Seeds were rubbed on a

rough surface. Finally, the seeds were sown in organic substrate and taken to a nursery at the Universidad de Cuenca and to a private nursery in the Tarqui parish of the city of Cuenca (Image 3 - 4).

### 1.3. In vitro Propagation

#### 1.3.1. Protocol for seed disinfection

Scarified seeds of *M. parvifolia* were soaked for 24 hours in a solution of gibberellic acid (GA3). In a laminar flow chamber, a solution of Tween 20, 70% ethanol and 2.5% sodium hypochlorite were used for seed disinfection. Subsequently, the aseptic seeds were placed in sterile Murashige & Skoog (MS) culture medium supplemented with Gamborg's vitamins, sucrose and agar. Finally, the samples in a growth chamber of the Universidad Técnica de Manabí were placed, and the growth conditions were photoperiod 12 hours light - 12 hours dark and temperature 25 °C  $\pm$ 2 (Image 5).

#### 1.3.2. Protocol for disinfection of explants

The apical shoots and nodal parts of *M. parvifolia* were washed with liquid soap and running water. In a laminar flow chamber, Tween 20 solution, 70% ethanol and sodium hypochlorite solution were used to disinfect explants. Subsequently, the aseptic explants were placed in sterile culture medium (MS), supplemented with Gamborg's vitamins, antioxidants (cysteine, polyvinylpyrrolidone, and activated charcoal), sucrose and agar. Finally, the samples were deposited in the growth chamber of the Universidad Técnica de Manabí and the Universidad Politécnica Salesiana, the growth conditions were photoperiod 12 hours light - 12 hours dark and temperature 25 °C  $\pm$ 2 (Image 6 - 7).



Image 1: Immature fruits of *M. parvifolia*. © José Zhunio



Image 2. Mature fruits of *M. parvifolia*. © Juan Pablo Iñamagua



Image 3. Germination of *M. parvifolia* seeds after pretreatment 1, Universidad de Cuenca nursery. © Mateo León



Image 4. Germination of *M. parvifolia* seeds after pretreatment 2, private nursery in the Tarqui parish of the city of Cuenca. © Mateo León



Image 5. *M. parvifolia* seeds in MS medium with Gamborg vitamins. © Miryam Pinoargote



Image 6. Apical shoots of *M. parvifolia* on MS medium with Gamborg vitamins, cysteine, fungicide, and antibiotic. © Miryan Pinoargote



Image 7. Disinfection of nodal segments of *M. parvifolia*, sowing in MS medium with Gamborg vitamins and polyvinylpyrrolidone. © Mateo León

## 2. Intensify actions that involve the Jima Community and the teams of the collaborating universities (researchers – lecturers and students)

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### 2.1 Local trips to the Tambillo Community Protected Area (TCPA)

October 10th, 2021: a prospecting visit was made with Dr Juan Pablo Iñamagua, professor at the Universidad de Cuenca.

November 13th, 2021: a field visit was conducted with Dr Juan Pablo Iñamagua and undergraduate students of agronomy at the Universidad de Cuenca. The objective was to explore the protected forest and recognise the forest species that will be used in the implementation of each project (Image 1).

November 26th, 2021: a prospecting visit was made with Dr Diana Fernández, a researcher from the National Institute of Biodiversity - Ecuador (INABIO in Spanish). The objective was to recognise a potential plot within the protected forest where a floristic inventory will be carried out (Image 2).

January 12th, 2021: a field visit was conducted with undergraduate students of biotechnology at the Universidad Politécnica Salesiana. The objective was to collect botanical samples of *Myrica* sp., *Clusia* sp., and *Hedyosmum* sp. for micro propagation trials (Image 3).

### 2.2 Participatory workshops and meetings with the Jima Community

Participatory workshops

November 13th, 2021: a diagnostic workshop was held, entitled "Tell us the Tambillo story", which was planned and executed by the Tambillo Forest Initiative coordinators. The objective was to generate trust and cohesion between the parties (professionals and partners) for the strengthening and organisational participation of the members of



the Cooperativa de Desarrollo de la Comunidad Jima Ltda (CDCJL) (Image 4).

- Surveys for participatory identification of forest species to be used in reforestation November 1th3, 2021: Dr Juan Pablo Ñamagua and undergraduate students from the agronomy programme of the Universidad de Cuenca surveyed members of the CDCJL. The objective was to identify participatory forest species of interest for reforestation in degraded areas of the TCPA.

December 10th, 2021: Dr Juan Pablo Ñamagua and undergraduate students of the agronomy program of the Universidad de Cuenca surveyed members of the Carmen de Zhipta Community of the Jima parish. The objective was to identify forest species of interest for reforestation in degraded areas of the TCPA.

- Meetings with local stakeholders from Educational Institutions in Jima Parish. December 03rd, 2021: Mgs. María Elisa Durán and undergraduate students of the environmental programme of the Universidad de Cuenca participated in a meeting with representatives of the Jima Educational Unit and the Marco Antonio Toral School of the Jima parish. The objective was to present the environmental education proposal linked to the TCPA conservation and restoration project (Image 5).

## **Partial Results**

2.3 Intensify actions that involve the Jima Community and the teams of the collaborating universities (researchers – lecturers and students)

### 2.3.1 Local trips to the Tambillo Community Protected Area

Between the period September 2021 and January 2022, six local trips have been made to the TCPA; this represents 46% of the committed local trips.

#### 2.3.1.1 Participatory workshops

Workshop "Tell us the story of Tambillo"

CDCJL members recounted the beginning of the formation of the cooperative, the acquisition of the Tambillo area, internal problems within the organisation and external problems, particularly with illegal mining and invasion by outsiders. They also commented on the solutions they have given to the different problems over the years.

On the other hand, CDCJL members have high expectations for the presence of professionals and university students. They are identified with the TFI and hope that this major project will contribute to materialise part of their cooperative dreams.

2.3.2 Surveys for the participatory identification of forest species to be used in reforestation.

The data obtained from the surveys were tabulated and are in the process of analysis by members of the Universidad de Cuenca, the result of the forest species of ecological and economic interest for the reforestation of degraded areas in the TCPA is detailed in Table 1.

Table 1: Forest species of ecological and economic interest to be used in TCPA reforestation

Common name in Ecuador	Botanical classification			Ecological ranking	Economic ranking
	Family	Genus	Species		
Duco	Clusiaceae	<i>Clusia</i>	sp	1st	5th
Laurel de cera	Myricaceae	<i>Myrica</i>	sp	2nd	1st
Garau	Proteaceae	<i>Lomatia</i>	sp	3rd	7th
Joyapa	Ericaceae	<i>Macleania</i>	sp	4th	8th
Sarar	Cunoniaceae	<i>Weinmannia</i>	sp	5th	2nd
Jalo	Rosaceae	<i>Hesperomeles</i>	sp	6th	4th
Toronjil de cerro	Chloranthaceae	<i>Hedyosmum</i>	sp	7th	3rd
Chachaco	Escalloniaceae	<i>Escallonia</i>	sp	8th	6th



Image 1: Field visit to the Community Protected Area with Juan Pablo Iñamagua, professor and students of Agronomy at the Universidad de Cuenca. © Mateo León



Image 2. Prospecting visit to Tambillo Community Protected Area with Diana Fernández, INABIO researcher. © Franklin Chacha



Image 3. Field visit to Tambillo Community Protected Area with students of the Biotechnology program of the Universidad Politécnica Salesiana. © Mateo León



Image 4. Participants of the first diagnostic workshop "Tell us the story of Tambillo". © Diana Cordero



Image 5. Presentation of the Environmental Education proposal by María Elisa Durán from the Universidad de Cuenca to the representatives of the educational institutions of the Jima Community. © María Domínguez