UNIVERSIDAD MIGUEL HERNÁNDEZ DE ELCHE

Programa de Doctorado en Recursos y Tecnologías Agrarias, Agroambientales y Alimentarias

TESIS DOCTORAL

POTENCIAL DEL MORTIÑO (*Vaccinium floribundum* Kunth) COMO ALTERNATIVA DE CULTIVO EN EL PÁRAMO ECUATORIANO

JORGE MARCELO CARANQUI ALDAZ

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La presente Tesis Doctoral, titulada **"POTENCIAL DEL MORTIÑO (*Vaccinium floribundum* Kunth) COMO ALTERNATIVA DE CULTIVO EN EL PÁRAMO ECUATORIANO"** se presenta bajo la modalidad de **tesis por compendio** de las siguientes **publicaciones:**

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PUBLICACIÓN 1

The mortiño (*Vaccinium floribundum* kunth): a review of its suitability as a promissory crop in the ecuadorian paramo and its potential uses, environmental role, and health benefits

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El **Dr. D. Rafael Todos Santos Martínez Font**, Titular de Universidad, director, y **Dña. Francisca Hernández García**, Catedrática de Universidad, codirectora de la tesis titulada **"Potencial del mortiño (*Vaccinium floribundum* kunth) como alternativa de cultivo en el páramo ecuatoriano"**

CERTIFICAN:

Que **D. Jorge Marcelo Caranqui Aldaz**, ha realizado bajo nuestra supervisión la Tesis Doctoral titulada **"Potencial del mortiño (*Vaccinium floribundum* kunth) como alternativa de cultivo en el páramo ecuatoriano"**, conforme a los términos y condiciones definidos en su Plan de Investigación y de acuerdo al Código de Buenas Prácticas de la Universidad Miguel Hernández de Elche, cumpliendo los objetivos previstos de forma satisfactoria para su defensa pública.

Y para que conste a los efectos oportunos se firma el presente certificado en Orihuela a 10 de julio de 2024

Firmado:

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Dr. Dña. Juana Fernández López, Catedrática de Universidad y Coordinadora del Programa de Doctorado en Recursos y Tecnologías Agrarias, Agroambientales y Alimentarias (ReTos-AAA) de la Universidad Miguel Hernández de Elche (UMH),

CERTIFICA:

Que la Tesis Doctoral titulada **"Potencial del mortiño (*Vaccinium floribundum* kunth) como alternativa de cultivo en el páramo ecuatoriano",** de la que es autor el Ingeniero Agrónomo **D. Jorge Marcelo Caranqui Aldaz**, ha sido realizada bajo la dirección del **Dr. D. Rafael Todos Santos Martínez Font** y la codirección de la **Dra. Dña. Francisca Hernández García**, actuando como tutor/a de la misma el **Dr. D. Ricardo Abadía Sánchez**. Considero que la Tesis es conforme, en cuanto a forma y contenido, a los requerimientos del Programa de Doctorado ReTos-AAA, siendo por tanto apta para su exposición y defensa pública.

Y para que conste a los efectos oportunos firmo el presente certificado en Orihuela a 10 de julio de dos mil veinticuatro.

Dra. Dña. Juana Fernández López

Coordinadora del Programa Doctorado ReTos-AAA

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ESTRUCTURA DE LA TESIS DOCTORAL

Esta Tesis Doctoral se ha redactado siguiendo el reglamento interno vigente de la Universidad Miguel Hernández de Elche para la presentación de la Tesis Doctoral bajo la modalidad de tesis por compendio de publicaciones.

Esta memoria se ha estructurado de la siguiente forma:

- **Resumen y Abstract:** breve resumen donde se detallan los principales objetivos y los resultados más relevantes (en castellano e inglés).
- **Introducción:** contextualización del estado del arte del mortiño (*Vaccinium floribundum* Kunth). Importancia, origen, taxonomía y usos del mortiño.
- **Objetivos:** objetivo principal y objetivos específicos de la investigación.
- **Resumen de la metodología:** breve descripción de la metodología empleada para la consecución de los objetivos.
- **Publicaciones científicas:** transcripción literal de las tres publicaciones científicas incluidas en esta Tesis.

Publicaciones:

1. Reproductive phenology of *Vaccinium floribundum* Kunth (Ericaceae) and codification according to the BBCH scale based on evidence from the volcano Chimborazo paramo (Ecuador). 2022. *Scientia Horticulture*. *303 (111207).* https://doi.org/10.1016/j.scienta.2022.111207

2. Chemical Composition and Polyphenol Compounds of *Vaccinium floribundum* Kunth (Ericaceae) from the Volcano Chimborazo Paramo (Ecuador). 2022. *Horticulturae. 8 (10), 956.* https://doi.org/10.3390/horticulturae8100956

3. The Mortiño (*Vaccinium floribundum* Kunth): a review of its suitability as a promissory crop in the Ecuadorian Paramo and its potential uses, environmental role, and health benefits. 2024. *European Food Research and Technology.* https://doi.org/10.1007/s00217-024-04546-4

- **Resumen de los resultados, discusión y conclusiones:** en esta sección se hace, de cada publicación, un breve resumen de los principales resultados obtenidos, una discusión general de los mismos y las conclusiones.
- **Conclusiones generales**: conclusiones de la Tesis Doctoral.
- **Investigaciones futuras:** breve descripción de futuras investigaciones que pueden desarrollarse a partir de los resultados obtenidos sobre el mortiño.
- **Referencias bibliográficas:** en esta sección se indican las referencias utilizadas para la redacción y justificación de esta Tesis Doctoral siguiendo la 7ª edición del formato APA.

RESUMEN

ABSTRACT

RESUMEN

Los páramos forman parte de una notable biodiversidad a escala de ecosistemas que se presentan en el Ecuador. El mortiño (*Vaccinium floribundum* Kunth), de la familia Ericaceae, es una especie nativa de los páramos ecuatorianos con hábito arbustivo y frutos comestibles. Las poblaciones han sufrido procesos de pérdida por deforestación, reconversión productiva, fragmentación, perturbación por su extracción, por lo que es necesario: (1) Realizar una concienzuda revisión bibliográfica sobre el "estado del arte" del mortiño; demostrando su importancia agroalimentaria y potencial contribución a la mejora de los entornos socioeconómicos deprimidos; (2) Caracterizar fenológicamente el material vegetal seleccionado mediante fenogramas de cada una de las distintas etapas del desarrollo reproductivo anual del mortiño y, (3) Caracterización bioquímica del material vegetal, resaltando las propiedades, principios activos y otros beneficios de los frutos del mortiño.

ABSTRACT

The páramos are part of a notable biodiversity at the ecosystem level that occurs in Ecuador. The mortiño (*Vaccinium floribundum* Kunth), from the Ericaceae family, is a native species of the Ecuadorian páramos with a shrub-like growth habit and edible fruits. Its populations have suffered losses due to deforestation, productive conversion, fragmentation, and disturbances from extraction. Therefore, it is necessary to: (1) Carry out a thorough literature review on the "state of the art" of mortiño, demonstrating its agri-food importance and potential contribution to the improvement of depressed socioeconomic environments; (2) Phenologically characterize the selected plant material through annual phenograms of the different development reproductive stages of mortiño; and (3) Biochemical characterization of the plant material, highlighting the properties, active principles, and other benefits of mortiño fruits.

1. INTRODUCCIÓN

1. INTRODUCCIÓN

El mortiño (*Vaccinium floribundum* Kunth), es una especie nativa de los páramos ecuatorianos que destaca por su valor cultural y nutricional. En el neotrópico se concentra en los Andes de Colombia y Ecuador donde crece en ambientes húmedos hasta los 4.350 m s.n.m. de altitud (Luteyn, 2002). Desde épocas inmemoriales las bayas moradas de esta especie, conocidos como "mortiño", han sido utilizadas, principalmente, para la elaboración de la tradicional colada morada en el día de los Difuntos (Coba-Santamaria et al., 2012).

Además de sus propiedades medicinales, el cultivo de mortiño es una alternativa sostenible y rentable para las comunidades que habitan en los páramos ecuatorianos, ya que, es una especie adaptada a los suelos ácidos y a las condiciones climáticas adversas prevalentes a muy elevada de alta altitud.

En los últimos años su potencial como cultivo comercial ha suscitado un interés creciente, impulsado por la alta demanda de alimentos saludables y funcionales en el mercado mundial. Las bayas del mortiño se consideran como un 'superfruto' debido a su capacidad antioxidante y posibles beneficios para la salud. Sin embargo, en los páramos ecuatorianos, el mortiño, ha comenzado a desaparecer debido a la dificultad para su propagación. Además, las poblaciones han sufrido procesos de pérdida por deforestación, reconversión productiva, fragmentación y perturbación de su ecosistema. El incremento en la demanda de frutos de mortiño ha ocasionado su extracción de forma intensiva del medio natural, lo cual, unido a la deforestación de los bosques, está causando que la especie se encuentre en un proceso de desaparición.

Por ello, pensamos que el conocimiento de la biología reproductiva del mortiño, así como de su propagación en la Reserva de Producción Faunística de Chimborazo, RPFCH (Ecuador), es fundamental para conservar su variabilidad genética, tanto con fines productivos, como de manejo sostenible de las poblaciones naturales. Además, el conocer la composición en compuestos bioactivos de las bayas de mortiño puede contribuir a mejorar las ventas en el mercado del mortiño y, al mismo tiempo, puede proporcionar oportunidades económicas sostenibles para los agricultores del páramo.

La finalidad última de esta Tesis Doctoral ha sido contribuir a mejorar el conocimiento sobre esta especie frutal, lo que indudablemnte ayudará a mejorar la sostenibilidad y preservación de este valioso recurso natural.

1.1 Características generales del mortiño

En el neotrópico, el mortiño, se concentra en los Andes de Colombia y Ecuador y crece en ambientes húmedos hasta los 4.350 m.s.n.m. (Luteyn, 2002). En Ecuador, *Vaccinium floribundum* Kunth es considerada una planta silvestre que crece en las partes altas de la cordillera, desde los páramos del Ángel en el Carchi hasta Tambo en Cañar. Además, se conocen datos proporcionados por el Parque Nacional Cotopaxi que ubican la zona de adaptación del mortiño desde los 2.000 m s.n.m. hasta los 4.500 m s.n.m. Pero la realidad es que son pocos los páramos que poseen un número considerable de plantas debido a la extensión de las áreas agrícolas, que han relegado a esta especie a zonas de páramo comprendidas entre los 3.400 - 3.500 hasta los 4.500 m s.n.m. (MAGAP, 1988).

En base a colectas realizadas, *Vaccinium floribundum* se encuentra en la Sierra en las provincias de Carchi, Imbabura, Pichincha, Cotopaxi, Tungurahua, Bolivar, Chimborazo, Cañar, Azuay y Loja. De igual manera y, en base a colectas realizadas, se indica que *Vaccinium distichum* y *Vaccinium crenatum* se encuentran localizados en las provincias del Azuay y Loja (Luteyn, 2002).

1.2 Taxonomía y descripción botánica del mortiño

La clasificación taxonómica de *V. floribundum* Kunth, de acuerdo al Sistema de Clasificación de APG IV (2016), es la siguiente:

Clase: Equisetopsida C. Agardh.

Subclase: Magnoliidae Novák ex Takht.

Superorden: Asteranae Takht.

Orden: Ericales Bercht. & J. Presl.

Familia: Ericaceae Juss.

Género: *Vaccinium* L.

Especie: *V. floribundum* Kunth.

El mortiño es un arbusto a menudo rizomatosos. Presenta hojas alternas, persistentes o caducifolias, de margen entero o aserrado, generalmente con nervios pinnados, raramente plinervias y pecíolo corto. Inflorescencia axilar, que se desarrolla a partir de las yemas de la temporada anterior, racemosa o rara vez con sólo 1 o 2 flores. Las flores nacen en la axila de la bráctea floral o de una hoja; pedicelo generalmente bibracteolado. Flores de 4 a 5-meras, generalmente inodoras; imbricada (*V. crenatum, V. floribundum*); cáliz articulado con el pedicelo (en Ecuador) o continuo; hipantio cilíndrico a globoso; los lóbulos rara vez están obsoletos; corola gamopétala, cilíndrica, urceolada o campanulada, blanca, verdosa, roja o amarillenta, los lóbulos rara vez se separan casi hasta la base; estambres 8 o 10, iguales, aproximadamente igualando la corola; filamentos distintos, más largos que las anteras, con o sin espolones, a veces en *V. floribundum* con espolones aparentemente vestigiales; tecas lisas o papiladas; túbulos dehiscentes por poros terminales o, raramente, hendiduras oblicuas; ovario total o parcialmente inferior, 4-5 (raramente 10) loculares; estigma pequeño, simple o algo capitado.

Imagen 1: Flores de mortiño

Fuente: elaboración propia

El fruto es una baya, con 5 o muchas semillas, coronada por los persistentes lóbulos del cáliz y coronada por el conspicuo disco nectarífero; semillas a veces con mucilago. *Vaccinium* es un género de quizás 300 especies principalmente del hemisferio norte, en su mayoría montano en los trópicos; tres especies se encuentran en Ecuador (Luteyn, 2002).

Imagen 2: Frutos de mortiño

Fuente: elaboración propia

1.3 Compuestos funcionales del mortiño y beneficios para la salud

Según Gaviria et al. (2009) el género *Vaccinium* (Ericaceae) tiene cerca de 400 especies y sus frutos han atraído el interés de muchos investigadores alrededor del mundo, debido al alto contenido en compuestos polifenólicos, tales como ácido cinámico, flavonoles, antocianinas y antocianidinas.

Teniendo en cuenta el creciente interés en las propiedades nutraceúticas de los frutos del género *Vaccinium* y la gran demanda de aditivos naturales en los mercados internacionales, varios investigadores han centrado sus estudios en las propiedades antioxidantes del mortiño como una forma de iniciar el proceso de selección de germoplasma, para la selección de poblaciones con viabilidad para ser domesticadas (Prior et al., 1998).

La actividad antioxidante de las bayas del género *Vaccinium*, en especies cultivadas comercialmente como arándano azul y arándano rojo, así como de otras especies que son recolectadas de los bosques, se ha encontrado que está altamente influenciada por: (i) el contenido de antocianinas y fenoles totales, (ii) el genotipo, (iii) la variación en las condiciones ambientales, (iv) el estado de madurez de las frutas y, (v) las condiciones de almacenamiento en poscosecha (Kähkönen et al., 2001).

El arándano andino (*Vaccinium floribundum* Kunth.) es un fruto que se encuentra en Ecuador a gran altitud y casi exclusivamente en forma silvestre. El uso comercial de los arándanos andinos aún es bajo. Vasco et al. (2009) determinaron, por primera vez, la composición fenólica de esta baya e informaron que se caracteriza principalmente por la presencia de antocianinas y proantocianidinas, en menor cantidad.

La capacidad antioxidante de *V. floribundum* ha sido cuantificada a través de varios ensayos, proporcionando información sobre su eficacia en la eliminación de radicales libres. Ramírez et al. (2015), determinaron la actividad antioxidante de los extractos de *V. floribundum* por los métodos DPPH (2,2-difenil-1-picrilhidrazilo) y el ABTS (2,2'-azino-bis (3-etilbenzotiazolin-6-sulfónico) (ABTS), y sus resultados demostraron una relación dosis-dependiente, lo que pone de manifiesto la capacidad de la fruta para combatir el estrés oxidativo.

Una de las propiedades medicinales destacadas atribuidas a *V. floribundum* es su efecto antiinflamatorio. La inflamación crónica está implicada en diversas enfermedades, incluida la artritis y enfermedades cardiovasculares. El estudio realizado por Ramírez et al. (2015), exploró el potencial antiinflamatorio de extractos a base de *V. floribundum* utilizando modelos *in vitro*. Los resultados indicaron una reducción significativa de los marcadores inflamatorios, lo que sugiere que el mortiño puede poseer propiedades antiinflamatorias y, por tanto, podría ser utilizado con fines terapéuticos. Dicha actividad antiinflamatoria del *V. floribundum* suele estar relacionada con su contenido en flavonoides como la quercetina y los derivados de miricetina identificados en el fruto. Este estudio subraya el potencial de *V. floribundum* como agente antiinflamatorio natural. Las propiedades antioxidantes y antiinflamatorias de la fruta pueden contribuir a mejorar la salud cardiovascular al reducir el estrés oxidativo y la inflamación, ambos implicados en enfermedades del corazón.

El perfil polifenólico varia dependiendo de la especie de *Vaccinium*. Esquivel-Alvarado et al. (2020) analizarón el contenido en polifenoles y los perfiles de antocianinas y proantocianidinas de diferentes especies de *Vaccinium* recolectadas en las tierras altas de Costa Rica. Los resultados obtenidos pusieron de manifiesto que los contenidos en polifenoles están influenciados por la etapa de desarrollo del fruto. Dichos investigadores sugieren que esta información puede utilizarse para mejorar el mercado de las bayas de *Vaccinium*, lo que puede brindar oportunidades económicas sostenibles a los agricultores.

Tradicionalmente el mortiño se consume en un plato especial con miel de caña, especias y otros pedazos de frutas en el Día de los Difuntos, sin olvidar a la tradicional “colada morada”, un plato y bebida típicos de la cultura popular.

Los campesinos utilizan este arbusto para calmar el reumatismo, fiebres y cólicos; se usa también para sanar la gripe, la borrachera y las dolencias del hígado y los riñones. Además, de para tratar dolencias pulmonares y la debilidad (CESA, 1993).

El mortiño es conocido como un arbusto ideal para fines ornamentales gracias a que posee hojas con características brillantes, lisas, de color granate y rosadas. Estas son usadas para adornar ambientes; es un arbusto que, con la poda, adquiere formas decorativas que suelen ser muy llamativas. Sus hojas se usan como forraje para alimentación animal. Asimismo, el mortiño también se usa como combustible y como planta regeneradora de zonas quemadas empleándose en las reforestaciones (Noboa, 2010; Torres et al., 2010).

Con respecto a los diferentes usos del *V. floribundum,* el consumo de los frutos de esta especie, ayuda a restablecer los niveles de azúcar en la sangre en personas con problemas de hipoglucemia, diabetes e incluso sirve para problemas digestivos. En la industria es utilizado como colorante natural por ser rico en pigmentos antocianínicos; en su potencial alimenticio se resalta que es rico en antioxidantes, contiene un alto contenido de vitamina C y vitaminas del complejo B, también minerales como potasio (K), calcio (Ca), fósforo (P) magnesio (Mg), además de proteínas, fibra, un alto contenido de agua y de buen sabor. A pesar de su importancia, se debe resaltar que estas plantas por ser endémicas de un lugar tan importante como son los páramos, se debe buscar la mejor forma de explotar *ex situ* su potencial sin causar inconvenientes en su hábitat para mantener el equilibrio de estos ecosistemas (Medina, 2019).

2 . OBJETIVOS

2. OBJETIVOS

El objetivo principal de la Tesis Doctoral fue determinar las épocas de floración y fructificación (fenología reproductiva), caracterizar bioquímicamente los frutos de esta especie nativa del páramo (*Vaccinium floribundum* Kunth), facilitándose así su conservación en la Reserva de Producción Faunística del Chimborazo (RPFCH); así como una revisión de literatura destacando los usos, beneficios y aplicaciones de la especie en estudio.

Para alcanzar dicho objetivo principal se establecieron los siguientes objetivos específicos:

I. Realizar una revisión bibliográfica sobre el mortiño enfocada a la idoneidad del mismo como un cultivo promisorio en el Páramo ecuatoriano y sus potenciales usos, papel medioambiental y beneficios para la salud.

II. Estudiar la fenología reproductiva de *Vaccinium floribundum*, en el páramo del volcán Chimborazo, en Ecuador, analizando la periodicidad en su floración y fructificación (biología reproductiva).

III. Caracterización bioquímica de los frutos del mortiño.

3. RESUMEN DE LA METODOLOGÍA

4. RESUMEN DE LA METODOLOGIA

Para el artículo de **revisión bibliográfica** la metodología utilizada se basó en el enfoque PRISMA Extension (PRISMA-ScR) (Page et al., 2021). Se llevó a cabo una exhaustiva búsqueda bibliográfica en Scopus y ScienceDirect. Se utilizaron palabras de texto y vocabulario controlado para varios conceptos (*Vaccinium floribundum* Kunth, taxonomía, compuestos bioactivos, reproducción, etnobotánica, industria) dentro de los títulos, resúmenes y palabras clave. Se dio prioridad a los estudios publicados en revistas incluidas en el Journal Citation Reports. Sólo se seleccionaron artículos de investigación que incluyeran el diseño experimental y el tratamiento de datos. La estructura de la revisión permite una disección de: (i) clasificación taxonómica y descripción botánica, (ii) evaluación etnobotánica, florística y ambiental, (iii) biología reproductiva, (iv) propiedades antioxidantes y beneficios para la salud, y (v) aplicaciones ambientales y uso industrial.

Para el **estudio de la fenología reproductiva** del mortiño se utilizó la escala BBCH (Biologische Bundesantalt, Bundessortenamt und Chemische Industrie) propuesta por Bleiholder et al. (1991) and Hack et al. (1992). Para el seguimiento fenológico se seleccionaron un total de 47 plantas adultas de *V. floribundum* en tres diferentes localidades del páramo del volcán Chimborazo, Ecuador (Tabla 1). **El monitoreo** de las plantas se realizó desde enero de 2017 a junio de 2018 (18 meses).

Tabla 1. Localidades donde se monitoreó la fenología reproductiva de mortiño en el páramo del volcán Chimborazo, Ecuador.

Localidad	Provincia	Nº. individuos	Coordenadas	Altitud (m)	Tipo de vegetación
Mindala Loma	Bolívar	17	01◦27′45′′ S 78◦56′05′′ W	4261	Páramo herbáceo
Polylepis	Chimborazo	19	01◦32′41′′ S 78◦53′05′′ W	4076	Páramo herbáceo
Mechahuasca	Tungurahua	11	01◦25′26′′ S 78◦47′53′′ W	4370	Páramo herbáceo

El registro de la evolución de los diferentes estados fenológicos se hizo mediante visitas quincenales a las diferentes localidades. En cada visita, se contaron el número de órganos fenológicos reproductivos en cada individuo, utilizando las siguientes categorías: botón floral, flor, fruto verde inmaduro, fruto rojo en maduración y fruto negro maduro. En base a la información cuantitativa obtenida se construyó una escala semicualitativa siguiendo el sistema BBCH, centrándose únicamente en las etapas reproductivas. Para cada etapa del desarrollo fenológico reproductivo, se elaboró una descripción acompañada de un registro fotográfico y un resumen gráfico de la presencia o ausencia de las diferentes etapas durante el período de monitoreo.

El **material vegetal** utilizado para determinar la **composición en compuestos bioactivos** del *V. floribundum* consistió en bayas frescas de mortiño recolectadas en tres localidades del páramo del volcán Chimborazo, Ecuador: Culebrillas, Polylepis y Cubillín, cuyas condiciones de altitud, tipo de vegetación, temperatura y precipitación media se muestra en la Tabla 2.

Tabla 2. Áreas de muestreo donde se recolectaron las bayas de mortiño en el páramo del volcán Chimborazo, Ecuador.

Localidad	Provincia	Coordenadas	Altitud (m)	Tipo de vegetación	Tª media (ºC)	Precipitación media (mm)
Culebrillas	Bolívar	01°34.20´S 78.55.5´W	4000	Páramo herbáceo	3.1	967
Polylepis	Chimborazo	01°32.41´S 78°53.5´W	4076	Páramo herbáceo	3.1	967
Cubillín	Chimborazo	01°45´S 78°31´W	3500	Bosque de alta montaña	7.0	1000

Las bayas se clasificaron según su estado de madurez en: (i) Estado 7: Desarrollo del fruto; las bayas comenzaron a desarrollar antocianinas, lo cual se identificó por su coloración rojiza desde la parte apical hasta la basal del fruto, y (ii) Estado 8: Maduración del fruto; el 100% de las bayas del racimo muestra un epicarpio púrpura (Caranqui-Aldaz et al., 2022).

La extracción para determinar el contenido de polifenoles totales y actividad antioxidante en las bayas de mortiño se realizó de acuerdo al protocolo descrito por Wojdyło et al. (2008). A medio gramo de bayas de mortiño liofilizadas se le añadieron 5 mL de extractante (metanol/agua (80:20, v/v) + 1% HCl). Esta mezcla se sonicó a 20 ºC durante 20 minutos; luego, la mezcla se dejó reposar durante la noche a 4 ºC. Las muestras se sonicaron nuevamente durante 20 minutos y luego se centrifugaron durante 10 minutos a 15.000 rpm. Las extracciones se realizaron por triplicado.

La actividad antioxidante como es bien sabido, debe medirse utilizando varios métodos de determinación, ya que cada uno de ellos tiene un mecanismo de acción distinto. En esta Tesis Doctoral se emplearon los métodos ABTS (Re et al., 1999) y DPPH (Brand-Williams et al., 1995).

Método ABTS (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid). El catión radical ABTS ($ABTS^{•+}$) se generó mediante la reacción de una solución acuosa de ABTS (7 mmol L^{-1}) con persulfato de potasio (2,45 mmol L^{-1}, concentración final) y se mantuvo en la oscuridad a temperatura ambiente durante 12-16 horas antes de su uso. En el análisis se empleó una solución diluida de $ABTS^{•+}$ con una absorbancia de 0,70 ± 0,02 a 734 nm. Las reacciones se realizaron añadiendo 990 µL de solución de $ABTS^{•+}$ a 10 µL de cada solución de extracto de bayas de mortiño. La disminución de absorbancia se midió 6 minutos después de la mezcla inicial. La absorbancia se midió con un espectrofotómetro UV-visible (Termospectromic Helios Gamma UVG 1002 E, Cambridge, Reino Unido). Todas las determinaciones se realizaron por triplicado y los resultados se expresaron en milimoles de Trolox por kilogramo de materia seca de la muestra (mmol Trolox kg^{-1} de MS).

Método DPPH (2,2-diphenyl-1-picrylhydrazyl). Se mezclaron 10 µL de cada solución de extracto de bayas de mortiño con 40 µL de MeOH y se añadieron a 950 µL de solución de DPPH. La mezcla se agitó vigorosamente y se colocó en una habitación oscura durante 10 minutos. La disminución de absorbancia se midió a 515 nm utilizando un espectrofotómetro UV-visible (Termospectromic Helios Gamma UVG 1002 E, Cambridge, Reino Unido). La solución madre del radical $DPPH^{•}$ se preparó diariamente diluyendo 100 µM del radical $DPPH^{•}$ en etanol al 80%, agitándolo y manteniéndolo en la oscuridad durante 30 minutos. Todas las determinaciones se realizaron por triplicado y los resultados se expresaron en milimoles de Trolox por kilogramo de materia seca de la muestra (mmol Trolox kg^{-1} de MS).

El contenido de polifenoles totales (CPT) se determinó utilizando el método colorimétrico de Folin-Ciocalteu descrito por Singleton et al. (1999), con ligeras modificaciones. En resumen, en una muestra de extractos de bayas (100 µL) se añadieron 200 µL (1/10) de reactivo de Folin-Ciocalteu, 2 mL de agua destilada y se incubaron durante 3 minutos a temperatura ambiente. Luego, se añadió 1 mL de carbonato de sodio (20%, p/v) y se incubó nuevamente durante una hora. La absorbancia del complejo azul formado se leyó a 765 nm utilizando un espectrofotómetro UV-visible (Termospectromic Helios Gamma UVG 1002 E, Cambridge, Reino Unido). Se utilizaron curvas de calibración en un rango de concentración entre 0 y 0,25 g de equivalente de ácido gálico por litro (g GAE L^{-1}) para la cuantificación del CPT, mostrando una buena linealidad ($r^2 \geq 0,996$). Todas las determinaciones se realizaron por triplicado y los resultados se expresaron como miligramos de equivalente de ácido gálico por 100 gramos de materia seca de la muestra (mg de GAE 100 g^{-1} de MS).

El perfil de ácidos orgánicos y azúcares se identificó y cuantificó de acuerdo al protocolo puesto a punto por Hernández et al. (2016), con ligeras modificaciones. En resumen, se mezcló medio gramo de bayas de mortiño liofilizadas con 5 mL de tampón fosfato (50 mmol L^{-1}) pH 7,8 y la mezcla se homogeneizó con un homogeneizador Ultra Turrax T18 Basic (IKA®-Werke GmbH & Co. KG Janke & Kunkel, Staufen, Alemania), se centrifugó (Sigma 3–18 K; Sigma Laborzentrifugen, Osterode am Harz, Alemania) y se filtró (filtro de membrana de 0,45 µm; Millipore, Billerica, MA, EE.UU.). Luego, se inyectaron 10 µL del sobrenadante en un cromatógrafo líquido de alta eficacia serie 1100 de Hewlett Packard (Wilmington, DE, EE.UU.) equipado con un detector de índice de refracción para la detección de azúcares y un detector UV/Vis para el análisis de ácidos orgánicos. El eluyente (isocrático a 30ºC) consistió en un tampón de ácido fosfórico 1 g L^{-1} a una velocidad de flujo de 0,5 mL min^{-1}. Se utilizó una columna Supelcogel TM C-610H (30 cm

× 7,8 mm) con una pre-columna (Supelguard 5 cm × 4,6 mm; Supelco, Bellefonte, PA, USA.) para los análisis tanto de ácidos orgánicos como de azúcares. La absorbancia de los ácidos orgánicos se midió a 210 nm. Se construyeron curvas de calibración utilizando diferentes estándares de ácidos orgánicos y azúcares proporcionados por Sigma (Poole, UK) en un rango de concentración entre 1 y 10 g L^{-1}. Los análisis se realizaron por triplicado y los resultados se expresaron en g kg^{-1} de materia seca (MS).

Para determinar el contenido de minerales en las bayas de mortiño, se pesaron 0,1 g de polvo liofilizado en vasos de teflón (TFM) de 75 mL. Luego, se agregaron 4 mL de HNO_3 (69% vol.%) y 2 mL de agua Milli-Q, dejando reposar durante 15 minutos para pre-digerir las muestras. Posteriormente, las muestras fueron digeridas por microondas (CEM Mars One 240/50, Matthews, NC, EE.UU.). La temperatura se elevó a 180°C en 30 minutos y se mantuvo durante 20 minutos, enfriándose finalmente a temperatura ambiente. Luego, 3 mL del digestado se filtraron a través de un filtro de membrana de 0,45 µm y se transfirió una alicuota de 2 mL a un vial de polipropileno de 10 mL, al que se añadieron 2 mL de agua Milli-Q (dilución 1:1). Las diluciones se almacenaron refrigeradas (4ºC) hasta el análisis. La cuantificación de los macro-elementos [calcio (Ca), sodio (Na), potasio (K) y magnesio (Mg)] y micro-elementos [hierro (Fe), cobre (Cu), manganeso (Mn) y zinc (Zn)] se realizó utilizando un Espectrómetro de Masas con Plasma de Acoplamiento Inductivo (ICPMS-2030, Shimadzu, Kyoto, Japón). Se utilizaron curvas de calibración para la cuantificación de minerales, mostrando una buena linealidad ($R^2 \geq 0,998$). Los análisis se realizaron por triplicado y los resultados se expresaron como g kg^{-1} para los macro-elementos y mg kg^{-1} para los micro-elementos.

Extracción y determinación de compuestos fenólicos No-antocianínicos mediante HPLC-DAD-ESI-MSn. La extracción y posterior determinación de los compuestos fenólicos no antocianínicos se realizó de la siguiente forma:

Extracción: A 50 mg de muestra liofilizada de bayas de mortiño se le añadió 1 mL de extractante (metanol/agua (80:20, v/v) + 1% ácido fórmico), esta mezcla fue agitada durante 5 minutos. Pasado ese tiempo, las muestras fueron centrifugadas durante 10 minutos a 12.000 rpm a 4 °C. El sobrenadante se filtró a través de un filtro de PTFE de 0.45 µm (Millipore, Billerica, MA, USA.) y se almacenó a -20ºC hasta su uso. Todas las extracciones se realizaron por triplicado.

Determinación de los compuestos fenólicos No-antocianínicos: Para el análisis de las diferentes muestras de bayas de mortiño, se utilizó un sistema HPLC-ESI-DAD-MSn Ion Trap (Agilent 1100 series System), que permite realizar rupturas sucesivas del ion precursor para su identificación de desconocidos. Este sistema HPLC con detector DAD de la serie 1100 está acoplado a un espectrómetro de masas equipado con una trampa de iones y una interfaz de ionización por electrospray (ESI). La temperatura y el voltaje de la capilar para la fuente ESI se configuraron en 350°C y 3500V, respectivamente. La fragmentación inducida por colisión se llevó a cabo dentro de la trampa de iones utilizando helio como gas de colisión y una energía de colisión del 50%. El rango de masas para los iones precursores (MS) y sus fragmentaciones posteriores (MS-MS) fue de 100 a 1000 m/z y los datos se adquirieron en modo de ionización negativa, donde se observa la desprotonación de las moléculas. La separación cromatográfica se realizó en una columna C18 (Poroshell 120, 100 mm x 3 mm i.d., tamaño de partícula de 2.7 µm). Las fases móviles consistieron en dos solventes: agua/ácido fórmico (95:1, v/v) como solvente A y acetonitrilo como solvente B a un flujo de 1 mL min^{-1}. Para la determinación de polifenoles, el gradiente comenzó con un 5% de B y alcanzó un 60% de B a los 37 minutos, aumentando al 98% de B a los 40 minutos y se mantuvo durante 2 minutos antes de volver a las condiciones iniciales. El volumen de inyección fue de 20 µL. La cuantificación relativa de los compuestos fenólicos presentes en las muestras se realizó mediante comparación cromatográfica con estándares puros (ácido cafeico, rutinosido de quercetina, pelargonidina y cianidina) y sus espectros de absorción en cuatro longitudes de onda emitidas a 280, 320, 360 y 520 nm a través de un detector UV de matriz de diodos (DAD) integrado en el HPLC y conectado en línea al espectrómetro de masas.

Extracción, identificación y cuantificación de antocianinas mediante HPLC-DAD-ESI-MSn. La extracción y posterior, identificación y cuantificación de antocianinas se realizó de la siguiente forma:

Extracción: La extracción y análisis de antocianinas se realizó utilizando el método propuesto por Hong et al. (2020) con ligeras modificaciones. A medio gramo de muestra liofilizada de bayas de mortiño se le añadieron 4 mL de extractante [metanol/agua/ácido fórmico (80:19.9:0.1, v/v/v)] y se matuvieron en agitación en un baño orbital durante 10 minutos. Pasado ese tiempo la mezcla se sometió a ultrasonido en un baño ultrasónico durante 10 minutos. Posteriormente, las muestras se centrifugaron durante 10 minutos a 4.000 rpm a 4°C, se recolectó el sobrenadante y se re-extrajo el residuo del pellet dos veces más siguiendo los mismos pasos, combinando los sobrenadantes. Dos mililitros del sobrenadante se filtraron a través de un filtro de membrana de nylon de 0.45 µm de Millipore y se almacenaron a -20ºC hasta su uso posterior. Todas las extracciones se realizaron por triplicado.

El perfil de antocianinas se determinó mediante cromatografía líquida de alta resolución acoplada a espectrometría de masas triple cuadrupolo (LC-MS/MS 8050; Shimadzu, Kyoto, Japón). Las moléculas se ionizaron utilizando ionización de presión atmosférica (Ionización por Electrospray-ESI). Los parámetros de análisis ESI-MS fueron: gas nebulizador (N2): 3 L min^{-1}; gas de secado (N2): 10 L min^{-1}; flujo de gas calefactor (10 L min^{-1}); temperatura de la línea de desolvatación (DL): 250°C y temperatura del bloque de calentamiento: 400°C. Los iones moleculares intactos se generaron en modo positivo con el modo de análisis MRM (Monitorización de Múltiples Reacciones) en el rango de m/z 100-1200. Se utilizó el software LabSolutions LCMS Ver.5.98 (Shimadzu) para el control del instrumento y el procesamiento de datos. Las separaciones cromatográficas se realizaron con una columna C18 (Mediterranean SEA 18, 10 mm x 0.21 mm i.d., tamaño de partícula de 2.2 µm) de Teknokroma (Barcelona, España). La fase móvil A consistió en 0.1% (v/v) de ácido fórmico (AF) en agua (Milli-Q) y la fase móvil B consistió en 0.1% (v/v) de ácido fórmico en acetonitrilo (ACN) a un flujo de 0.4 mL min^{-1}, un volumen de inyección de 10 µL y temperatura del horno de 50ºC. El gradiente de condiciones fue de 0-2 minutos 5% B, 2-10 minutos 95% B, 10-11 minutos 95% B, 11-12 minutos 5% B y 12-16 minutos 5% B. Para la cuantificación de las antocianinas (delphinidina 3-*O*-glucósido; cianidina-3,5-di-*O*-glucósido; cianidina 3-*O*-glucósido; cianidina-3-*O*-arabinósido; petunidina-3-*O*-glucósido; peonidina-3-*O*-glucósido; pelargonidina-3-*O*-rutinosido), se preparó una solución stock de 100 ppm y a partir de ella se obtuvo una curva de calibración con concentraciones de 0,1; 0,3; 0,5; 0,8 y 1 ppm. Todos los análisis se realizaron por triplicado.

Análisis estadístico

En el estudio de **la fenología reproductiva del mortiño** se llevó a cabo un análisis de regresión lineal para detectar cualquier posible influencia de los parámetros climáticos en la fenología reproductiva del mortiño. Para este análisis se utilizó el software XLSTAT Premium 2016 (Addinsoft, Nueva York, NY, EE.UU.) y Statgraphics Plus (versión 3.1, Statistical Graphics Corp., Rockville, MA, EE.UU.).

En el estudio de los **compuestos bioactivos de las bayas de mortiño** los datos fueron sometidos a un análisis de varianza de una vía (ANOVA) y un test de rango múltiple de Tukey para comparar las medias obtenidas. El intervalo de confianza fue del 95%, y se consideró una diferencia significativa cuando $p < 0,05$. Se utilizó el análisis de componentes principales (ACP) para reducir este conjunto de datos complejo a una dimensión inferior y revelar estructuras simplificadas y relaciones entre localidades y estados de madurez. Para realizar estos análisis estadísticos, se utilizó el software XLSTAT Premium 2016 (Versión 9 - Addinsoft, Nueva York, NY, EE.UU.).

4. PUBLICACIONES

4.1. PUBLICACIÓN 1

Publicación 1 (Open Access)

The Mortiño (*Vaccinium floribundum* Kunth): a review of its suitability as a promissory crop in the Ecuadorian Paramo and its potential uses, environmental role, and health benefits.

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REVIEW ARTICLE

The Mortiño (*Vaccinium floribundum* Kunth): a review of its suitability as a promissory crop in the Ecuadorian Paramo and its potential uses, environmental role, and health benefits

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Abstract
The mortiño (*Vaccinium floribundum* Kunth), belonging to the Ericaceae family, is a native species of the Ecuadorian paramos. It has a shrub-like habit and produces edible fruits. In the Neotropics, it is primarily found in the Andes of Colombia and Ecuador, thriving in humid environments up to an elevation of 3700 m above sea level. In this review, a selection of studies was carried out that evaluated the taxonomy, reproduction biology, and nutraceutical, environmental properties, and industrial use of the mortiño (*V. floribundum*). The data gathered from various bioassays were essential in determining the appropriate techniques for tissue differentiation and assessing the quality of resulting plants. This work aims to generate a deep knowledge of the cultivation of mortiño, as well as the properties of its fruits and the benefits they provide for health. These are rich in compounds with antioxidant activity, so the consumption of *V. floribundum* fruits is related to health benefits. Besides, the environmental role of *V. floribundum* and its applications in various industries, especially in the development of nanoparticles contributes to the valorization of this plant. Overall, this research contributes to establishing sustainable methods for the propagation of *Vaccinium floribundum*, ensuring its successful cultivation and utilization for both commercial purposes and ecological preservation.

Keywords Mortiño · *Vaccinium floribuntum* · Reproduction · Bioactive compounds · Environment · Industry

Introduction

The Mortiño (*Vaccinium floribundum* Kunth), belonging to the Ericaceae family, is an indigenous species found in the Ecuadorian paramos, characterized by a shrub-like structure and edible fruits. Within the Neotropics, its distribution is concentrated in the Andean regions of Colombia and Ecuador, thriving particularly in humid environments up to an elevation of 4350 m above sea level [1]. Across generations in the Ecuadorian paramos, the fruits of this species, commonly referred to as "mortiño," have been predominantly utilized in the preparation of the traditional 'colada morada' on the Day of the Dead [2]. However, factors such as traditional consumption practices, recent promotion for winemaking, and challenges associated with propagation have contributed to the rapid decline of this species [3]. Additionally, populations have faced setbacks due to deforestation, alterations in land usage, habitat fragmentation, and disturbance resulting from its extraction [2]. These fruits boast significant levels of sugars, minerals, antioxidants, B and C complex vitamins, potassium, calcium, and phosphorus [2, 3].

As outlined by Gutierrez & Camacho [4], inhabitants of high Andean ecosystems, acknowledging the potential of this species, have engaged in irrational exploitation. This behavior involves uprooting plants from their natural habitats and cultivating them in unsuitable conditions, thereby inducing a systemic imbalance within the original zone and disrupting the natural development of other plant species dependent on it. Such practices extend to numerous native Andean species due to inadequate knowledge regarding proper propagation techniques. Despite the significance of these plant species

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to the nation, research in this domain is limited, and available information concerning their genera and species remains scarce. There exists a dearth of studies investigating propagation mechanisms or strategies within Ecuador.

According to Pedraza-Peñalosa et al. [5], the Ericaceae family is present in temperate, subtemperate zones, and tropical mountain forests, predominantly thriving in conditions of elevated humidity and moderate temperatures. Remarkably diverse in the Neotropics, this family comprises approximately 900 species, with nearly 94% being endemic. Concentrated mainly in Colombia and Ecuador within the Neotropics, most Ericaceae species are closely associated with the Andes, especially the cloud forests situated between 1000 and 3000 m above sea level. To a lesser extent, they are also found in lower cloud forests (below 1000 m) and paramos (above 3000 m) regions. In Ecuador, there are 22 genera and 221 species, making it one of the most endemic families in the country. Around 44% or 98 species are exclusive to Ecuador, with the sole family species found in Galápagos being *Pernettya howellii*. Due to their specific climatic requirements, the distributions of Ericaceae are severely constrained, both latitudinally and altitudinally. They prefer forest ecotones and are abundant in roadside areas, trails, ravines, and clearings, playing a pivotal role in the Andean Forest succession process. Apart from their ecological significance, several Ericaceae species hold ornamental or edible value in temperate regions. In Ecuador, the fruits of certain species of Ericaceae, such as *V. floribundum* or "mortiño," are consumed locally, in a special drink called "colda morada", typical of the Day of the Dead.

Concerning the conservation status of Ericaceae in the country, a substantial majority of species (89.7%) are under threat, with six species critically endangered, 33 endangered, and 49 classified as vulnerable. Among the non-endangered species, four are categorized as near threatened, and six are classified under the least concern category. The primary threats to the conservation of these species, ranked in order of significance, include deforestation, habitat fragmentation, colonization, and agricultural expansion. For certain paramo species, grazing and human-induced fires pose substantial threats. The percentage of species protected by the SNAP (State Protected Areas Network) is notably low, considering the locations where these species have been recorded: only 21 out of 98 species are found within state-protected areas [5].

Thus, this review intends to give an overview of the importance of *V. floribundum* to Ecuadorian Paramo.

Scientific literature review

This review is organized as a research paper. A scoping review was used to synthesize the evidence and assess the scope of the studies on the topic. This review was based on the PRISMA Extension (PRISMA-ScR) approach [6] for Scoping Reviews. A comprehensive literature search—Scopus and ScienceDirect—was performed in September 2023 (Fig. 1). Text words and controlled vocabulary for several concepts (*Vaccinium floribundum* Kunth, taxonomy, bioactive compounds, reproduction, ethnobotanical, industry) within the titles, abstracts and keywords were used. The focus has been given to studies published in journals included in the Journal Citation Reports. Only research papers that included the experimental design and data treatment were selected. The structure of the review allows a dissection of (i) taxonomic classification and botanical description, (ii) ethnobotanical, floristic and environmental assessment, (iii) reproductive biology, (iv) antioxidant properties and health benefits, and (v) environmental applications and industrial use.

Taxonomic classification and botanical description of the Mortiño

The taxonomic classification of *V. floribundum*, as documented by the APG IV Classification System [7] is detailed as follows:

Class: Equisetopsida C. Agardh
Subclass: Magnoliidae Novák ex Takht.
Superorder: Asteranae Takht.
Order: Ericales Bercht. & J. Presl

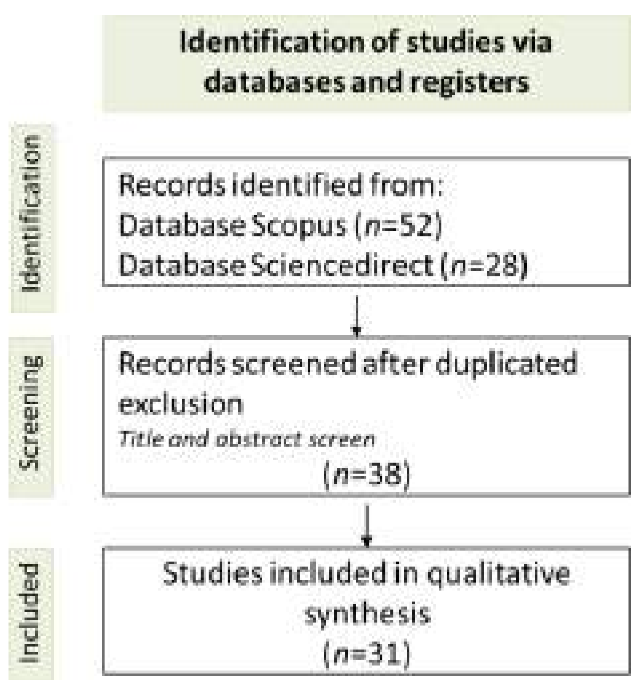

Fig. 1 Flow diagram describing the study selection process of scientific literature

Family: Ericaceae Juss.

Genus: *Vaccinium* L.

These plants typically manifest as shrubs, occasionally as trees, often exhibiting rhizomes. Their leaves, either persistent or deciduous, are arranged alternately, featuring an entire or serrated margin, commonly displaying pinnate veins, occasionally parallel veins, and possessing a short petiole. The inflorescence develops axillary, emerging from the previous season's buds, either in a racemose arrangement or, at times, comprising only 1–2 flowers, borne in the axil of the floral bract or a leaf. Usually, the pedicel includes two bracts. The flowers, typically 4–5-merous, often lack scent; their arrangement is imbricate (as observed in *V. crenatum* and *V. floribundum*). The calyx may either articulate with the pedicel (in Ecuador) or remain continuous. The hypanthium varies from cylindrical to globose, with lobes seldom absent. The corolla, gamopetalous in nature, may appear cylindrical, urceolate, or campanulate, displaying white, greenish, red, or yellowish hues, with lobes occasionally partially divided near the base. The stamens, numbering 8 or 10, are comparable in length to the corolla; their filaments are distinct, longer than the anthers, occasionally possessing spurs (as observed in *V. floribundum*), which may seem vestigial. The anthers lack disintegration tissue, with smooth or papillate thecae; tubules dehisce through terminal pores or, occasionally, oblique clefts. The pollen is devoid of viscin strands. The ovary, entirely or partly inferior, typically contains 4–5 (falsely 10) locules; the stigma is small, simple, or somewhat capitate. The fruit takes the form of a berry, housing 5-many seeds, crowned by persistent calyx lobes and enveloped by a noticeable nectar-producing disc; occasionally, seeds may feature a mucilaginous covering. The *Vaccinium* genus encompasses approximately 300 species, primarily distributed in the Northern Hemisphere, predominantly inhabiting mountainous regions within the tropics; specifically, three species are found in Ecuador [8].

Ethnobotanical, floristic, and environmental assessment

The climatic conditions conducive to the thriving of *Vaccinium* genus species are associated with a specific life zone: humid montane regions characterized by predominantly young and minimally disturbed soils [1].

Most *Vaccinium* species have evolved within mineral-rich soils abundant in organic matter and featuring an acidic pH. The primary limiting factor for blueberry growth is soil pH, with the optimal range falling between 4.2 and 5.2. Soil consistency should be loose and suitably porous to facilitate the superficial and delicate root system's efficient exploration [9].

For the optimal development of *V. floribundum*, ideal agroecological conditions necessitate temperatures ranging from 8 to 16 °C (considered cold), humidity levels between 60 and 80%, an annual precipitation range from 800 to 2000 mm, and an altitude spanning from 3200 to 3800 m above sea level. Soil prerequisites for the propagation of these materials align with characteristics found in high-mountain ecosystems: sandy, humic, loose soils, rich in organic matter, with a slightly acidic to neutral pH [10].

Considering the flourishing nature of Ericaceae in paramo conditions, it is crucial to note that Colombia, with an extensive distribution of paramos mainly across its three mountain ranges, holds substantial potential for the sustainable cultivation and utilization of the Ericaceae family, particularly within the *Vaccinium* genus. Paramos in Colombia are recognized as natural ecosystems with limited and localized human-induced impact [11]. Paramos experience a cold and humid climate characterized by sudden atmospheric changes. While the annual temperature fluctuation remains small (2–10 °C), daily temperature variations range from freezing point to 30 °C. These fluctuations give rise to a daily cycle of freezing, temperature elevation, and intense solar radiation, described by some authors as a "summer every day—winter every night" [12]. The diurnal seasonality in paramos bears a superficial resemblance to the annual seasonality typical of temperate and polar latitudes. The climate is unstable, cold, cloudy, and rainy, interspersed with periods of intense sunlight [13]. In broad terms, blueberries require 650–850 h of cold temperatures below 7.2 °C to ensure abundant and uniform flowering. Additionally, a minimum duration of 160 days is essential for this process. Flowers suffer damage at temperatures below – 1 °C and above 30 °C; in leaves, vegetative growth halts, leading to fruit dehydration [14, 15].

In Ecuador, *V. floribundum* thrives as a wild plant in the elevated regions of the mountain range, spanning from the paramos of El Ángel in Carchi to Tambo in Cañar. Information sourced from the Cotopaxi National Park delineates the adaptation zone for the "mortiño" within the range of 2000 m above sea level to 4500 m above sea level. However, only a few paramos accommodate a significant population of these plants due to the encroachment of extensive agricultural areas on their habitat, confining this species to paramo zones ranging between 3400 and 3500 up to 4500 m above sea level [16].

Based on documented collections, *V. floribundum* is prevalent in the Sierra region across the provinces of Carchi, Imbabura, Pichincha, Cotopaxi, Tungurahua, Bolivar, Chimborazo, Cañar, Azuay, and Loja. Likewise, records indicate the presence of *Vaccinium distichum* and *Vaccinium crenatum* in the provinces of Azuay and Loja [1]. Estrella [17] lists Macleania ecuadoriensis "hualicon," Macleania laurina

Blake "Chaqui-lulu," Macleania popenoei "Joyapa" as edible berries with similar characteristics.

Traditionally, "mortiño" is partaken in a special dish with cane honey, spices, and assorted fruit pieces on the Day of the Dead, often accompanied by the traditional "colada morada," a customary culinary delight in popular culture [17]. Historically, few were acquainted with its specific name, and it is likely that a substantial segment of the urban populace remained oblivious to its existence, associating it more with the family of "agraz" or blueberries.

The "mortiño" is esteemed as a sacred product due to its wild attributes, thereby requiring no specific treatment. Rural communities utilize this shrub to alleviate conditions like rheumatism, fevers, and colic. It is also employed in treating flu, intoxication, liver, and kidney ailments, along with addressing pulmonary issues and weakness [18]. Notably, the "mortiño" stands out as an ideal shrub for ornamental use owing to its shiny, smooth, reddish-purple, and pinkish leaves, often employed to adorn spaces. Through skilled pruning, the shrub can assume decorative shapes that are strikingly appealing. Its leaves serve as fodder for animal feed. Furthermore, the "mortiño" finds use as fuel and in the regeneration of burnt areas as part of reforestation efforts [19, 20].

Reproductive biology

In Colombia, the incorporation of *Vaccinium meridionale* and *V. floribundum* into production systems has been limited, unlike the blueberry crop, which also belongs to the *Vaccinium* genus and serves as a reference. Due to its wild nature, there is a lack of understanding regarding its propagation methods [21]. However, propagation efforts have been conducted with species other than *V. floribundum*, providing a foundation for further research.

A practical and viable method for asexual propagation of *V. floribundum* is through cuttings. This approach offers economic advantages and simplicity, addressing issues of plant incompatibility and low vigor while ensuring greater uniformity and quality in production [22]. Additionally, the natural spread of plants in the Ericaceae family through rhizomes in their native habitat supports the feasibility of this propagation method [22]. In the case of blueberry (*Vaccinium corymbosum*), it is directly propagated through hard cuttings with a diameter greater than 5 mm, or young cuttings with leaves [23]. Rooting capacity varies among species and cultivars, necessitating empirical tests to determine optimal conditions.

Despite the advantages, a challenge in the asexual propagation of mortiño is the low rooting potential of cuttings under controlled conditions. Nonetheless, research indicates that treatments with 3-aceitc acid (IIA) and indole-3-butyric acid (IBA) can enhance the viability of *V. floribundum* cuttings [22].

While there are in vitro propagation techniques for *Vaccinium* sp. [24, 25], these methods are not accessible to fruit producers. In contrast, micropropagation or in vitro propagation has been successfully employed with blueberry (*V. corymbosum*), demonstrating its practical efficacy in obtaining homogeneous plants. This method involves the proliferation of cuttings cultured in a nutritional medium with growth regulators [14, 23]. Notable works by Torres et al. [20] and Debnath [26] have explored protocols for the in vitro propagation of *V. floribundum.*

In terms of sexual reproduction of *V. meridionale*, challenges arise due to difficulties in handling and germination of seeds. Issues include tiny seed size, variations, low germination percentages, potential photoblasticity, and dependence on light for germination [22]. Germination of *V. floribundum* seeds under ambient conditions is not viable without hormones, but in vitro conditions have shown successful germination, albeit with long-term results [4]. *V. floribundum* seeds exhibit innate dormancy under in situ conditions, requiring rest periods exceeding six months to break dormancy. Hypochlorite, when combined with Gibberellic Acid in low concentrations, can disrupt the dormancy conditions of *V. floribundum* seeds [4].

Despite these challenges, aspects related to species propagation and phytosanitary issues with imported propagules limit agricultural production. Therefore, efforts are directed towards meeting market demand with native species due to the similar organoleptic characteristics of *V. corymbosum* and *V. floribundum* [27]. In tropical latitudes, the fruits of the *Vaccinium* genus are marketed on a small scale, and their potential as food or medicine remains to be fully explored. Limited publications exist regarding the use of species from this genus, emphasizing the significance of *V. floribundum* and *V. meridionale* as the most utilized native species [10].

Antioxidant properties and health benefits

According to different studies [2, 28–30], *V. florubindum* berries have relatively high concentrations of sugars, antioxidants such as vitamin C and the vitamin B complex, minerals such as potassium, calcium, and phosphorus. About antioxidants, the mortiño fruit shows a high content of polyphenolic compounds, such as cinnamic acid, flavonols, anthocyanins and anthocyanidins. These compounds have been extensively studied for their ability to neutralize free radicals and protect cells from oxidative stress. The high concentration of anthocyanins contributes significantly to the potent antioxidant capacity of *V. floribundum* [28, 31–33]. Anthocyanins, the pigments responsible for the deep blue color of *V. floribundum* berries, have garnered significant attention in antioxidant research. A study by Ortiz et al. [3] investigated the anthocyanin profile of *V. floribundum* and found a diverse range of anthocyanins, including cyanidin and delphinidin derivatives. The study highlighted the correlation between the anthocyanin content and the fruit's antioxidant activity, emphasizing the potential health benefits associated with these compounds. Caranqui-Aldaz et al. [28] determined that the main constituents of mortiño berries include hydroxycinnamic acids (5-O-caffeoylquinic acid), flavonols (quercetin derivatives), and anthocyanins, and reported three anthocyanins (petunidin, peonidin, and pelargonidin) for the first time in mortiño berries.

The antioxidant capacity of *V. floribundum* has been quantified through various assays, providing insights into its efficacy in scavenging free radicals. In a study by Ramírez et al. [34], the antioxidant activity of *V. floribundum* extracts was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays. The results demonstrated a dose-dependent scavenging effect, underscoring the fruit's ability to combat oxidative stress.

One of the prominent medicinal properties attributed to *V. floribundum* is its anti-inflammatory effect. Chronic inflammation is implicated in various diseases, including arthritis and cardiovascular conditions. The study conducted by Ramírez et al. [34] explored the anti-inflammatory potential of *V. floribundum* extracts using in vivo models. The results indicated a significant reduction in inflammatory markers, suggesting that the fruit may possess anti-inflammatory properties that could be harnessed for therapeutic purposes. The anti-inflammatory activity of *V. floribundum* is often linked to its flavonoid content. Flavonoids, such as quercetin and myricetin derivatives identified in the fruit, are known for their ability to modulate inflammatory pathways. This study [34] underscores the potential of *V. floribundum* as a natural anti-inflammatory agent. The antioxidant and anti-inflammatory properties of the fruit may contribute to cardiovascular health by reducing oxidative stress and inflammation, both implicated in heart diseases.

V. floribundum has also been investigated for its antimicrobial properties. In a study by Llivisaca et al. [35], researchers examined the inhibitory effects of *V. floribundum* extracts against various bacterial strains. The results revealed a significant antimicrobial activity, suggesting that the fruit may have applications in the treatment of microbial infections. The antimicrobial potential of *V. floribundum* is attributed to the presence of bioactive compounds that interfere with microbial growth and survival.

Environmental applications and industrial use

V. floribundum plays a crucial environmental and ecological role, particularly in paramo ecosystems, as it stands out as one of the initial species to rebound following deforestation and man-made fires [36]. This resilience is attributed, in part, to its robust regenerative capacity, facilitated by propagation from roots and other woody structures [37]. Numerous studies have focused on Mortiño's ability to regenerate areas affected by fire, underscoring its significant contribution to restoring ecosystem structure and acting as a pioneer in ecological succession [36, 37].

Various investigations [37, 38], including those by Ramsay & Oxley (1996) and Llivisaca-Contreras et al. (2022), have demonstrated Mortiño's impressive regenerative activity of *V. floribundum*. These studies highlight its capacity to colonize regions impacted by fires, playing a pivotal role in soil recovery. The shallow roots and horizontally spreading root growth of *V. floribundum*, coupled with prolific sprouting, designate it as a pioneer species adept at regenerating damaged ecosystems in the paramo. Consequently, it has garnered attention as a species essential for ecosystem restoration in the Andes. For effective conservation programs targeting native *Vaccinium* species, it is imperative to consider more efficient utilization of natural environments. This involves creating protected areas for wild plants and fostering ongoing research to understand their benefits and potential applications [39].

Ripe *V. floribundum* fruits exhibit properties that are of interest across various industries, particularly in the synthesis of photocatalytic nanocomposite materials, as demonstrated Vizuete et al. [40]. The study successfully synthesized silver nanoparticles (Ag Nps) using Mortiño berry extract, introducing a novel dimension to Andean fruits in the realm of nanotechnology. This environmentally friendly approach not only underscores the significance of Mortiño berries in green nanotechnology but also opens up new possibilities for other Andean fruits in engineering applications. Characterization techniques revealed that the generated silver nanoparticles were stable, non-aggregated, monodispersed, with a spherical shape and an average size of 20.5 ± 1.5 nm, showcasing a face-centered cubic nature. The anthocyanin molecules present in *V. floribundum* and other *Vaccinium* species' fruits, with hydroxyl groups effectively binding with TiO_2 nanoparticles, hold significance for the nanoparticle industry [41, 42].

A study by Taco-Ugsha et al. [42] confirms that pigments in mortiño are flavonoids of the anthocyanidin group, such as cyanidin-3-galactoside and cyanidin-3-arabinoside. Due to these compounds, mortiño dyes emerge as a potential alternative to artificial sensitizers for solar cell technologies, given their harmless and abundant nature. Dyes extracted from mortiño fruits were utilized as sensitizers in DSSC and characterized through chromatography (HPLC and TLC), FT-IR spectroscopy, and MALDI mass spectrometry analysis. The most abundant compounds in mortiño extracts correspond to cyanidin derivative anthocyanins. Mortiño dye-sensitized solar cells demonstrated a power conversion efficiency between 0.18 and 0.26%, significantly influenced by the acid extraction medium, with the highest value achieved using TFA-acidified methanol [42].

In a study by Vizuete et al. [40], a simple, cost-effective, and environmentally friendly method was reported for the synthesis of Ag-G nanocomposite using Mortiño berry extract as the reducing agent. This green synthesis of Ag-G nanocomposite, involving the reduction of Ag + and graphene oxide, showcases enhanced photocatalytic properties. The resulting material holds promise for addressing environmental concerns and is poised to play a significant role in various industrial applications. The eco-friendly and green reduction method avoids the use of toxic reagents, making it a potential choice for biocompatible materials in future engineering applications [40].

Conclusions

This work provides comprehensive information about taxonomy and environmental development of *V. floribundum*. About reproductive biology, cuttings are the more viable propagation method for *V. floribundum,* due to their simplicity and low cost compared with other methods. Besides, berries of *V. floribundum* showed high amounts of bioactive compounds, mainly polyphenolic compounds, and anthocyanins, which provide this fruit an excellent source of antioxidants that are related to medicinal properties and health benefits. Furthermore, *V. floribundum* presents other uses, such as environmental restoration after deforestation and fires, due to their ecological role and regenerative activity. About the potential industrial uses, some investigations demonstrate that *V. floribundum* could be used for the synthesis of nanoparticles, with the advantage of the elimination of harmful reagents, positioning it as a promising option for biocompatible materials in upcoming engineering applications. All these characteristics and properties make the mortiño a potentially promising plant for the development of medicines, cosmetics, functional foods and new materials, among others, as well as an ideal plant for the regeneration of soils and the environment.

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Declarations

Conflict of interest The authors declare no confict of interest.

Compliance with ethics requirements The authors declare this study was conducted in accordance with ethical guideline and principles.

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4.2. PUBLICACIÓN 2

Publicación 2 (Open Access)

Reproductive phenology of *Vaccinium floribundum* Kunth (Ericaceae) and codification according to the BBCH scale based on evidence from the volcano Chimborazo paramo (Ecuador).

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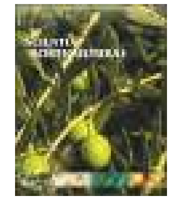

Reproductive phenology of *Vaccinium floribundum* Kunth (Ericaceae) and codification according to the BBCH scale based on evidence from the volcano Chimborazo paramo (Ecuador)

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Vaccinium floribundum

A B S T R A C T

Vaccinium floribundum Kunth (Ericaceae) is a native Andean species with shrubby habit and edible fruits. It is mostly distributed in the paramos of the Andes, from Venezuela to Bolivia, where it is commonly known as "mortiño". In this study, we describe the reproductive phenological growth stages of *V. floribundum* in the paramo of the Chimborazo volcano in Ecuador, using an adaptation of the BBCH scale system, and according to semi-monthly observations conducted in three localities from January 2017 to June 2018 (18 months). In addition, a linear regression analysis was performed to detect any potential influence of climatic parameters on the reproductive phenology of “mortiño”. Throughout the monitoring period, different stages of reproductive growth such as bud, flower and fruit were simultaneously found among individuals and, sometimes, even in the same plant. The reproductive phenology of sampled individuals from sites 1 and 2 (Mindala Loma and Polylepis, respectively) were relatively synchronized, whereas individuals from site 3 (Mechahuasca) showed a different phenological pattern. A significant, apparently quick, loss of flowers was evident in all localities during the study period. The patterns observed could reflect physiological constraints imposed by the extreme weather conditions of the paramo.

1. Introduction

Paramos are high-altitude humid mountain environments, where high number of species co-exist and show homoplasy traits adapted for the survival in this environment characterized by volcanic orography and extreme climatic conditions (Gentry, 1988; Luteyn, 2002). One emblematic species of the paramos is *Vaccinium floribundum* Kunth (Ericaceae), locally known as "mortiño". This species is particularly common in the Andes from Venezuela to Bolivia, and has been used since immemorial times. Since the syncretism between the Catholic faith and the indigenous culture, "mortiño" became the main ingredient of a traditional, relatively thick, beverage called “colada morada”, that is prepared to commemorate “All Souls’ Day” (Torres et al., 2010). The widespread use of "mortiño" is a healthy custom because its fruits have relatively high concentrations of sugar, antioxidants such as vitamin C and vitamin B complex, and minerals such as potassium, calcium, and phosphorus (Freire, 2004; Santamaría et al., 2012). Yet, over the years, human consumption of "mortiño" in Ecuador has decreased, as well as its availability in the local markets, probably because of the decimation of its natural populations, the technical difficulties to cultivate it, and the lack of knowledge about its health benefits (Freire, 2004). Indeed, "mortiño" populations are being continuously fragmented by anthropogenic processes such as deforestation, productive land reconversion, and overexploitation (Torres et al., 2010).

Reproductive phenology tends to respond to the local climate, which assures that gene flow occurs between individuals from the same or nearby populations (Jianwu et al., 2016). Furthermore, depending on the local climate variability, plant species can adopt different reproductive phenological strategies to guarantee the maintenance of their genetic diversity (Körner et al., 2016). In tropical regions, the variability and intensity of rain is generally considered as an environmental signal and evolutionary force that explains the variation of phenological events

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among species (Morellato et al., 2006; Martínez-Adriano et al., 2016; Medina-Cano et al., 2019). In Ecuador, phenological studies of paramo species has been historically relegated, despite the importance of these complex ecological systems in terms of the ecosystem services they provide (Jianwu et al., 2016). Ideally, assessing the variation of the reproductive growth stages (flowering and fruiting) of each species in different paramos would allow the design of effective repopulation or management plans (Prado and Valdebenito, 2000; Caiza, 2011).

The genus Vaccinium includes a group of species to which the "mortiño" and blueberry belong. Reproductive phenology and growth developmental stages of both species are quite similar (Prado and Valdebenito, 2000), but it has not been evaluated or described to date in the ecological conditions of Ecuadorian central Andes.

For this species, information on phenological studies has been obtained. Gómez (2004) described some reproductive and growth developmental stages as leaf sprouting, floral bud, open flower, anthesis, fruit set and green and mature fruits. Other studies even reported up to six different stages of berry ripening (Buitrago et al., 2015). Furthermore, the peasant communities of Guarne (Antioquia, Colombia) revealed that the "agraz" (*Vaccinium meridionale*) yielded biannual fruiting because of the bimodal rain pattern in the area (Medina et al., 2019). For the area of the Antilles, flowering occurred from the beginning of winter in the Ericaceae family. This reproductive stage of development extended throughout the spring until the beginning of summer. Likewise, the fruiting stage lasted from the beginning of fall, continuing through the winter, and to reach its peak during the spring-summer period. These plants showed abundant flowering and fruiting all over the year (Berazaín, 2006).

Meanwhile, the "mortiño" also played an important and ecological role in the high Andes. Because of its high regenerative capacity, the species was vital for vegetation preservation in the paramos following man-made destructive fires (Ramsay and Oxley, 1996). And despite its commercial interest, cultural and ecological significance, "mortiño" remains as an endangered wild species due to the ongoing fragmentation of its natural habitat (Coba et al., 2012).

Therefore, the main goal of the current study was to describe the reproductive phenological growth stages (flowering and fruiting) of *Vaccinium floribundum* Kunth, as observed in a natural paramo conditions in the Andes and through an adaptation of the BBCH extended scale. To ensure the accuracy of observations in the scale development, 18 months of observations on a semi-monthly basis, from January 2017 to June 2018, were taken in three paramo areas of the Chimborazo volcano in central Ecuador, South America.

2. Materials and methods

2.1. Study area

Three different localities with presence of *V. floribundum* were sampled in the paramo of the Chimborazo volcano (Sierra, 1999) in the central Andes of Ecuador. The localities where to observe *V. floribundum* were selected based on verbal information from park rangers. In each locality, different adult plants were randomly selected. More information about each locality is shown in **Tables 1 and 2** and **Fig. 1**.

Table 2
Monthly maximum, minimum and mean air temperatures (°C) and precipitation (mm) for the three monitored sites (Mindala Loma, Polylepis and Mechahuasca). Climatic data was provided by the meteorological station of the R. P. F. Chimborazo from January 2017 to June 2018.

	Air temperature (°C)			Precipitation(mm)
Month	Max.	Min.	Mean	
2017				
January	4.9	3.5	4.2	56.7
February	5.7	3.1	4.4	22.9
March	5.2	2.9	4.1	114.5
April	4.6	3.5	4.1	163.8
May	4.8	3.3	4.0	45.7
June	3.63	2.1	2.9	70.7
July	4.0	2.2	3.1	8.7
August	4.0	2.3	3.1	4.4
September	3.6	2.1	2.8	42.2
October	3.9	2.7	3.3	66.1
November	4.2	2.9	3.6	25.0
December	3.4	2.1	2.1	81.5
2018				
January	3.5	2.3	2.9	94.1
February	3.4	2.2	2.8	137.6
March	3.1	2.1	2.6	235.9
April	3.8	2.7	3.3	108.1
May	4.1	2.7	3.4	90.3
June	3.4	2.3	2.8	88.2

2.2. Plant material

Phenological observations and measurements were recorded on 47 adult and healthy plants in three different localities (Table 1) belonging to the paramo of Chimborazo volcano (Ecuador). *Vaccinium floribundum* is considered a wild plant species in Ecuador that grows in the highlands of the mountain range. The adaptation zone of "mortiño" are the Andean Paramos located at altitudes ranging from 1000 to 4500 m.a.s.l. But the truth is that few of them have enough plant populations to carry out *in situ* studies. Because of the extension of the agricultural areas, this plant species has been relegated to paramo areas between the 3400 and 3500 up to 4500 m.a.s.l. (MAGAP, 1998).

This deciduous perennial shrub is extraordinarily resistant to drought and frost. It grows successfully in tundra-like ecosystems, commonly known as Paramos (Coba et al., 2012). And since the "mortiño" is a wild and endemic fruit species of the high Andes, more studies on basic biology and ecology are necessary to establish efficient conservation programs for sustainable agriculture (Cobo et al., 2016).

2.3. Monitoring of reproductive phenology

The monitoring of the different reproductive phenological growth stages of *V. floribundum* was conducted from January 2017 to June 2018 (18 months). The reproductive phenological phases were monitored in order to ensure that the phenological information would cover the entire period of manifestation of the characteristic, from the beginning to the decline. This was achieved through semi-monthly visits to the different localities. In each visit, the number of reproductive phenological organs in each individual was counted, using the following categories: flower bud, flower, green immature fruit, red maturing fruit and black mature fruit. Using this quantitative information, a semi-qualitative scale following the BBCH system was adaptively constructed, focusing on the

Table 1
Localities where the reproductive phenology of *Vaccinium floribundum* "mortiño" was monitored on a semi-monthly basis from January 2017 to June 2018 (18 months) in the paramo of the Chimborazo volcano, Ecuador.

Locality Name	Province	No. of individuals sampled	Coordinates	Altitude (m)	Vegetation type
1. Mindala Loma	Bolívar	17	01°27'45″ S 78°56'05″ W	4261	herbaceous paramo
2. Polylepis	Chimborazo	19	01°32'41″ S 78°53'05″ W	4076	herbaceous paramo
3. Mechahuasca	Tungurahua	11	01°25'26″ S 78°47'53″ W	4370	herbaceous paramo

Fig. 1. The three paramo locations where "mortiño" reproductive phenology was monitored and described on the Chimborazo volcano area (Ecuador).

reproductive stages only.

For each reproductive phenological growth stage, we then elaborated a description accompanied by a photographic record and a graphical summary of the presence or absence of the different stages during the monitoring period. Once the monitoring of the selected individuals was completed, the best photographs were chosen to illustrate the main developmental stages of "mortiño" and, then, to successfully codify its reproductive phenology (Sakar et al., 2019).

2.4. Local climatic patterns

Climatic data from January to December 2017 and from January to September 2018 was obtained from a temporary weather station that was installed near the study sites for the purpose of a different research project (Table 2). Note that the phenological monitoring period of this study lasted from January 2017 to June 2018. The cumulative rainfall during the period from January to December 2017 was 702.2 mm, while from January to September 2018 was 794.4 mm. In 2017, average monthly rainfall (12 months) was 58.5 mm, with only two months, March and April, showing a cumulative monthly rainfall >100 mm (114.5 and 163.8 mm, respectively). In 2018, the average monthly rainfall (9 months) was 88.3 mm, with monthly rainfall >100 mm occurring in February, March and April (138.4, 235.1 and 108.8 mm, respectively). In both years, the period from July to September tended to be relatively dry, with an average monthly rainfall of <20 mm. Relative humidity during a day-night cycle can vary quickly—in any case, daily relative humidity average (24 h) ranged from 77.3% to 92.4% in 2017, and from 82% to 92.8% in 2018; although a few hourly records <20% existed, the great majority of hourly records were >80%. The average daily temperature was around 4 °C, with relatively low maximum to minimum temperature variation.

2.5. Statistical analysis

Linear regression analysis was carried out to detect any possible influence of climatic parameters on the reproductive phenology of the "mortiño". XLSTAT Premium 2016 software (Addinsoft, New York, NY, USA) and Statgraphics Plus (version 3.1, Statistical Graphics Corp., Rockville, MA, USA) were used for this analysis.

3. Results

3.1. BBCH scale codification for vaccinium floribundum reproductive phenology

Bleiholder et al. (1991) and Hack et al. (1992) proposed the BBCH scale (Biologische Bundesantalt, Bundessortenamt und Chemische Industrie), which describes a uniform coding system for the phenological identification of the growth stages of all species of mono- and dicotyledonous plants. It uses a decimal code that is basically divided into principal and secondary plant growth stages, including those reproductive ones.

The BBCH scale for mono and dicotyledonous plants considers 10 principal growth stages, numbered from 0 to 9 (Hack et al., 1992). In addition to the principal growth stages, secondary stages are defined using a second digit from 0 to 9, which represent short developmental steps that occur within the principal stages of development. For the *V. floribundum* BBCH scale, as adapted for this study, only the principal stages corresponding to the reproductive phenology were used, starting at flower development (stage 5) and ending at the ripening or maturity of fruit (stage 8). For the secondary stages, we did not always use all the digits from 0 to 9, but instead defined secondary stages that better matched the official descriptions of the BBCH scale (Hack et al., 1992). Fig. 2 portrays, using photographs, representative reproductive phenological stages observed in 47 individuals of *V. floribundum* from the three different localities studied.

Below we describe each reproductive phenological growth stage, and the period in the year at which each stage was observed.

Principal growth stage 5: Inflorescence emergence

51: Inflorescence buds swelling: buds closed, light brown scales visible (Fig. 2).

This stage represents the beginning of the inflorescence and basically occurs throughout the year in localities 1 and 2 (Figs. 3 and 4). However, in locality 3, it was only observed from May to August (Fig. 5).

53: Bud burst: green sepals tips enclosing flowers visible.

This stage is similar to the 51 stage, except that the inflorescence shows a higher degree of maturity. It occurred at more or less the same time as stage 51.

Principal growth stage 6: Flowering

Fig. 2. Representative photographs portraying the primary and secondary reproductive growth stages observed in 47 *Vaccinium floribundum* individuals, according to the BBCH-scale and focusing only on the reproductive stages. Note that it is common that in a given individual more than one stage is occurring at the same time.

61: Beginning of flowering: about 10% flowers open (Fig. 2).

This stage is present in very few individuals. In locality 1, it was observed in August 2017 and from February to March 2018 (Fig. 3); while in locality 2, it was only observed in January 2018 (Fig. 4). This stage was not observed in locality 3 (Fig. 5).

62: About 20% of flowers open.

This stage was only observed in locality 1 during May 2017 (Fig. 3), and in the month of March 2018 in locality 2 (Fig. 4). This stage was not observed in locality 3 (Fig. 5).

63: About 30% of flowers open.

This stage was monitored on only a few plants (Fig. 2). It was observed in locality 1 in April and June 2017 (Fig. 3), and in March 2017 in locality 2 (Fig. 4). This stage was not observed in locality 3 (Fig. 5).

64: About 40% of flowers open.

This stage was rarely observed. It was only observed in locality 2 in February 2018 (Fig. 4).

65: Full flowering: at least 50% of flowers open, many visible styles and stigmas (Fig. 2).

This stage was rarely observed. It was detected in locality 2 in January 2017 and March 2018 (Fig. 4).

66: Full flowering: at least 60% of flowers open, many visible styles and stigmas. This stage was rarely observed. It was only detected in locality 2 in December 2017 (Fig. 4).

67: Full flowering: at least 70% of flowers open, many visible styles and stigmas (Fig. 2). This stage was observed in locality 2 in May and October 2017 and in January 2018 (Fig. 4); while in locality 3, it was only detected in June 2017 (Fig. 5). This stage was not observed in locality 1 (Fig. 3).

69: End of flowering: numerous fruits have already set (Fig. 2).

In locality 2, this stage was observed almost throughout the study period (Fig. 4). In locality 3, this stage was also registered during many months, from April to October 2017 (Fig. 5). There is no record of this stage in locality 1 (Fig. 3).

Principal growth stage 7: Fruit development

70: Immature fruits visible (Fig. 2).

In localities 1 and 2, this stage was commonly observed throughout the monitoring period (Figs. 3 and 4). In locality 3, it was observed sporadically, from January to March 2017, and from August to October 2017 (Fig. 5).

Principal growth stage 8: Ripening or maturity of fruit

81: Beginning of maturation and color change: fruit skin appears orange. Approximately 10% of the final color (Fig. 2).

In localities 1 and 2, this stage was commonly observed throughout the monitoring period (Figs. 3 and 4). In locality 3, it was observed from January to March 2017, and from September 2017 to January 2018 (Fig. 5).

85: Coloring advanced: first fruits ripe black (Fig. 2).

This stage was not commonly observed in the three localities. In locality 1, it was observed in the months of February, June-July and December 2017 (Fig. 3). In locality 2, it was observed in November 2017 (Fig. 4); while in locality 3, it was observed in January and November 2017 (Fig. 5).

87: Beginning of softening of the fruits (Fig. 2).

This stage was observed almost throughout the monitoring period in localities 1 and 2 (Figs. 3 and 4). It was not observed in locality 3 (Fig. 5).

88: Beginning of the decrease of the consistency of the fruits (Fig. 2).

This stage is similar to the 87 stage, except that the fruit shows a higher degree of maturity. It occurred at more or less the same time as stage 87.

3.2. Chronology and timing of phenological events

The reproductive phenology of sampled individuals from sites 1 and 2 (Mindala Loma and Polylepis, respectively) were relatively synchronized, whereas individuals from site 3 (Mechahuasca) showed a different phenological pattern. Though the distance that separates the three sampled sites is 15 km at most (Fig. 1), site 3 reproductive phenology differed from the others since it is a special ecological niche surrounded by a huge lagoon.

A statistical analysis was performed using a linear regression model to detect potential influence of climatic parameters (independent variables) on the appearance frequency of each monitored reproductive

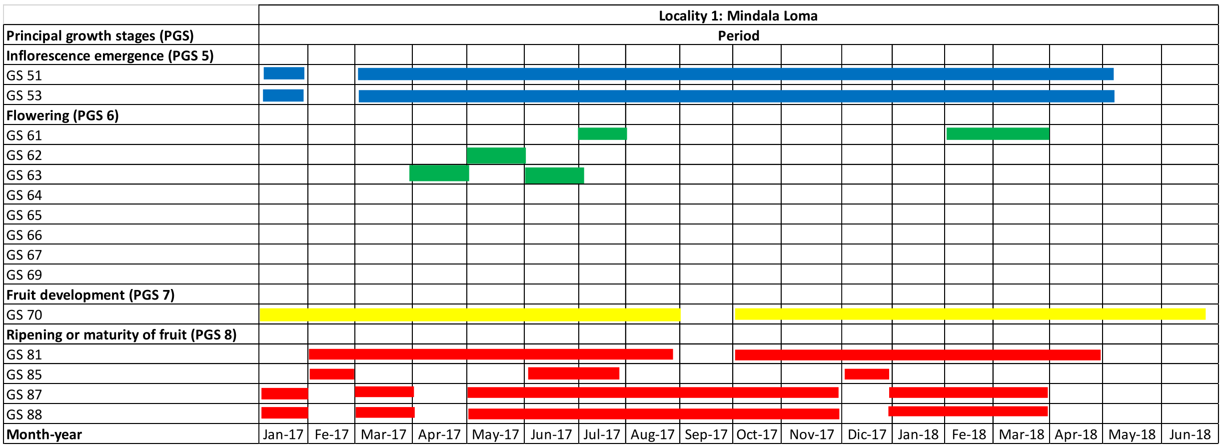

Fig. 3. Schematic representation of the chronological progression of reproductive phenological growth stages of *V. floribundum* ("mortiño") in Mindala Loma locality, paramo of the Chimborazo volcano.

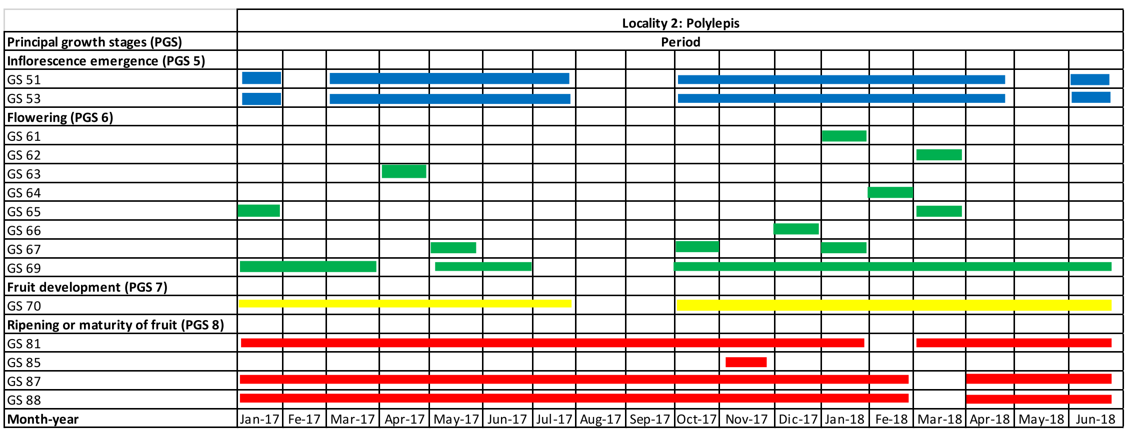

Fig. 4. Schematic representation of the chronological progression of reproductive phenological growth stages of *V. floribundum* ("mortiño") in Polylepis locality, paramo of the Chimborazo volcano.

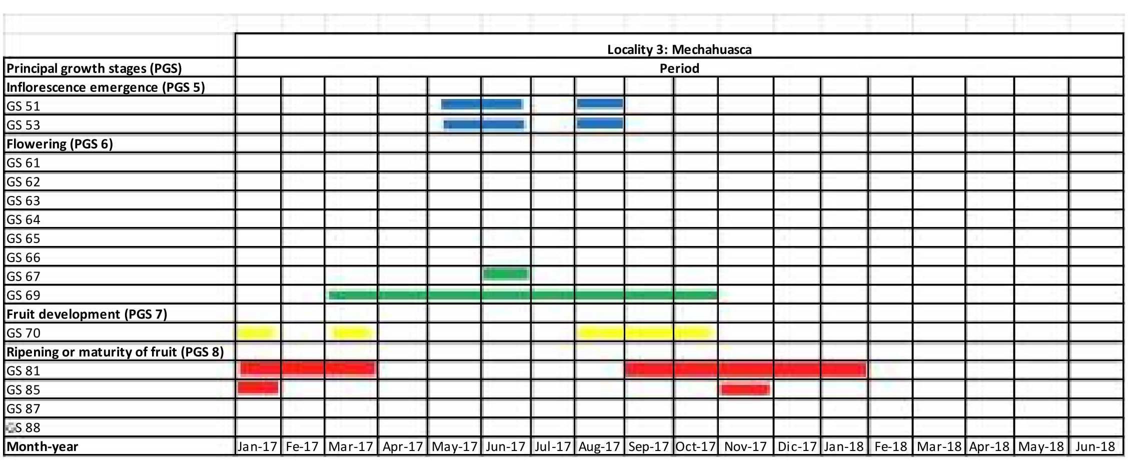

Fig. 5. Schematic representation of the chronological progression of reproductive phenological growth stages of *V. floribundum* ("mortiño") in Mechahuasca locality, paramo of the Chimborazo volcano.

growth stage (dependent variable). There was no significant influence of any climatic variable on the reproductive phenological patterns described (Figs. 3–5).

The chronological evolution of principal growth stages for the reproductive phenology of "mortiño" is shown in Figs. 3–5. Localities 1 and 2 (Mindala Loma and Polylepis, respectively) practically showed similar phenograms (Figs. 3 and 4), being the growth stages (GS) 51, 53, 70, 81, 87 and 88 the most frequent ones. This could be attributed to the fact that both locations are very close within the same area. In contrast, Mechahuasca (locality 3) showed a different trend since it is much further away and because the type of vegetation (alpine grasses) differed from sites 1 and 2 (herbaceous paramos). Here, the reproductive growth stages were rather irregular (Fig. 5).

4. Discussion

The BBCH codification scale has been used in different fruit crops, more commonly in northern latitudes (Legua et al., 2013; Hernández et al., 2015; Sánchez-Salcedo et al., 2017; Kishore, 2018). In the Neotropics, the BBCH scale was applied to *Vaccinium meridionale* in the Colombian paramos (Medina et al., 2019) and for *Vaccinium floribundum* in the Ecuadorian paramos (Mendoza, 2018; Rivera, 2019). However, it is difficult to compare the *V. floribundum* studies with the present study because of the different, or convoluted, approaches used in them. In this sense, it is important to realize that not all growth stages that are usually easily distinguishable in a crop, grown under semi-controlled conditions, are also observable or perceptible when a wild species in its natural setting is monitored, as is the case for this study. Another confounding factor, for the direct application of the BBCH scale on *V. floribundum* individuals monitored in this study, could be that they are growing at very high altitudes (>4000 m.a.s.l.), which could enforce particular physiological constraints imposed by the extreme weather conditions. On the other hand, in other studies with *Vaccinium spp.* (Medina et al., 2019; Mendoza, 2018; Rivera, 2019), observations were made at lower altitudes (3500–3600 m.a.s.l.).

Based on the general BBCH phenological scale (Meier, 1997), and along with the observations of mortiño reproductive growth phenology over the 18-months period and across the three monitored sites, we established and extended BBCH scale specific to *Vaccinium floribundum*. The use of the BBCH scale is very appropriate for the study of the phenological behavior of a plant species under different ecological conditions (Leather, 2010). The specific scale, obtained to detail the reproductive phenology of mortiño, definitely agreed with the general key proposed by Finn et al. (2007) for tree and woody plants description.

Morellato et al. (2006 and 2013) stated that rainfall is usually considered as an environmental signal responsible for the phenological variation of plant growth stages in tropical zones. And according to Mendoza et al. (2017), the most determining factor on plant phenology in high-altitude areas is certainly temperature. But in the tropics it is really the periodicity between dry and rainy periods. In our study, in fact, the occurrence and duration of the reproductive phenological growth stages, monitored over 18 months, did not show a clear relation with the precipitation pattern (i.e., with the strong wet season from March to April, and with the strong dry season around July-August) (Figs. 3–5). And this does not mean that climate could have an influence on the intensity or abundance of reproductive structures. Future studies should attempt to assess this possible relationship. In addition, it can be observed that throughout the monitoring period, there was a constant production of flowers and fruits.

Zapata (2002) reported that fruiting of *V. floribundum* occurs in eastern Antioquia in two main periods: from April to June and from September to December. However, Gómez (2004) found that in natural populations fruiting occurred throughout the year, with percentages ranging from 17% to 39%. In our study, we observed fruits throughout the whole monitoring period (18 months), but not necessarily at the time when the rainy season occurred, from February-March to April-May.

Regarding the time span that different reproductive organs can last (in days) during the reproductive phase of *V. floribundum*, we could gain some insight from the studies conducted in Colombia with *V. meridionale*. In this species, Chamorro and Nates-Parra (2015), in Guachetá (Cundinamarca) and San Miguel de Sema (Boyacá), found that the time it takes from a flower bud to become a full open flower was 18 days; and six days later, the senescence of this organ started. In addition, Chamorro (2014) stated that flowers only lasted for six to ten days, which was already considered a long floral longevity for the Ericaceae family (Primack, 1985). In the future, it will be worthwhile to embark in detailed floral biology studies for the *V. floribundum* populations of Ecuador, in order to allow comparisons with the numerous studies in Colombia.

Regarding the relative flower vs. fruit production, Rathcke (2003), Torres-Díaz et al. (2011), Chamorro (2014) and Chamorro and Nates-Parra (2015) found that *V. meridionale* produces a large number of flowers but low fruit set, which could be evidence of a selective abortion process of self-pollinated fruits. Though in our study we did find fruit production throughout the monitoring period, particularly in locations 1 and 2, while flowers were just sporadically observed because of a relatively fast flower loss.

5. Conclusions

For the first time, the reproductive phenology specific to “mortiño” (*Vaccinium floribundum*) was established according to BBCH phenological scale, based on evidence from the volcano Chimborazo paramo of Ecuador. The reproductive growth stages (GS) can be described in more detail using this specific scale. And based on the phenological observations carried out in the three monitored areas, we conclude that in this paramo there was not an ordered chronological progression of the reproductive phenology of “mortiño”, probably because of the adverse and continuously changing climatic conditions. Furthermore, it was relatively common to observe different growth stages concomitantly occurring in the same individual.

The main limiting factor for the cultivation and production of “mortiño” on a large scale is the lack of knowledge about its particular ecology, climatic requirements, soils and rhizosphere. This leads to a lack of domestication of the Andean fruit crop and, then, limiting its significant nutritional potential. And because of that, it is necessary to learn more about the conditions in which this fruit species grows in order to domesticate it for agro-industrial purposes. In addition, “mortiño” can also be used as biofuel and for regeneration and reforestation of paramos devastated by the great fires.

The results reported in this study can provide insight for: (i) climate change studies for preservation of the species on fragile habitats, (ii) adaptation studies of cultivars to different environmental conditions, (iii) propagation trials of the species under quasi *in situ* conditions, (iv) development of germoplasm collections for programing agronomic practices and (v) establishment of programs for “mortiño” commercial breeding.

CRediT authorship contribution statement

Jorge M. Caranqui-Aldaz: Data curation, Investigation, Writing – original draft, Writing – review & editing. **Hugo Romero-Saltos:** Software, Writing – original draft, Writing – review & editing. **Francisca Hernández:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Rafael Martínez:** Methodology, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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4.3. PUBLICACIÓN 3

Publicación 3 (Open Access)

Composition and Polyphenol Compounds of *Vaccinium floribundum* Kunth (Ericaceae) from the Volcano Chimborazo Paramo (Ecuador).

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Article

Chemical Composition and Polyphenol Compounds of *Vaccinium floribundum* Kunth (Ericaceae) from the Volcano Chimborazo Paramo (Ecuador)

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Abstract: Mortiño (*Vaccinium floribundum* Kunth) is considered a "superfruit" due to its antioxidant capacity and possible health benefits. To date, there is no known study that addresses the biochemical characterization of mortiño berries from the paramo of the Chimborazo volcano (Ecuador). So, the aim of this research was to evaluate for the first time the effect of the stage of development of the mortiño berries (two stages) and environment of origin (three sampling areas) on fruit quality. Polyphenol compounds were identified by high-performance liquid chromatography (HPLC) coupled to electrospray ionization mass spectrometric (ESI-MS^n) and quantified by high-performance liquid chromatography with a diode array detector (HPLC-DAD). Moreover, antioxidant properties ($ABTS^{\bullet+}$, and DPPH), sugar and organic acids, and minerals were examined. The main organic acids were quinic and citric acid, while glucose, fructose, sucrose, mannose, and sorbitol were the main sugars determined in the mortiño fruits. The main constituents of the mortiño berries included hydroxycinnamic acids (5-*O*-caffeoylquinic acid), flavonols (quercetin 3-hexoside, quercetin 5-hexoside, quercetin 3-pentoside, and quercetin-3-*O*-rhamnoside) and anthocyanins. Seven anthocyanins were identified: glycosides of cyanidin, delphinidin, petunidin, peonidin, and pelargonidin. The research confirms that the mortiño berries produced in the Ecuadorian paramo area are a valuable source of polyphenolics, rich in sugars and organic acids, and can be classified as a good source of microelements.

Keywords: antioxidant activity; polyphenolic; anthocyanins; mortiño; minerals

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1. Introduction

Mortiño (*Vaccinium floribundum* Kunth)—also known as Andean blueberry—is a deciduous perennial shrub endemic to the high Andes of South America and it is considered a "superfruit" due to its antioxidant capacity and possible health benefits [1,2]. This species is particularly common in the Andes from Venezuela to Bolivia and has been used since immemorial times. The berry is consumed fresh, dried, in sausages, jellies, jams, desserts, and in a special beverage called "colada morada" [3,4]. Mortiño berries are used by local communities in Ecuador for medical uses (allegedly as an aliment for rheumatism, fevers, colics, common colds, hangovers, and liver and kidney problems), as well as for ornamental and other uses as dye, fodder, or firewood [5]. One of the current limitations of *V. floribundum* fruit production is that the plant has not been domesticated and the technical difficulties to cultivate it, in addition to the continuous fragmentation experienced by mortiño populations due to anthropogenic processes such as deforestation, productive land reconversion, and overexploitation [6]. Mortiño berries can be found at local markets in the northern Andes (Colombia and Ecuador), where these fruits are usually collected from wild plants. Actually, with the advancement in food processing technologies, commercial mortiño products are available in the markets in different presentations such as capsules, powder, and wines, known as Wine of the Andes [7].

Mortiño, which can be found in Ecuador in the paramos at high altitudes ranging from 3000 to 4500 m above sea level (masl) [3], is a source of anthocyanins, proanthocyanidins, and polyphenolic compounds, which have been shown to possess antioxidant and anti-inflammatory properties, as well as lipid accumulation inhibition activity in adipocytes [8]. Freire [9] and Coba et al. [10] reported that berries of mortiño have relatively high concentrations of sugar, antioxidants such as vitamin C and the vitamin B complex, and minerals such as potassium, calcium, and phosphorus. The concentration of phytonutrients is influenced by many factors, such as variety, state of maturity, location, environmental conditions, agricultural practices, and pre-/postharvest handling [11–13]. During fruit ripening several biochemical and physiological processes take place, producing changes in fruit quality parameters [14]. Thus, due to oxidative stress in advanced stages of development, an increase in the content of phenol levels and a high content of antioxidant compounds have been observed [10]; however, during berry storage, the content of anthocyanins is reduced, as temperature is the main factor for destabilization of molecular structure [15]. Studies carried out show that the lyophilized extract of *Vaccinium floribundum* does not present toxicity and could be safely included as an ingredient in food. Because of its functional properties, the mortiño extract can also be handled as an antioxidant or natural colorant in the food industry, or even for the development of nutraceuticals in the pharmaceutical industry [16]. Thus, Guijarro-Fuertes et al. [17] developed bread with healthier properties by adding mortiño pulp. Moreover, it has even been used for the synthesis of nanoparticles and solar cells [18]. Additionally, the mortiño bagasse also contains a high amount of gallic acid, chlorogenic acid, caffeic acid, epicatechin, and coumaric acid, which makes it a very interesting source of antioxidant compounds [19]; the berries present as well antimicrobial activity, which makes the blueberry a potential source of bioproducts that can be used to develop new antimicrobials [1]. In addition, medicinal properties have been attributed to *V. floribundum*, such as potential applications in managing the symptoms of diabetes [20] and protection against oxidative stress [21].

Biochemical, nutraceutical and functional evaluation of *mortiño* plant material is essential to acknowledge its potential as a health-promoting aliment. To our knowledge, there are no studies that have addressed the biochemical characterization of *Vaccinium floribundum* from the paramo of the Chimborazo volcano (Ecuador).

For all the above-mentioned reasons, the objective of this research was the novel evaluation of the effect of the stage of development of mortiño berries (two stages) and location of origin (three sampling areas) on: (i) antioxidant activity, (ii) mineral composition, (iii) profile of sugars and organic acids, (iv) total phenolic content, and (v) anthocyanin profile and non-anthocyanin phenolic profile of mortiño (*Vaccinium floribundum* Kunth) produced in the volcano Chimborazo paramo (Ecuador). This information can be used to improve the market for mortiño, which can provide sustainable economic opportunities for farmers, and can be useful in promoting the conservation and sustainable use of this natural resource.

2. Materials and Methods

2.1. Plant Material

Three different local habitats with presence of *V. floribundum* were sampled in the paramo of the Chimborazo volcano in the central Andes of Ecuador. The sampling areas were to observe "mortiño" individuals selected based on verbal information from park rangers. In each study area, different adult plants were randomly selected.

Fresh berries of *V. floribundum* were harvested in three sampling areas: Culebrillas, Polylepis, and Cubillín, in the paramo of the Chimborazo volcano (Ecuador), the native habitat of the species. All growing environments showed loamy-sandy texture soils, with fairly poor percentages of organic matter (0.90% in Polylepis and Culebrillas, and 0.80% in Cubillín), acidic pH values of 5.27 (Culebrillas and Polylepis) and 5.63 (Cubillín). The predominant vegetation in the three monitored areas was the herbaceous paramo, and only Cubillín also showed an Alpine steppe (alpine grassland).

Berries were randomly picked from different parts of wild bushes on mountain slopes at an altitude between 3500 and 4100 masl. More information about each environmental area is shown in Table 1 and Figure 1. The berries were classified according to their maturity state into: (i) Stage 7: Fruit development; the berries began to develop anthocyanins, which was identified by their reddish coloration from the apical to the basal part of the fruit, and (ii) Stage 8: Ripening or fruit maturity; 100% of cluster berries shows a purple epicarp [22].

Table 1. Sampling areas where the mortiño berries were harvested in the paramo of the Chimborazo volcano, Ecuador.

Locality Name	Province	Coordinates	Altitude (m)	Vegetation Type	Mean Temperature(°C)	Mean Precipitation(mm)
Culebrillas	Bolívar	01°34.20′ S 78.55.5′ W	4000	Herbaceous paramo	3.1	967
Polylepis	Chimborazo	01°32.41′ S 78°53.5′ W	4076	Herbaceous paramo	3.1	967
Cubillín	Chimborazo	01°45′ S 78°31′ W	3500	High mountain forest	7.0	1000

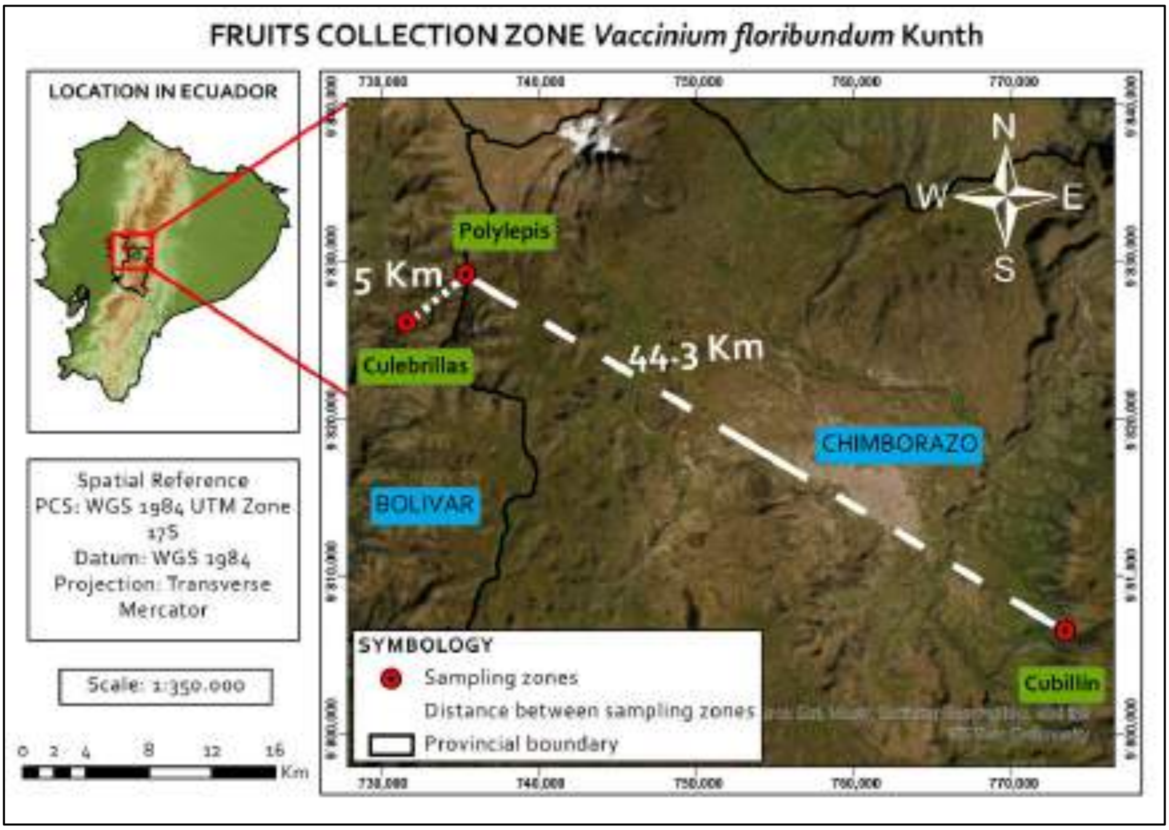

Figure 1. The three paramo zones where the mortiño berries were harvested.

2.2. Sample Preparation

After sorting, the berries were cleaned by removing leaves, stems, and damaged berries and were washed with drinking water to reduce the microbial load, dirt, and organic matter. Then, the berries were immediately frozen with liquid nitrogen and later freeze-dried in an Alpha 2–4 freeze drier (Alpha 2–4; Christ, Osterode am Harz, Germany) for 24 h at a pressure reduction of 0.220 mbar. The temperature in the drying chamber was −25 °C, while the heating plate reached 15 °C. At the end of freeze-drying, the samples were powdered, and vacuum packed at −20 °C until analyzed. Moisture content in freeze-dried mortiño was 5 g 100 g^{-1}. For conversion from DM to FW, the moisture contents in fresh and freeze-dried mortiño were used.

2.3. Extraction Procedure for Total Polyphenols Content (TPC) and Antioxidant Activity (AA)

The extraction procedure for TPC and AA quantification was prepared as described by Wojdyło et al. [23]. The extractions were performed in triplicate.

2.3.1. Quantification of Total Polyphenols Content (TPC)

The TPC was determined using the Folin–Ciocalteu colorimetric method described by Singleton et al. [24], with some modifications. The absorbance of the blue complex formed was read at 765 nm using a UV–visible spectrophotometer (Termospectromic Helios Gamma UVG 1002 E, Cambridge, UK). Calibration curves, with a concentration range between 0 and 0.25 g GAE L^{-1}, were used for the quantification of TPC and showed good linearity ($r^2 \geq 0.996$). All determinations were performed in triplicate, and results were expressed as milligrams of gallic acid equivalent per 100 g of sample dry matter (mg of GAE 100 g^{-1} of DM).

2.3.2. Determination of Antioxidant Activity by Two Different Methods

ABTS Method

The ABTS [2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) assay was performed according to Re et al. [25] with some modifications. The absorbance was measured by UV–visible spectrophotometer (Termospectromic Helios Gamma UVG 1002 E, Cambridge, UK). All determinations were performed by triplicate, and the results were expressed in millimoles of Trolox per kilogram of sample dry matter (mmol Trolox kg^{-1} of DM).

DPPH Method

The radical scavenging activity was evaluated using the $DPPH^{\bullet}$ radical (2,2-diphenyl-1-picrylhydrazyl) method, as described by Brand-Williams et al. [26], with a modification in the reaction time. The decrease in absorbance was measured at 515 nm using a UV–visible spectrophotometer (Termospectromic Helios Gamma UVG 1002 E, Cambridge, UK). All determinations were performed by triplicate, and the results were expressed in millimoles of Trolox per kilogram of sample dry matter (mmol Trolox kg^{-1} of DM).

Calibration curves in the range 0.01–5.00 mmol Trolox L^{-1} were used for the quantification of the two methods of antioxidant activity, both showing good linearity ($r^2 \geq 0.998$).

2.4. Determination of Sugars and Organic Acids Profile

Organic acids and sugars profile were identified and quantified according to Hernández et al. [27], with some modifications. Briefly, half a gram of freeze-dried mortiño berry was mixed with 5 mL of phosphate buffer (50 mmol L^{-1}) pH 7.8; the mixture was homogenized, centrifuged, and filtered. Then, 10 µL of the supernatant was injected into a Hewlett Packard (Wilmington, DE, USA) series 1100 high-performance liquid chromatography equipped with a refractive index detector for sugars detection, and UV–Vis detector for organic acids analysis. A Supelcogel TM C-610H column (30 cm × 7.8 mm) with a pre-column (Supelguard 5 cm × 4.6 mm; Supelco, Bellefonte, PA, USA) was used for the analyses of both organic acids and sugars. Absorbance of organic acids was measured at 210 nm. Analyses were run in triplicate and the results expressed as g kg^{-1} dry matter (DM).

2.5. Minerals Analysis

To determine mineral content in the mortiño berries weighed accurately to a weight of 0.1 g of freeze-dried powdered into 75 mL Teflon (TFM) vessels. Then, 4 mL HNO_3 (69 vol.%) and 2 mL of ultra-high-purity deionized water were added and left to stand for 15 min to pre-digest the samples. Next, samples were microwave (CEM Mars One 240/50, Matthews, NC, USA) digested. The quantification of macro-elements [calcium (Ca), sodium (Na), potassium (K), and magnesium (Mg)] and micro-elements [iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn)] was carried using an Inductively Coupled Plasma Mass Spectrometer (ICPMS-2030, Shimazu, Kyoto, Japan). Calibration curves were used for the quantification of minerals and showed good linearity ($R^2 \geq 0.998$). The analyses were run in triplicate and results expressed as g kg^{-1} and mg kg^{-1} for macro and micro-elements, respectively.

2.6. Identification and Quantification of Phenolic Compounds by HPLC-DAD-ESI-MS^n

2.6.1. Extraction and Determination of Phenolic Compounds Non-Anthocyanin

Extraction method: Samples (50 mg) were mixed with 1 mL of extractant (methanol/water (80:20, *v*/*v*) + 1% formic acid), and this mix was stirred during 5 min. After that time, the samples were centrifuged for 10 min at 12,000 rpm and 4 °C. The supernatant was filtered through a 0.45 μm PTFE filter (Millipore Billerica, MA, USA) and then was stored at −20 °C until further use. All extractions were carried out in triplicate.

Phenolic compounds non-anthocyanin: For the analysis of the different samples of mortiño berries, HPLC-ESI-DAD-MS^n Ion Trap (Agilent 1100 series System) was used, which allows us to make successive breaks of the precursor ion for its identification of unknowns. Chromatographic separation was carried out on a C18 column (Poroshell 120, 100 mm × 3 mm i.d., 2.7 μm particle size). The mobile phases consisted of two solvents: water/formic acid (95:1, *v*/*v*) as solvent A and acetonitrile as solvent B at a flow rate of 1 mL min^{-1}. For the determination of polyphenols, the gradient started with 5% B to reach 60% B at 37 min, at 40 min the percentage of B increased to 98% and was maintained for 2 min before returning to the initial conditions. The injection volume was 20 μL. Relative quantification of the phenolic compounds present in the samples was performed by chromatographic comparison with pure standards (caffeic acid, rutinoside quercetin, pelargonidin and cyanidin), and their absorbance spectra at four wavelengths emitted at 280, 320, 360, and 520 nm through a diode array UV detector (DAD) integrated in the HPLC and connected on-line to the mass spectrometer.

2.6.2. Identification and Quantification of Anthocyanins

Extraction method: Anthocyanins extraction and analysis were determined using the method proposed by Hong et al. [28] with some modifications. Briefly, half a gram of freeze-dried samples of mortiño berries was mixed by shaking in an orbital bath, with 4 mL cold extractant [methanol/water/formic acid (80:19.9:0.1, *v*/*v*/*v*)] for 10 min. The mixture was sonicated by ultrasonic bath for 10 min. Next, the samples were centrifuged for 10 min, 4000 rpm at 4 °C, and the supernatants were collected; the pellets residue was re-extracted twice using the same steps, being the supernatants definitely combined. Following, two milliliters of the supernatant were filtered through a 0.45 μm nylon Millipore membrane filter, and then stored at −20 °C until further use. All extractions were carried out in triplicate.

Analytical method: Anthocyanins profile was determined by high-performance liquid chromatography triple quadrupole mass spectrometer (LC-MS/MS 8050; Shimadzu, Kyoto, Japan). The molecules were ionized using Atmospheric Pressure Ionization (Electrospray Ion-ization-ESI). Chromatographic separations were performed with a C18 column (Mediterranean SEA 18, 10 mm × 0.21 mm i.d., 2.2 μm particle size) from Teknokroma (Barcelona, Spain). The mobile phase A consisted of 0.1% (*v*/*v*) formic acid (FA) in water (Milli-Q), and the mobile phase B consisted of 0.1% (*v*/*v*) formic acid in acetonitrile (ACN) at a flow rate of 0.4 mL min-1, an injection volume of 10 μL and oven temperature of 50 ° C. The gradient condition was 0–2 min 5% B, 2–10 min 95% B, 10–11 min 95% B, 11–12 min 5%B, and 12–16 min 5% B. For the quantification of the anthocyanins (Delphinidin 3-*O*-glucoside; Cyanidin-3,5-di-*O*-glucoside; Cyanidin 3-*O*-glucoside; Cyanidin-3-*O*-arabinoside; Petunidin-3-*O*-glucoside; Peonidin-3-*O*-glucoside; Pelargonidin-3-*O*-rutinoside), a stock of 100 ppm was made, and from it, a calibration plot of concentrations 0.1, 0.3, 0.5, 0.8 and 1 ppm was obtained. All analyses were performed in triplicate.

2.7. Statistical Analyses

The data were subjected to one-way analysis of variance (ANOVA) and later to Tukey's multiple-range test to compare the means. The confidence interval was 95%, and the significant difference was defined as $p < 0.05$. Principal component analysis (PCA) was used to reduce this complex dataset to a lower dimension and reveal simplified structures and relations between localities and states of maturity. To perform these statistical analyses, the software XLSTAT Premium 2016 (Version 9-Addinsoft, New York, NY, USA) was used.

3. Results and Discussion

Since the processed experimental data are so diverse, the results are differently presented into main subheadings.

3.1. Antioxidant Activity (AA) and Total Polyphenol Content (TPC)

The results for AA and TPC obtained in mortiño berries are summarized in Table 2. These parameters are important from a functional point of view because oxidative stress is reported to be the key factor for many diseases such as cardiovascular, hypertension, atherosclerosis, neurodegenerative or cancer, mainly caused by an imbalance between reactive oxygen species (ROS) and the antioxidative defense system [29]. The AA of mortiño berries was evaluated using two spectrophotometric assays: ABTS and DPPH, as each antioxidant compound has different mechanism of action. Significant differences were observed for "sampling area", "stage" and the interaction "sampling area x stage" factors. The highest value of AA by ABTS method was found in mortiño berries collected in the zone of Culebrillas (65.74 mmol Trolox kg^{-1} DM) while for DPPH method the highest value was obtained in mortiño berries collected in Polylepis (77.22 mmol Trolox kg^{-1} DM). These results were slightly higher than those reported by Vasco et al. [3] in mortiño berries and in Andean blackberry, and that those reported by Garzon et al. [4] for Colombian bilberries (*V. meridionale*). Furthermore, our AA values were higher compared to the values reported by Sellappan et al. [30] for other *Vaccinium* species as *V. corymbosum* L. hybrids and *V. ashei*. On the other hand, and with respect to the stage of fruit development, the AA for both methods decreased during fruit development, ranging from 63.34 (stage 7) to 39.74 (stage 8) mmol Trolox kg^{-1} DM for ABTS method, and from 72.16 (stage 7) to 55.37 (stage 8) mmol Trolox kg^{-1} DM for DPPH method. The same trend was reported by Esquivel-Alvarado et al. [31] for berries of *V. consanguineum, V. poasanum and V. floribundum* collected between 3000 and 3200 masl. Analyzing the interaction "sampling area x stage" factors (Table 2), it can be seen that the highest AA content, measured by both methods, was found in the mortiño fruits collected in the Polylepis and Culebrillas areas (>4000 masl). The results indicate that mortiño berries have a great inhibitory activity against free radicals, as can be seen when contrasted with other reports in the literature. Therefore, it can be concluded that mortiño berries are an accessible source of antioxidants.

Table 2. Antioxidant activity (mmol Trolox kg^{-1} dry matter, DM) and total polyphenol content [mg gallic acid equivalent (GAE) 100 g^{-1} DM] in Mortiño berries as affected by sampling area and stage of fruit development.

Factor	ABTS	DPPH	TPC
	(mmol Trolox kg^{-1} DM)		(mg GAE 100 g^{-1} DM)
	ANOVA Test †		
Zone	***	***	***
Stage	***	***	***
Sampling zone x Stage	***	***	***
	Tukey's multiple range test ‡		
	Zone		
Polylepis	47.76 b	77.22 a	2231.45 b
Cubillín	41.12 c	44.85 c	1649.69 c
Culebrillas	65.74 a	69.22 b	2651.57 a
	Stage ¥		
Stage 7	63.34 a	72.16 a	2559.12 a
Stage 8	39.74 b	55.37 b	1796.02 b
	Study zone × Stage		
Polylepis * Stage 7	79.53 a	86.64 a	3089.38 a
Polylepis * Stage 8	16.00 e	67.80 b	1373.52 e
Cubillín * Stage 7	41.43 d	50.97 c	1717.46 d
Cubillín * Stage 8	40.81 d	38.72 d	1581.91 d
Culebrillas* Stage 7	69.07 b	78.86 a	2870.53 b
Culebrillas* Stage 8	62.42 c	59.57 bc	2432.62 c

† NS: not significant at $p < 0.05$; * and ***, significant at $p < 0.05$ and 0.001, respectively. ‡ Values (mean of 3 replications) followed by the same letter, within the same column was not significantly different ($p < 0.05$), Tukey's least significant difference test. ¥ Stage 7: Fruit development; the berries began to develop anthocyanins; Stage 8: Ripening or maturity of fruit; 100% of cluster berries epicarp is purple.

With respect to the TPC, significant differences were also observed for the "sampling zone", "stage" and the interaction "sampling zone × stage" factors for $p < 0.001$. For the "sampling area" factor, the highest values were found for mortiño berries collected in Polylepis and Culebrillas, ranging those from 2231.45 (Polylepis) to 2651.57 (Culebrillas) mg GAE 100 g^{-1} DM; while the lowest values were obtained in the location of Cubillín at lower altitude (values 0.67- fold lower). For the "stage" factor, mortiño berries presented the highest levels of TPC in stage 7 (2559.12 mg GAE 100 g^{-1} DM); and for the interaction "sampling zone × stage" factors, the highest levels were found for the "Polylepis × Stage 7" (3089.38 mg GAE 100 g^{-1} DM) and the "Culebrillas × Stage 7" (2870.53 mg GAE 100 g^{-1} DM). TPC decreased during fruit development by 0.70-fold for stage 8 (Table 2). A similar trend was reported by Esquivel-Alvarado et al. [31] for *V. floribundum* collected at an altitude of 3200 m, who reported values from 1787 mg GAE 100 g^{-1} DM for early fruit development stage to 1441 mg GAE 100 g^{-1} DM for the late fruit development stage. This trend can be attributed to changes such as the hydrolysis of glycosides, the oxidation of phenols by polyphenol oxidases, and the polymerization of free phenols [32]. In the current study, the content of total polyphenol was higher than those reported by Prior et al. [11], who reported mean TPC values for *V. corymbosum.*, *V. angustifolia*, and *V. myrtillus* of 347, 398, and 525 mg GAE 100 g^{-1} FW, respectively, and were also higher than those found in other fruits as pomegranate (777 to 1660 g GAE kg^{-1} DM), plum (440 mg GAE 100 g^{-1} FW) and strawberry (238 mg GAE 100 g^{-1} FW) [3,33], whereas were lower than those reported by Llerena et al. [34] for blackberry (6352.28 mg GAE 100 g^{-1} DM).

In this study, total polyphenols content and antioxidant activity were clearly influenced by altitude. The higher the altitude, the higher the contents of TPC and AA presented by mortiño berries. These results demonstrate that altitude is an important factor affecting the antioxidant activity and total polyphenols content in *V. floribundum*. Similarly, previous studies have indicated that AA and TPC levels can vary significantly depending on the geographical location of the mortiño plants, as well as the growing conditions such as altitude, radiation, and temperature [1]. The high content of TPC in mortiño berries can significantly contribute to the use of this material as a source of natural antioxidants.

3.2. Sugars and Organic Acids Profile

Table 3 summarizes the effects of environmental origin and stage of fruit development on organic acids (OA) and sugars profile and content. The main organic acids found in *V. floribundum* were quinic acid, followed by citric and malic acids. The same main organic acids were reported by Wang et al. [35] in 10 populations of *Vaccinium uliginosum* from the Changbai Mountains of China. The "Sampling zone" factor significantly ($p < 0.001$) affected the three acids content.

Table 3. Organic acids and sugar content (g kg^{-1} dry matter, DM) in Mortiño berries as affected by location and stage of fruit development.

Factor		Organic Acids				Sugars		
	Citric	Malic	Quinic	Sucrose	Glucose	Fructose	Mannose	Sorbitol
ANOVA Test †								
Zone	***	***	***	***	***	***	***	***
Stage	***	***	***	***	NS	***	***	***
Zonex Stage	***	***	***	***	***	***	***	***
Tukey's multiple range test ‡								
Zone								
Polylepis	41.98 b	22.30 c	108.39 c	9.96 b	110.05 a	60.80 b	31.80 b	106.46 c
Cubillín	65.05 a	28.35 b	199.48 a	61.42 a	71.75 b	50.61 b	35.82 b	190.55 a
Culebrillas	58.06 a	36.71 a	155.99 b	13.05 b	106.94 a	79.19 a	134.04 a	157.76 b
Stage ¥								
Stage 7	63.10 a	26.22 b	177.32 a	41.56 a	91.91	39.08 b	79.30 a	178.97 a
Stage 8	46.96 b	32.02 a	131.92 b	14.73 b	100.58	87.99 a	55.14 b	124.20 b
Zone × Stage								
Polylepis * Stage 7	50.76 b	10.21 e	153.20 b	12.90 bc	116.43 a	64.35 b	34.14 cd	145.12 b
Polylepis * Stage 8	33.20 c	34.96 b	63.57 c	7.02 c	103.66 ab	57.25 b	29.45 cd	67.80 c
Cubillín * Stage 7	75.98 a	25.23 cd	216.84 a	104.74 a	60.04 c	24.19 c	21.00 d	217.02 a
Cubillín * Stage 8	54.11 b	31.47 bc	182.12 ab	18.10 b	83.46 bc	77.03 b	50.64 c	164.07 b
Culebrillas * Stage 7	62.56 ab	19.04 de	161.91 b	7.04 c	99.27 ab	28.69 c	182.76 a	174.77 ab
Culebrillas * Stage 8	53.56 b	54.37 a	150.07 b	19.07 b	114.61 a	129.69 a	85.32 b	140.75 b

† NS: not significant at $p < 0.05$; * and ***, significant at $p < 0.05$ and 0.001, respectively. ‡ Values (mean of 3 replications) followed by the same letter, within the same column were not significantly different ($p < 0.05$), Tukey's least significant difference test. ¥ Stage 7: Fruit development; the berries began to develop anthocyanins; Stage 8: Ripening or maturity of fruit; 100% of the epicarp of the cluster berries is purple.

Quinic and citric acids predominated over malic acid in all environmental areas. This result agreed with that obtained by Mikulic-Petkovsek et al. [36], who indicated that fruit of the Ericaceae family generally contained very little malic acid. Mikulic-Petkovsek et al. [37] reported that wild bilberry fruits (*V. myrtillus*) from high altitude (up to 636 masl) had more organic acids content, compared with bilberry fruit from lower altitudes (up to 217 m). While mortiño berries collected in Cubillín (at an altitude of 3500 m and at a mean temperature of 7 °C) showed the highest content of total OA (287.88 g kg^{-1}), Culebrillas and Polylepis (at more than 4000 m altitude and at a mean temperature of 3.1 °C) showed total OA contents of 250.76 g kg^{-1} and 172.67 g kg^{-1}, respectively. It is important to note that changes in organic acids in response to temperature also depend on other factors as plant age or fruit type. The temperature at which fruits are grown affects both their titratable acidity and the content of stored organic acids [38]. Our results agree with those found by Mikulic-Petkovsek et al. [37]. However, the results obtained in this study suggest that above 3500 m of altitude the content of organic acids decreases strongly; from 3500 m of altitude (Cubillín) to 4076 (Polylepis), the total AO content decreased to 60%.

Different studies indicate that the organic acid content of the flesh of fruits is affected by environmental factors and cultivation practices such as temperature, light intensity, cultivar, rootstock, mineral nutrition, water availability, and fruit load/pruning. Nonetheless, how these factors alter metabolism to bring about changes in organic acid content is in most cases uncertain [39]. Thus, Wang et al. [35] reported that in *V. uliginosum* the organic acid content was not related to altitude. These differences may be due to a combination of different growing environments (e.g., microclimate), genetics, or other environmental factors. The "stage" and the interaction "zone x stage" factors also affected significantly ($p < 0.001$) the organic acid content (Table 3). Organic acids accumulate in the flesh of many types of fruits at certain stages of their development [38]. Organic acids are related to maturation, in particular malic acid which confers a bitter taste. Thus, the results obtained in this study showed that malic acid increases with maturation, ranging from 26.22 g kg^{-1} DM (Stage 7) to 32.02 g kg^{-1} DM (Stage 8), while both quinic and citric acids decreased. Ayaz et al. [40] also reported in *V. artostaphylos* and *V. myrtillus* that the level of malic acid increased gradually during the maturation of fruits. Compared to previous results reported by Kafkas et al. [41] for blackberries and Correia et al. [42] for Highbush blueberries (*V. corymbosum*), our study showed much lower contents of malic acid for *V. floribundum*.

Regarding the sugar content, glucose, fructose, sucrose, mannose, and sorbitol were the main sugars determined in the fruits of mortiño (Table 3). The sugar content was significantly ($p < 0.001$) affected by the "location", "stage" and the interaction "location × stage" factors. The higher values of glucose and fructose were obtained in the areas of higher altitudes (>4000 m), Polylepis, and Culebrillas. Additionally, Wang et al. [35] reported that the higher the location altitude (>1200 m considered high altitude), the higher the contents of glucose and fructose in bog bilberry (*V. uliginosum*). On the other hand, during fruit development of mortiño, fructose and glucose increased while sucrose, mannose, and sorbitol decreased; the low sucrose content may be due to enzymatic hydrolysis or its transformation into other sugars during the ripening process. Our values agreed with the results reported by Kalt and McDonal [43] for lowbush blueberry (*V. angustifolium*), Correia et al. [42] for highbush blueberry (*V. corymbosum*), and Ayaz et al. [40] for *V. arcostaphylos* and *V. myrtillus*. However, Mikulic-Petkovsek et al. [37] indicated that bilberries grown at low altitude sites (217 m considered low altitude) contained higher levels of total sugars compared to bilberries grown at higher altitudes (636 m considered high altitude). Additionally, since the sucrose content of mortiño was quite low, this fruit should be recommended for low-carbohydrate diets.

Sorbitol is a sugar alcohol characteristic of higher plants. It is a major final product of photosynthesis and, together with sucrose, represents the main forma of carbon translocated in some fruit species [36]. While the highest sorbitol content (190.55 g kg^{-1} DM) was obtained in mortiño fruits grown at low altitudes (Cubillín), the lowest content was found in those fruits grown at higher altitudes (>4000 m). Though Mikulic-Petkovsek [36] also detected sorbitol in chokeberry, rowanberry, and eastern shadbush, this sugar was not detected at all in other berry species [44]. Our results suggest that climate factors such as altitude and temperature play an important role in the sugar content of mortiño berries. Likewise, Cobo et al. [45] also observed this variability in the chemical composition of *V. floribundum* fruits, as a result of climatic and geographic influences.

3.3. Mineral Content

The content of the macronutrients and micronutrients was significantly affected by the "growing environment" and the interaction "growing environment × stage" factors.

Only the macronutrients potassium (K), sodium (Na), and magnesium (Mg) and the micronutrient iron (Fe) were significantly affected by the "stage" factor (Table 4). While the highest quantity of macronutrients (K > Ca > Mg > Na) was found in the mortiño fruits grown in Polylepis, the highest content of micronutrients (Fe > Mn) was observed in fruits grown in Cubillín. Unfortunately, to date there are no studies that provide complete information on the content of minerals in mortiño fruits, and neither how these contents can be influenced by the factors previously outlined such as altitude, stage of development of the fruit, temperature, etc. Vasco et al. [3] reported that mortiño berries are rich in potassium; a serving of 100 g could provide 13% of the recommended adequate intake (AI) of 4.7 g/day for adults. Our results revealed that the macronutrient (K, Na, and Mg) contents decrease with fruit development, while calcium (Ca) and iron (Fe) are the only macro and micronutrients that increase with fruit development. Several studies showed that Ca is an effective pressure-lowering agent [46]; thus, a high Ca content can be beneficial for health. Likewise, it was observed that mortiño immature berries (development stage 7) grown at high altitudes (>4000 m) had a higher potassium content (10.13 and 8.27 g kg^{-1} DM for Polylepis and Culebrillas, respectively). Our results indicated that the mineral content in mortiño fruits is clearly influenced by the growing conditions, the state of fruit development, and the altitude. Karlsons et al. [47] studied the mineral composition of four species of *Vaccinium* (*V. corymbosum, V. myrtillus, V. macrocarpon,* and *V. oxycoccos*), and reported that the berries of these species were characterized by having a high content of Fe, Ca, Mg, and Mn. In our study, mortiño berries showed levels of macro and micronutrients comparable to those obtained by Karlsons et al. [47] and by Miljkovič et al. [48] in Serbia for *V. myrtillus*. The mineral composition shown by mortiño fruits indicates that these berries are an excellent source of K, Ca, and Fe. In addition, due to their low levels of sodium, mortiño fruits could be properly recommended for low-sodium diets.

3.4. Identification and Quantification of Phenolic Compounds Non-Anthocyanin and Anthocyanins

A total of sixteen different compounds, nine non-anthocyanin (Table 5) and seven anthocyanins (Table 6) have been identified in mortiño berries. To make the discussion easy to follow, phenolic compounds non-anthocyanin and anthocyanins were discussed separately. Quantification of each identified compound is shown in Table 7.

Table 4. Minerals content (g or mg kg^{-1} dry matter, DM) in Mortiño berries as affected by environmental zone and stage of fruit development.

Factor	K	Na	Ca	Mg	Cu	Mn	Fe	Zn
		Macro-Elements (g kg^{-1})				Micro-Elements (mg kg^{-1})		
ANOVA Test †								
Zone	**	***	***	***	**	***	***	***
Stage	**	***	NS	***	NS	NS	***	NS
Zonex Stage	**	***	***	***	**	***	***	***
Tukey's multiple range test ‡								
Zone								
Polylepis	8.85 a	0.30 a	4.88 a	1.24 a	5.63 a	47.63 b	80.95 b	29.61 a
Cubillín	6.83 b	0.24 b	1.80 c	0.41 c	4.74 b	93.05 a	126.89 a	14.40 c
Culebrillas	8.10 a	0.22 b	3.47 b	0.93 b	4.84 ab	22.96 c	70.90 b	17.79 b
Stage ¥								
Stage 7	8.52 a	0.28 a	3.18	0.92 a	5.11	56.76	70.84 b	21.60
Stage 8	7.33 b	0.23 b	3.58	0.79 b	5.03	52.34	114.99 a	19.60

Table 4. *Cont.*

Factor	K	Na	Ca	Mg	Cu	Mn	Fe	Zn
	Macro-Elements (g kg^{-1})				Micro-Elements (mg kg^{-1})			
Zone × Stage								
Polylepis * Stage 7	10.13 a	0.36 a	3.92 b	1.28 a	5.06 ab	48.97 b	69.81 bc	32.42 a
Polylepis * Stage 8	7.57 b	0.25 bc	5.84 a	1.20 a	6.20 a	46.30 b	92.10 b	26.80 a
Cubillín * Stage 7	7.15 b	0.27 b	1.87 c	0.43 c	5.02 ab	93.04 a	61.29 c	15.82 bc
Cubillín * Stage 8	6.50 b	0.21 bc	1.72 c	0.39 c	4.42 b	93.07 a	192.49 a	12.99 c
Culebrillas * Stage 7	8.27 ab	0.19 a	3.74 b	1.06 a	5.22 ab	28.26 c	81.41 bc	16.55 bc
Culebrillas * Stage 8	7.93 b	0.24 ab	3.20 bc	0.81 b	4.46 b	17.67 c	60.38 c	19.02 b

† NS: not significant at $p < 0.05$; *, **, and ***. significant at $p < 0.05$, 0.01, and 0.001, respectively. ‡ Values (mean of 3 replications) followed by the same letter, within the same column, were not significantly different ($p < 0.05$), Tukey's least significant difference test. ¥ Stage 7: Fruit development; the berries began to develop anthocyanins; Stage 8: Ripening or fruit maturity; 100% of cluster berries epicarp is purple.

Table 5. Phenolic compounds (non-anthocyanin) identified by HPLC-DAD-ESI-MS^n in Mortiño berries.

Peak No.	[a] Rt (min)	[b] MS/MS (*m*/*z*)	Name of Compounds [c]	Chemical Family
P1	7.3	353,191	3-*O*-Caffeoylquinic acid	Hydroxycinnamic Acid
P2	9.1	337,163	3-Coumaroylquinic acid	Hydroxycinnamic Acid
P3	9.9	707,353,191	5-*O*-Caffeoylquinic acid	Hydroxycinnamic Acid
P4	12.6	335,179	Caffeoylshikimic acid	Hydroxycinnamic Acid
P5	15.9	433,323	Caffeic acid derivate	Hydroxycinnamic Acid
P6	16	463,301	Quercetin 3-hexoside	Flavonols
P7	17.8	463,301	Quercetin 5-hexoside	Flavonols
P8	18	433,301	Quercetin 3-pentoside	Flavonols
P9	18.1	447,301	Quercetin-3-O-rhamnoside	Flavonols

[a] Rt = retention time; [b] MS/MS = tandem mass spectrometry; [c] Compounds were numberer by their elution time.

Table 6. Anthocyanins identified by HPLC-ESI-MS^n in Mortiño berries.

Peak No.	[a] Rt (min)	Molecular Ion [M + H] (*m*/*z*)	[b] MS/MS (*m*/*z*)	Name of Compounds [c]
An1	2.3	627	303,465	Delphinidin 3-*O*-glucoside
An2	4.3	611	287,449	Cyanidin-3,5-di-*O*-glucoside
An3	4.8	449	287,213,137	Cyanidin 3-*O*-glucoside
An4	4.9	419	287,137,213	Cyanidin-3-*O*-arabinoside
An5	4.9	479	317,302,274	Petunidin-3-*O*-glucoside
An6	5.1	463	301,286,201	Peonidin-3-*O*-glucoside
An7	5.0	579	271,433	Pelargonidin-3-*O*-rutinoside

[a] Rt = retention time; [b] MS/MS = tandem mass spectrometry; [c] Compounds were numberer by their elution time.

Table 7. Phenolic compounds quantified in Mortiño berries as affected by sampling zone and stage of fruit development (mg 100 g^{-1} DM).

Factor	Hydroxycinnamic Acid					Flavonols				Anthocyanins							∑Total Polyphenols
	P1	P2	P3	P4	P5	P6	P7	P8	P9	An1	An2	An3	An4	An5	An6	An7	
ANOVA Test †																	
Zone	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Stage	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Zone × Stage	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Tukey's multiple range test ‡																	
Zone																	
Polylepis	1.56 c	1.56 c	291.68 c	140.89 c	69.73 c	61.40 c	75.54 c	35.08 b	24.34 c	0.07 a	0.04 a	6.48 b	12.30 b	0.00 b	0.35 b	0.03 a	721.14 c
Cubillín	247.37 a	95.05 b	595.05 b	343.98 a	221.44 a	165.64 b	224.88 b	149.97 a	367.79 b	0.00 b	0.001 c	10.71 a	18.69 a	0.91 a	0.67 a	0.006 c	2442.21 b
Culebrillas	192.67 b	142.15 a	2032.71 a	196.16 b	138.23 b	196.03 a	281.66 a	1.29 c	509.90 a	0.00 b	0.02 b	6.08 b	11.47 b	0.01 b	0.12 c	0.01 b	3708.58 a
Stage ¥																	
Stage 7	179.22 a	93.33 a	577.24 b	271.86 a	183.31 a	174.01 a	228.07 a	122.94 a	339.93 a	0.00 b	0.001 b	4.46 b	9.74 b	0.06 b	0.42 a	0.008 b	2184.64 a
Stage 8	115.18 b	65.85 b	1369.06 a	182.17 b	102.96 b	108.03 b	159.98 b	1.29 b	261.43 b	0.05 a	0.04 a	11.06 a	18.57 a	0.54 a	0.34 b	0.02 a	2396.65 a
Zone × Stage																	
Polylepis * Stage 7	1.56 d	1.56 d	581.80 b	280.22 bc	137.89 cd	105.71 c	124.17 c	68.88 b	47.40 c	0.00 b	0.00 c	5.55 c	10.44 c	0.00 c	0.70 b	0.02 b	1365.96 c
Polylepis * Stage 8	1.56 d	1.56 d	1.56 c	1.56 e	1.56 e	17.08 d	26.91 d	1.29 c	1.29 c	0.15 a	0.09 a	7.42 c	14.17 bc	0.00 c	0.00 e	0.04 a	76.32 d
Cubillín * Stage 7	302,36 a	110.06 b	667.27 b	363.20 a	235.87 a	172.4 b	226.14 b	298.64 a	370,36 b	0,00 b	0.00 c	6.77 c	12.97 bc	0.19 b	0.47 c	0.00 d	2766.40 b
Cubillín * Stage 8	192,39 bc	80.05 c	522.84 b	324.75 ab	207.01 ab	159.23 b	223.62 b	1.29 c	365.23 b	0.00 b	0.002 c	14.65 a	24.41 a	1.63 a	0.87 a	0.01 c	2118.03 b
Culebrillas * Stage 7	233,75 b	168.36 a	482.64 b	172.14 d	176.16 bc	244.28 a	333.90 a	1.29 c	602.03 a	0.00 b	0.004 c	1.06 d	5.81 d	0.00 c	0.08 de	0.00 d	2421.57 b
Culebrillas * Stage 8	151,59 c	115.95 b	3582.78 a	220.19 cd	100.31 d	147.78 bc	229.42 b	1.29 c	417.78 b	0.00 b	0.05 b	11.10 b	17.12 b	0.01 c	0.15 d	0.02 b	4995.60 a

† NS: not significant at $p < 0.05$; * and ***, significant at $p < 0.05$ and 0.001, respectively. ‡ Values (mean of 3 replications) followed by the same letter, within the same column, were not significantly different ($p < 0.05$), Tukey's least significant difference test. ¥ Stage 7: Fruit development; berries began to develop anthocyanins; Stage 8: Ripening or fruit maturity; 100% of the epicarp of cluster berries is purple.

The nine non-anthocyanin phenolic compounds were classified into two chemical families: (i) hydroxycinnamic acid (5 compounds), and (ii) flavonols (4 compounds). The content of the phenolic compounds non-anthocyanins was significantly affected by the "growing zone", "stage" and the interaction "growing zone x stage" factors (Table 7). Five hydroxycinnamic acids were detected, four caffeoyl acid derivates, and one coumaroylquinic acid. Baenas et al. [16] also identified in mortiño berries purchased at a local market in Machaci, Ecuador, the same four caffeoyl acid derivates, but at very low concentrations compared to our values; yet they could not identify the presence of coumaroylquinic acid. Furthermore, the highest content of hydroxycinnamic acids was noted for mortiño berries grown in Culebrillas (2701.92 mg 100 g^{-1} DM), followed by Cubillín (1502.89 mg 100 g^{-1} DM), while the lowest content was presented by mortiño fruits grown in Polylepis (505,42 mg 100 g^{-1} DM). From the hydroxycinnamic acids, the isomer of chlorogenic acid, 5-*O*-caffeoylquinic acid, was the most representative in mortiño berries. Our results agree with previous studies carried out by Vasco et al. [3] in *V. floribundum*, by Garzón et al. [4] in *V. meridionale*, by Prencipe et al. [49] in *Vaccinium* berries and by Baenas et al. [16] in *V. floribundum*. Moreover, Wojdyło et al. [50] reported that hydroxycinnamic acids such as 5-caffeoylquinic and caffeoylquinic acid are good sources of antioxidants in vitro that protect low-density lipoprotein (LDL) from oxidation and therefore, supposedly prevent various age-related diseases. Therefore, the consumption of mortiño berries would reduce the risk of cardiovascular disease since its antioxidants lower low-density lipoprotein (LDL) cholesterol levels.

The flavanol glycosides were the second group after the hydroxycinnamic acid derivatives that contributed to the final concentration of polyphenols in mortiño berries. Additionally, those fruits grown in Culebrillas presented the highest levels of flavonols, the quercetin 3-hexoside, quercetin 5-hexoside, and quercetin-3-*O*-rhamnoside were significantly high (Table 7). Other studies also reported these flavonols as the predominant ones in mortiño berries [3,16]. Garzón et al. [4] reported in *V. meridionale* that 93% of the total flavonoids are represented by quercetin derivatives. High content of flavonols may well reflect plant responses to biotic and abiotic stresses or just acclimation to environmental stressors such as heat, cold, UV radiation, drought, salinity, or an attack of herbivores or pathogens [51]. In this study, the highest content of flavonols and hydroxycinnamic acids was obtained for mortiño fruits at the early stages of fruit development (stage 7); the only exception was the 5-*O*-Caffeoylquinic acid, which showed a higher content in the late stage of development. According to Garzón et al. [4], a fairly common feature in the *Vaccinium* family is the presence of quercetin glycosides and hydroxycinnamic acids. Our results confirm that fruit maturity stage and altitude definitely influence the content of non-anthocyanin phenolic compounds; in such a way that above 4000 m of altitude there is a strong reduction in the content of these phenolic compounds. Jaakola and Hohtola [52] reported that flavonol accumulation in fruit skin, as a result of sunlight exposition, is well documented and is the most important environmental factor inducing flavonol biosynthesis, just like that; fruits with sun-exposed peel have higher levels of anthocyanins and flavonols than those grown in the shade. In the literature, there is a great variability regarding the non-anthocyanin phenolic compounds in mortiño berries and in other several species of the *Vaccinium* genus. This variability is due to several factors such as stage of maturity, agronomic factors, cultivars and varieties, geographic region, storage conditions, ripeness, and climate, among others [3,16,31].

Anthocyanins are coloring pigments that give a wide range of colors such as orange, red, purple, and blue in flowers, seeds, fruits, and vegetative tissues [53]. Blueberry and bilberry (*Vaccinium* spp.) are one of the richest sources of anthocyanins [3,16]. In this study, seven anthocyanins have been identified in mortiño berries: glycosides of cyanidin (peaks An_2, An_3, and An_4), delphinidin (peak An_1), petunidin (peak An_5), peonidin (peak An_6) and pelargonidin (peak An_7) (Table 6). Baenas et al. [16] and Esquivel-Alvarado et al. [31] reported the presence of six and five anthocyanins in mortiño berries, respectively, namely derivatives of delphinidin and cyanidin. Garzón et al. [4] and Vasco et al. [3] reported that Colombian bilberry and Andean blueberry contained only cyanidin and delphinidin glycosides. To our knowledge, the current study identifies the presence of petunidin, peonidin, and pelargonidin in *V. floribundum*. In addition, the anthocyanins contents were significantly affected by the "growing environment", "stage" and the interaction "growing environment x stage" factors (Table 7). Analyzing the environmental area factor, it was observed that the predominant anthocyanins were cyanidin-3-*O*-arabinoside (ranging from 18.69 mg 100 g^{-1} DM of Cubillín to 11.47 mg 100 g^{-1} DM of Culebrillas), followed by cyanidin 3-*O*-glucoside (ranging from 10.71 mg 100 g-1 DM of Cubillín to 6.08 mg 100 g^{-1} DM of Culebrillas). Likewise, higher contents of anthocyanins were shown when mortiño berries reached the late developmental stage (stage 8), being the cyanidin derivatives, followed by petunidin and peonidin, the main anthocyanins. The mortiño fruits showing the highest anthocyanin contents were those grown at an altitude of 3500 m and in a more advanced developmental stage (stage 8). Furthermore, our results indicate that the anthocyanin content slightly decreases above 3500 m of altitude.

It is fairly known that temperature plays a vital role in the synthesis of anthocyanins, and these are more prone to oxidation and relatively unstable. However, the mechanisms are not well understood [54]. Low temperature induces anthocyanin synthesis in various species [55]. However, the accumulation of anthocyanins in cold temperatures is light dependent; in the absence of light, low temperatures prevent anthocyanin biosynthesis. The regulation of cold induction of anthocyanins and the role of light are not well understood yet [52]. The higher the solar radiation at high altitudes, the greater the influence on the secondary metabolite profiles [52]. Li et al. [56] reported that warm weather was related to low levels of anthocyanins, and cool weather was associated with the rapid accumulation of anthocyanins in fruit skin. Maier and Hoecker [57] suggested that high light intensity stimulates anthocyanins production in most plants. In view of the results obtained in this research, well-designed long-term studies are necessary to better understand the plant–environment interaction regarding anthocyanin biosynthesis.

3.5. Principal Component Analysis (PCA)

PCA was used because it is one of the beneficial statistical tools for analyzing several samples and variables in order to establish their differences and similarities. Figure 2 shows that 64.19% of the total variance in the data are represented by PC1 and PC2. Of these two top principal components, PC1 described 42.29% of the total variation and PC2 explained 21.90% of the variation. It is important to note that the higher the distance between two parameters, the lower their correlation. Considering F1 as the dimension that explained the main differences among growing areas and fruit developmental stage Culebrillas-red and Culebrillas-green were positively linked with hydroxycinnamic acids (3-coumaroylquinic acid, and 5-caffeoylquinic acid), flavonols (quercetin derivatives), (antioxidant activity (ABTS), TPC, and organic acids (quinic and malic acids). Instead, Cubillín-red and Cubillín-green were positively linked with anthocyanins (cyanidin 3-*O*-glucoside, cyanidin-3-*O*-arabinoside, pelargonidin-3-*O*-rutinoside, and peonidin-3-*O*-glucoside), hydroxycinnamic acids (caffeoylshikimic acid), flavonols (quercetin-3-pentoside) and minerals (Mn and Fe).

On the other hand, whereas Polylepis-red was negatively linked with anthocyanins (cyanidin-3,5-di-*O*-glucoside, petunidin-3-*O*-glucoside, and delphinidin 3-*O*-glucoside), minerals (Cu) and sugar (fructose), Polylepis-green was positively linked with minerals (Ca, Na, Zn, K, and Mg), antioxidant activity (DPPH) and sugars (glucose).

The biplot (Figure 2) showed that Culebrillas-red and Cubillín-green were laid relatively close to each other along the *X*-axis (PC1). Polylepis-red had large negative scores on the PC2, and it was quite separated from the other locations across PC1. Polylepis-green had large positive scores on the PC2 axis, and it was opposed to Polylepis-red.

The results clearly indicated that the sampling zone and fruit developmental stage factors had a crucial effect on the chemical composition and polyphenol compounds of *Vaccinium floribundum*. Therefore, more research considering both the growing environmental factor and the stage of development of the fruit is a must to obtain mortiño berries with high contents of bioactive compounds, becoming an exceptional ingredient to be used in both the cosmetic and pharmacological industries, as well as in the agri-food one.

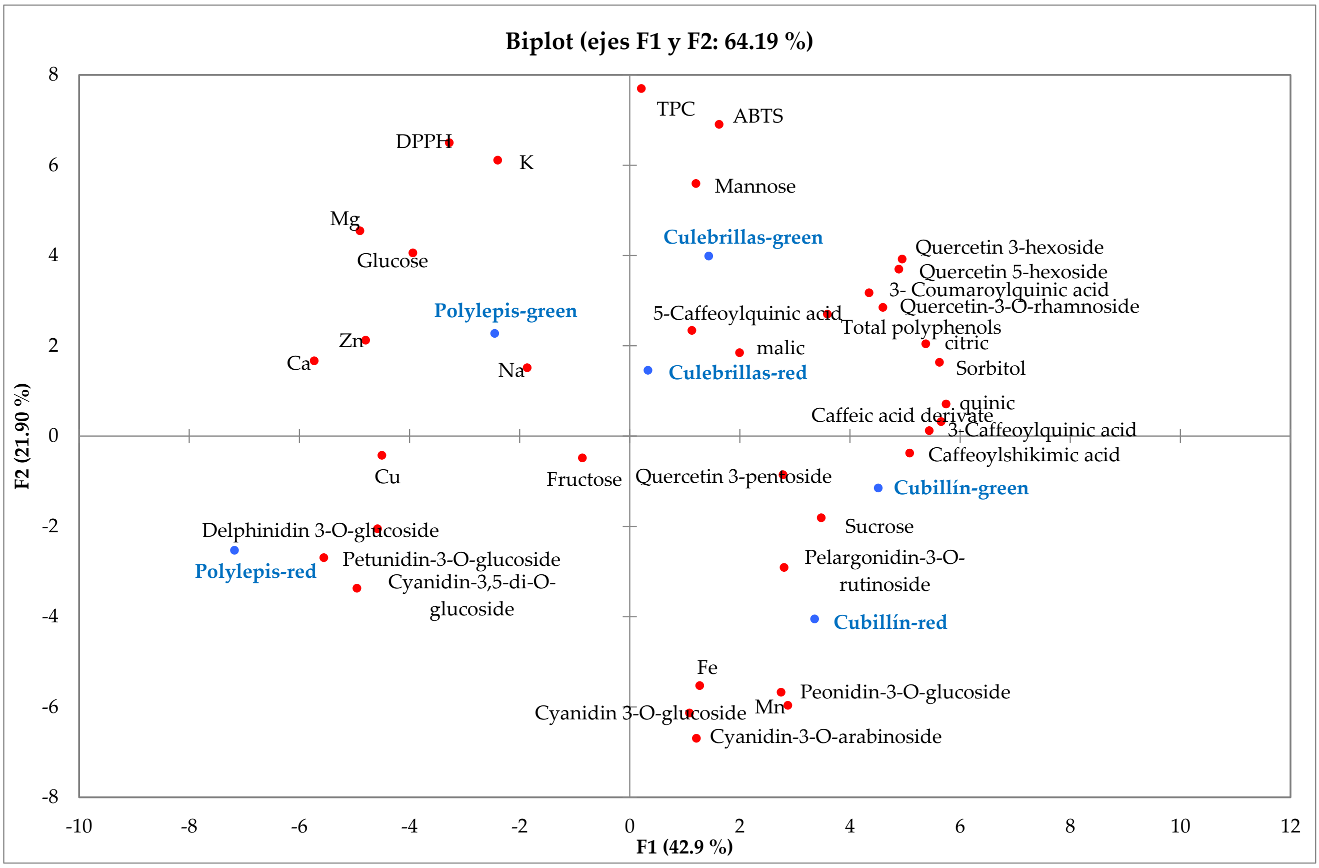

Figure 2. Biplot of principal components analysis (PCA) means showing the relationship among phytochemical parameters and effects of environmental zones (Polylepis, Cubillín and Culebrillas) and stage of development of the fruit of mortiño berries (stage 7 green and stage 8 red).

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4. Conclusions

The present study investigated the chemical composition and polyphenol compounds of *Vaccinium floribundum* produced in the volcano Chimborazo paramo, Ecuador. The study was carried out in three growing areas (Polylepis, Culebrillas, and Cubillín) located above 3500 m of altitude and with mortiño berries showing two different stages of fruit development. Despite the fact that the altitude at which this mountain fruit species is found is the main limitation for observation and monitoring, the research confirms that the mortiño berries produced in the Ecuadorian paramo area are a valuable source of polyphenols, rich in sugars and organic acids and can be classified as a good source of microelements, an excellent source of K, Ca and Fe. In addition, due to their low sodium levels, mortiño berries could be recommended for low-sodium diets. The main constituents of mortiño berries include hydroxycinnamic acids (5-*O*-caffeoylquinic acid), flavonols (quercetin derivatives), and anthocyanins. Three anthocyanins (petunidin, peonidin, and pelargonidin) were reported for the first time in mortiño berries, which have never been before identified and quantified in *V. floribundum*. Overall, our data indicate that altitude and stage of fruit development significantly affect mortiño berries quality. This research may successfully contribute to improving market sales for the mortiño and, at the same

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time, can provide sustainable economic opportunities for farmers. Finally, our contribution to improving knowledge about this wild fruit species will help improve the sustainability and preservation of this rich natural resource.

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5. RESUMEN DE RESULTADOS, DISCUSIÓN Y CONCLUSIONES

Esta sección incluye los principales resultados, discusión y conclusiones de los artículos publicados. Los resultados detallados se pueden consultar en las publicaciones incluidas en la sección anterior.

5.1 PUBLICACIÓN 1

The Mortiño (*Vaccinium floribundum* Kunth): a review of its suitability as a promissory crop in the Ecuadorian Paramo and its potential uses, environmental role, and health benefits. https://doi.org/10.1007/s00217-024-04546-4

El objetivo principal de esta revisión bibliográfica es dar una visión general de la importancia que el mortiño tiene para el páramo ecuatoriano. El principal factor limitante para el desarollo de un cultivo es la falta de conocimiento sobre su ecología, requerimientos edafo-climáticos, biología, propiedades funcionales de sus frutos, potenciales aplicaciones industriales, etc.

Tras el análisis de los 31 artículos seleccionados para la revisión, de acuerdo a los criterios marcados, los aspectos más destacables son que:

(i) en relación a la biología reproductiva, los esquejes son el método de propagación más viable para el mortiño, debido a su simplicidad y bajo costo en comparación con otros métodos de propagación;

(ii) tratamientos con ácido 3-acético y ácido indol-3-butírico pueden mejorar la viabilidad de los esquejes de mortiño;

(iii) para el desarrollo óptimo del mortiño las condiciones agroecológicas ideales requieren: temperaturas medias que oscilen entre los 8ºC y los 16ºC, la humedad relativa entre el 60% y el 80%, un rango de precipitación media anual entre los 800 a 2.000 mm y una altitud de entre los 3.200 a 3.800 m s.n.m.;

(iv) las bayas de mortiño presentan cantidades significativas de compuestos bioactivos, principalmente compuestos polifenólicos y antocianinas, lo que convierte a esta fruta en una excelente fuente de antioxidantes;

(v) el mortiño desempeña un papel ambiental y ecológico crucial en los ecosistemas de páramo, ya que, es una de las primeras especies en recuperarse después de la deforestación y de los incendios provocados por el hombre. Su resilencia se atribuye, en parte, a su sólida capacidad regenerativa, facilitada por la propagación desde las raíces y otras estructuras leñosas;

(vi) entre los posibles usos industriales el mortiño podría ser utilizado para la síntesis de nanopartículas, con la ventaja de eliminar reactivos dañinos, posicionándolo como una opción prometedora para materiales biocompatibles en futuras aplicaciones de ingeniería.

En conclusión, todas estas características y propiedades sugieren que el mortiño es una planta con un alto potencial para el desarrollo de alimentos funcionales, desarrollo de nuevos materiales, regeneración de suelos y del medio ambiente, en definitiva, es una especie que ayudará a mejorar la sostenibilidad del páramo del volcán Chimborazo en Ecuador.

5.2 PUBLICACIÓN 2

Reproductive phenology of *Vaccinium floribundum* Kunth (Ericaceae) and codification according to the BBCH scale based on evidence from the volcano Chimborazo paramo (Ecuador). https://doi.org/10.1016/j.scienta.2022.111207

El objetivo principal de este estudio fue describir las etapas fenológicas del desarollo reproductivo (floración y fructificación) de *Vaccinium floribundum* Kunth, observadas en condiciones naturales de páramo en los Andes, utilizando una adaptación de la escala extendida BBCH. Para garantizar la precisión de las observaciones en el desarrollo de la escala, se realizaron observaciones quincenales durante 18 meses, desde enero de 2017 hasta junio de 2018, en tres áreas (Mindala Loma, Polylepis y Mechahuasca) del páramo en el volcán Chimborazo, en el centro de Ecuador.

Resultados de los estados fenológicos

En el caso del mortiño de los 10 estados fenológicos recogidos en la escala BBCH, solo se aplicaron los estados principales correspondientes a la **fenología reproductiva**, comenzando por el *estado fenológico 5: desarrollo de la flor* y, finalizando en el *estado fenológico 8: maduración del fruto.*

Dentro del **estado fenológico 5: aparición de las inflorescencias**, se identificaron dos subestados:

(i) **51:** *Brotes de inflorescencia hinchados: brotes cerrados, escamas de color marrón claro visibles.*

Este subestado se observó durante todo el año en la zona de Mindala Loma y Polylepis. En la zona de Mechahuasca solo se observó en los meses de mayo a agosto.

(ii) **53:** *Brotación: puntas de sépalos verdes que encierran las flores visibles.*

Dentro del estado **fenológico 6: Floración**, se identificaron ocho subestados:

(i) **61:** *Inicio de la floración: alrededor del 10% de las flores se abren.*

Este subestado se observó en muy pocas plantas de mortiño y únicamente en las zonas de Mindala Loma y Polylepis.

(ii) **62:** *Alrededor del 20% de las flores se abren.*

Este subestado se observó solo en las zonas de Mindala Loma y Polylepis.

(iii) **63:** *Alrededor del 30% de las flores se abren.*

Este subestado se observó en muy pocas plantas de mortiño y únicamente en las zonas de Mindala Loma y Polylepis.

(iv) **64:** *Alrededor del 40% de las flores se abren.*

Este subestado se observó únicamente en la zona de Polylepis.

(v) **65:** *Plena floración: al menos el 50% de las flores abiertas, muchos estilos y estigmas visibles.*

Este subestado se observó únicamente en la zona de Polylepis.

(vi) **66:** *Plena floración: al menos el 60% de las flores abiertas, muchos estilos y estigmas visibles.*

Este subestado se observó únicamente en la zona de Polylepis.

(vii) **67:** *Plena floración: al menos el 70% de las flores abiertas, muchos estilos y estigmas visibles.*

Este subestado se observó únicamente en las zonas de Polylepis y Mechahuasca.

(vii) **69:** *Fin de la floración: ya han cuajado numerosos frutos.*

Este subestado se observó en la zona de Polylepis durante todo el período de estudio. En la zona de Mechahuasca se registró solo durante los meses de abril a octubre.

Dentro del **estado fenológico 7: Desarrollo del fruto,** se identificó un solo subestado:

(i) **70:** *Frutos inmaduros visibles.*

Este subestado se observó en las zonas de Mindala Loma y Polylepis durante todo el período de monitoreo. En la zona de Mechuhuasca se observó de forma esporádica en los meses de enero a marzo y de agosto a octubre.

Dentro del **estado fenológico 8**: *Maduración o madurez del fruto,* se identificaron cuatro subestados:

(i) **81:** *Inicio de maduración y cambio de color: la piel del fruto aparece de color naranja. Aproximadamente el 10% del color final.*

Este subestado se observó en las zonas de Mindala Loma y Polylepis durante todo el período de monitoreo. En la zona de Mechahuasca se observó de forma esporádica en los meses de enero a marzo y de septiembre a enero.

(ii) **85:** *Coloración avanzada: primeros frutos maduros de color negro.*

Este subestado se observó en la zona de Mindala Loma en los meses de febrero, junio, julio y diciembre. En la zona de Polylepis en los meses de noviembre y en la zona de Mechahuasca en los meses de enero y noviembre.

(iii) **87:** *Inicio del ablandamiento de los frutos.*

Este subestado se observó en las zonas de Mindala Loma y Polylepis durante todo el período de monitoreo.

(iv) **88:** *Inicio de la disminución de la consistencia de los frutos.*

Este subestado se observó en las zonas de Mindala Loma y Polylepis durante todo el período de monitoreo.

Cronología y sincronización de eventos fenológicos

La fenología reproductiva de las plantas de mortiño monitorizadas en la zona de Mindala Loma y Polylepis, estuvo relativamente sincronizada, mientras que las plantas de mortiño de la zona de Mechahuasca mostraron un patrón fenológico diferente. Esa diferencia se debió a que la zona de Mechahuasca se encuentra en un nicho ecológico especial rodeado por una enorme laguna.

El análisis estadístico realizado para detectar la influencia potencial de los parámetros climáticos en la frecuencia de aparición de cada estado fenológico puso de manifiesto que no hubo influencia significativa de ninguna variable climática sobre los patrones fenológicos reproductivos monitorizados.

De hecho, la ocurrencia y duración de las etapas de crecimiento fenológico reproductivo, monitoreadas durante 18 meses, no mostraron una relación clara con el patrón de precipitación. En nuestro estudio observamos frutos durante todo el período de monitoreo (18 meses), pero no necesariamente en el momento en que ocurrió la temporada de lluvias (febrero- mayo).

Las zonas de Mindala Loma y Polylepis mostraron prácticamente fenogramas similares, siendo los subestados: 51, 53, 70, 81, 87 y 88 los más frecuentes. Esto podría atribuirse a que ambas localidades se encuentran muy cerca dentro de la misma zona.

En el Neotrópico la escala BBCH ha sido aplicada a *Vaccinium meridionale* en los páramos colombianos por Medina et al. (2019) y a *Vaccinium floribundum* en los páramos ecuatorianos por Mendoza (2018) y Rivera (2019). Sin embargo, es difícil comparar los estudios de *V. floribundum* con el presente estudio debido a las condiciones mediambientales. Las plantas de mortiño monitorizadas en este estudio crecen a altitudes >4000 m.s.n.m., lo que podría imponer restricciones fisiológicas particulares impuestas por las condiciones climáticas extremas.

En conclusión, este es el primer estudio realizado sobre la fenología reproductiva del mortiño en el páramo del volcán Chimborazo de Ecuador de acuerdo a la escala BBCH.

En base a las observaciones fenológicas realizadas en las tres áreas monitoreadas: Mindala Loma, Polylepis y Mechahuasca, se concluye que en el páramo ecuatoriano no hubo una progresión cronológica ordenada de la fenología reproductiva del mortiño, probablemente debido a las condiciones climáticas adversas y continuamente cambiantes. Además, fue relativamente común observar diferentes etapas de desarrollo ocurriendo concomitantemente en el mismo individuo.

Se evidenció una pérdida significativa y aparentemente rápida de flores en las tres zonas durante el período de estudio.

5.3 PUBLICACIÓN 3

Chemical Composition and Polyphenol Compounds of *Vaccinium floribundum* Kunth (Ericaceae) from the Volcano Chimborazo Paramo (Ecuador).
https://doi.org/10.3390/horticulturae8100956

El objetivo de esta investigación fue evaluar el efecto que tienen la etapa de desarrollo de las bayas de mortiño (estados fenológicos 7 y 8) y la zona de cultivo (tres zonas de muestreo en el páramo del volcán Chimborazo: Culebrillas, Polylepis y Cubillín) tienen sobre los siguientes parámetros de calidad: (i) actividad antioxidante, (ii) composición mineral, (iii) perfil de azúcares y ácidos orgánicos, (iv) contenido fenólico total y, (v) perfil de antocianinas y perfil fenólico no antociánico.

Actividad antioxidante (AA.)

La AA de las bayas de mortiño se evaluaron mediante el método ABTS y DPPH. El análisis estadístico mostró diferencias significativas para los factores "zona de muestreo", "estado fenológico" e interacción "zona de muestreo x estado fenológico".

El mayor valor de AA determinado por el método ABTS se encontró en bayas de mortiño recolectadas en la zona de Culebrillas (65,74 mmol Trolox kg^{-1} MS), mientras que por el método DPPH el mayor valor se obtuvo en bayas de mortiño recolectadas en Polylepis (77,22 mmol Trolox kg^{-1} MS). Estos resultados fueron ligeramente superiores a los obtenidos por Vasco et al. (2009) en bayas de mortiño.

Con respecto al "estado fenológico", la AA disminuyó durante el desarrollo del fruto, oscilando entre 63,34 (estado 7) y 39,74 (estado 8) mmol Trolox kg^{-1} MS para el método ABTS, y entre 72,16 (estado 7) y 55,37 (estado 8) mmol Trolox kg^{-1} MS para el método DPPH. La misma tendencia fue reportada por Esquivel-Alvarado et al. (2020) para bayas de *V. consanguineum, V. poasanum* y *V. floribundum* recolectados entre los 3.000 y los 3.200 m s.n.m.

Con respecto a la “zona de muestreo”, los mayores valores de AA se obtuvieron en las bayas de mortiño recolectados en las zonas de Polylepis y Culebrillas (>4000 m s.n.m.).

Contenido en fenoles totales.

Respecto al contenido en fenoles totales también se observaron diferencias significativas entre la “zona de muestreo”, el estado fenológico” y la “interacción” entre ambas variables.

Con respecto a la “zona de muestreo”, los valores más altos se obtuvieron para las bayas de mortiño recolectadas en Polylepis y Culebrillas, que oscilaron desde los 2.231,45 (Polylepis) hasta los 2.651,57 (Culebrillas) mg GAE 100 g $^{-1}$ MS; mientras que los valores más bajos (0,67 veces inferiores) se obtuvieron en la localidad de Cubillín, a menor altitud que las anteriores.

Con respecto al “estado fenológico”, las bayas de mortiño que presentaron los niveles más altos en fenoles totales fueron las del estado 7 (2.559,12 mg GAE 100 g $^{-1}$ MS). Esquivel-Alvarado et al. (2020) obtuvieron una tendencia similar para *V. floribundum* recolectado a una altitud de 3.200 m s.n.m. Esta tendencia se puede atribuir a cambios como la hidrólisis de glucósidos, la oxidación de fenoles por polifenol oxidasas y la polimerización de fenoles libres (Remorini et al., 2008).

En nuestro estudio, tanto la actividad antioxidante como el contenido en fenoles totales estuvieron claramente influenciados por la altitud; así a mayor altitud, mayores fueron el contenido en fenoles totales y la AA presentados por las bayas de mortiño. Estos resultados demuestran que la altitud es un factor importante que afecta a la actividad antioxidante y al contenido total de polifenoles *en V. floribundum*.

Perfil de azúcares y ácidos orgánicos.

Los principales **ácidos orgánicos** encontrados en las bayas de mortiño fueron el ácido quínico, seguido del ácido cítrico y málico. Los ácidos quínico y cítrico predominaron sobre el ácido málico en las tres zonas de estudio. Estos resultados están en consonancia con los obtenidos por Mikulic-Petkovsek et al. (2012), quienes indicaron que el fruto de la familia Ericaceae, generalmente, contenía muy poco ácido málico. Las bayas de mortiño recogidas en Cubillín (a 3.500 m de altitud y a una temperatura media de 7ºC) mostraron el mayor contenido en ácidos orgánicos totales (287,88 g kg^{-1}), mientras que en Culebrillas y Polylepis (a más de 4000 m de altitud y a una temperatura media de 3,1ºC) mostraron menores contenidos totales en ácidos orgánicos de 250,76 g kg^{-1} y 172,67 g kg^{-1}, respectivamente. Los resultados obtenidos en este estudio sugieren que por encima de los 3.500 m de altitud el contenido de ácidos orgánicos disminuye fuertemente; desde 3.500 metros de altitud (Cubillín) a 4.076 m s.n.m. (Polylepis), el contenido total en ácidos orgánicos disminuyó hasta el 60%. Sin embargo, Wang et al. (2019) informaron que en *V. uliginosum*, el contenido en ácidos orgánicos no estaba relacionado con la altitud. Estas diferencias pueden deberse a una combinación de diferentes entornos de cultivo, por ejemplo, microclima, genética u otros factores ambientales.

Con respecto a la influencia del "estado fenológico" en el contenido en ácidos orgánicos los resultados obtenidos en este estudio demostraron que el ácido málico aumenta con la maduración del fruto, oscilando entre los 26,22 g kg-1 MS (estado 7) y los 32,02 g kg^{-1} MS (estado 8); mientras que tanto el ácido quínico como el cítrico disminuyeron. Ayaz et al. (2001) también indicaron en *V. artostaphylos* y *V. myrtillus* que el nivel de ácido málico aumenta gradualmente durante la maduración de los frutos.

En cuanto **al perfil de azúcares,** en las bayas de mortiño, se encontraron glucosa, fructosa, sacarosa, manosa y sorbitol. Los mayores valores de glucosa y fructosa se obtuvieron en las zonas de mayores altitudes Polylepis y Culebrillas (>4.000 m s.n.m.). Estos resultados están en consonancia con los reportados por Wang et al. (2019), quienes indicaron que a mayor altitud (>1200 m s.n.m. considerada gran altitud), mayor es el contenido de glucosa y fructosa en el arándano negro (*V. uliginosum*). Sin embargo, el mayor contenido de sorbitol (190,55 g kg^{-1} MS) fue obtenido en frutos de mortiño a bajas altitudes (Cubillín), y los menores contenidos se obtuvieron en los frutos recolectados de zonas de mayor altitud (>4.000 m s.n.m.).

Por otra parte, durante el desarrollo del fruto aumentaron los contenidos en fructosa y glucosa, mientras que disminuyeron los contenidos en sacarosa, manosa y sorbitol.

Nuestros resultados están en consonancia con los resultados obtenidos por Kalt y McDonal (1996) para el arándano enano (*V. angustifolium*), Correia et al. (2016) para el arándano azul (*V. corymbosum*) y Ayaz et al. (2001) para *V. arcostaphylos* (arándano caucásico) y *V. myrtillus* (mirtilo o arándano silvestre). Nuestros resultados sugieren que factores climáticos como la altitud y la temperatura juegan un papel importante en el contenido en azúcares de las bayas de mortiño.

Contenido mineral

El contenido en macronutrientes y micronutrientes se vió significativamente afectado por la “zona de muestreo” y la” interacción” entre la “zona de muestreo” y el estado fenológico”.

Sólo los macronutrientes potasio (K), sodio (Na) y magnesio (Mg) y el micronutriente hierro (Fe) se vieron significativamente afectados por el factor "estado fenológico”. Nuestros resultados pusieron de manifiesto que los contenidos de macronutrientes (K, Na y Mg) disminuyen con el desarrollo del fruto, mientras que el calcio (Ca) y el hierro (Fe) son los únicos macro y micronutrientes, respectivamente, que aumentan con el desarrollo del fruto.

En relación a la “zona de muestreo”, el mayor contenido en macronutrientes (K > Ca > Mg > Na) se encontró en los frutos de mortiño cultivados en Polylepis; mientras que el mayor contenido en micronutrientes (Fe, Mn) se observó en los frutos recolectados en Cubillín.

Asimismo, se observó que las bayas inmaduras de mortiño (estado 7) a elevadas altitudes >4.000 m s.n.m. presentaban un mayor contenido en potasio (10,13 y 8,27 g kg^{-1}, DM para Polylepis y Culebrillas, respectivamente).

No existen estudios previos que proporcionen una completa información sobre el contenido mineral de los frutos de mortiño, ni cómo estos contenidos, pueden verse influenciados por factores como: la altitud, la etapa de desarrollo del fruto y la temperatura. Nuestros resultados indicaron que el contenido mineral de los frutos de mortiño está claramente influenciado por las condiciones de cultivo, estado de desarrollo del fruto y la altitud.

La composición mineral que muestran los frutos de mortiño, en las zonas del páramo del volcán Chimborazo, indica que estas bayas son una excelente fuente de K, Ca y Fe. Además, debido a sus bajos niveles de sodio, los frutos de mortiño podrían recomendarse para dietas bajas en sodio.

Identificación y cuantificación de compuestos fenólicos No antocianinas y antocianinas

Un total de dieciséis compuestos fenólicos, nueve no antocianinas y siete antocianinas se han identificado en las bayas de mortiño.

Los nueve **compuestos fenólicos no antocianinas** identificados se clasificaron en dos familias químicas: (i) ácido hidroxicinámico (5 compuestos) y (ii) flavonoles (4 compuestos).

De los cinco ácidos hidroxicinámicos detectados cuatro fueron derivados del ácido cafeico y un ácido cumaroilquínico. Baenas et al. (2020) también identificaron en bayas de mortiño, compradas en un mercado local en Machachi (Ecuador), los mismos cuatro derivados del ácido cafeico, pero a concentraciones inferiores a las encontradas en nuestro estudio.

El mayor contenido en ácidos hidroxicinámicos se obtuvo en las bayas del mortiño de Culebrillas (2.701,92 mg 100 g^{-1} MS), seguidas del de Cubillín (1.502,89 mg 100 g^{-1} MS), mientras que el menor contenido lo presentaron los frutos de mortiño de Polylepis (505,42 mg 100 g^{-1} DM). De los ácidos hidroxicinámicos, el isómero del ácido clorogénico, el ácido 5-*O*-cafeoilquínico, fue el más representativo en las bayas de mortiño. Nuestros resultados coinciden con estudios previos realizados por Vasco et al. (2009) en *V. floribundum*, Garzón et al. (2010), en *V. meridionale* (arándano agraz), Prencipe et al. (2014), en bayas de *Vaccinium* y por Baenas et al. (2020) en *V. floribundum*.

Los glucósidos de flavanol fueron el segundo grupo en importancia tras los derivados del ácido hidroxicinámico, que contribuyó a la concentración final de polifenoles en las bayas de mortiño. Los frutos de Culebrillas presentaron los mayores niveles de flavonoles, la quercetina 3-hexósido, quercetina 5-hexósido y quercetina-3-*O*-ramnósido. El mayor contenido de flavonoles y ácidos hidroxicinámicos se obtuvo para frutos de mortiño en las primeras etapas de desarrollo del mismo (estado 7); la única excepción fue el ácido 5-*O*-cafeoilquínico, cuyo contenido fue mayor en la última etapa de desarrollo del fruto (estado 8). Nuestros resultados confirman que el estado de madurez del fruto y la altitud influyen en el contenido de compuestos fenólicos no antociánicos, de tal manera que a altitudes >4.000 m s.n.m. se produce una fuerte reducción en el contenido de estos compuestos fenólicos.

En la literatura existe una gran variabilidad respecto a los compuestos fenólicos no antocianos en bayas de mortiño y en otras especies del género *Vaccinium*. Esta variabilidad se debe a varios factores tales como: el estado de madurez, factores agronómicos, variedades, región geográfica, condiciones de almacenamiento y clima, entre otros (Vasco et al., 2009; Baenas et al.,2020; Esquivel-Alvarado et al., 2020).

En relación al **contenido en antocianinas,** se han identificado siete antocianinas en las bayas de mortiño: tres glucósidos de cianidina, delfinidina, petunidina, peonidina y pelargonidina. Hasta donde sabemos, este es el primer estudio que identificó la presencia de petunidina, peonidina y pelargonidina en *V. floribundum*.

Los contenidos de antocianinas se vieron significativamente afectados por los factores "zona de muestreo", "estado fenológico" y la interacción entre ambas variables.

Analizando el factor de "zona de muestreo", se observó que las antocianinas predominantes fueron la cianidina-3-*O*-arabinosido (varió de los 18,69 mg por 100 g^{-1} de Cubillín a los 11, 47 mg por 100 g^{-1} de Culebrillas), seguida por la cianidina-3-*O*-glucósido (varió de los 10,71 mg por 100 g^{-1} de Cubillín a los 6,08 mg por 100 g^{-1} de Culebrillas).

Teniendo en cuenta el "estado fenológico" se encontraron mayores contenidos de antocianinas cuando las bayas de mortiño alcanzaron el estado de desarrollo 8, siendo los derivados de cianidina, seguidos por petunidina y peonidina, las principales antocianinas.

Analizando los resultados de la “interacción” de ambas variables se observó que los frutos de mortiño que mostraron los mayores contenidos de antocianinas fueron aquellos cultivados a una altitud de 3.500 m s.n.m. y en la etapa de desarrollo 8. Los resultados obtenidos sugieren que el contenido en antocianinas disminuye ligeramente por encima de los 3.500 m de altitud.

Análisis de Componentes Principales (ACP).

Los resultados del ACP pusieron de manifiesto que la “zona de muestreo” y el “estado fenológico” influyeron en la composición química y en el perfil de compuestos polifenólicos de las bayas de mortiño.

En conclusión, a pesar de que la altitud a la que se encuentra esta especie frutal de montaña es la principal limitación para la observación y el monitoreo, los resultados obtenidos confirman que las bayas de mortiño producidas en el área del páramo ecuatoriano son una valiosa fuente de polifenoles, ricas en azúcares y ácidos orgánicos, y pueden clasificarse como una buena fuente de microelementos y una excelente fuente de K, Ca y Fe. Además, debido a sus bajos niveles de sodio, las bayas de mortiño podrían recomendarse para dietas bajas en sodio.

Los principales polifenoles en las bayas de mortiño son: ácidos hidroxicinámicos, flavonoles y antocianinas. Tres antocianinas (petunidina, peonidina y pelargonidina) se identificaron, por primera vez, en las bayas de mortiño.

En general, nuestros resultados indican que la altitud y la etapa de desarrollo del fruto afectan significativamente a la calidad de las bayas de mortiño.

6. CONCLUSIONES

CONCLUSIONES GENERALES

- En el páramo ecuatoriano la fenología reproductiva del mortiño no presenta una progresión cronológica ordenada debido a las condiciones climáticas adversas y continuamente cambiantes

- En las plantas de mortiño es común observar diferentes etapas de desarrollo ocurriendo concomitantemente en el mismo individuo.

- La elevada altitud a la que se desarrolla el mortiño hace que se produzca una pérdida rápida y significativa de flores, lo que conlleva una menor producción por planta.

- Las bayas de mortiño producidas en el área del páramo ecuatoriano son una valiosa fuente de polifenoles, ricas en azúcares y ácidos orgánicos, y pueden clasificarse como una buena fuente de microelementos y una excelente fuente de K, Ca y Fe.

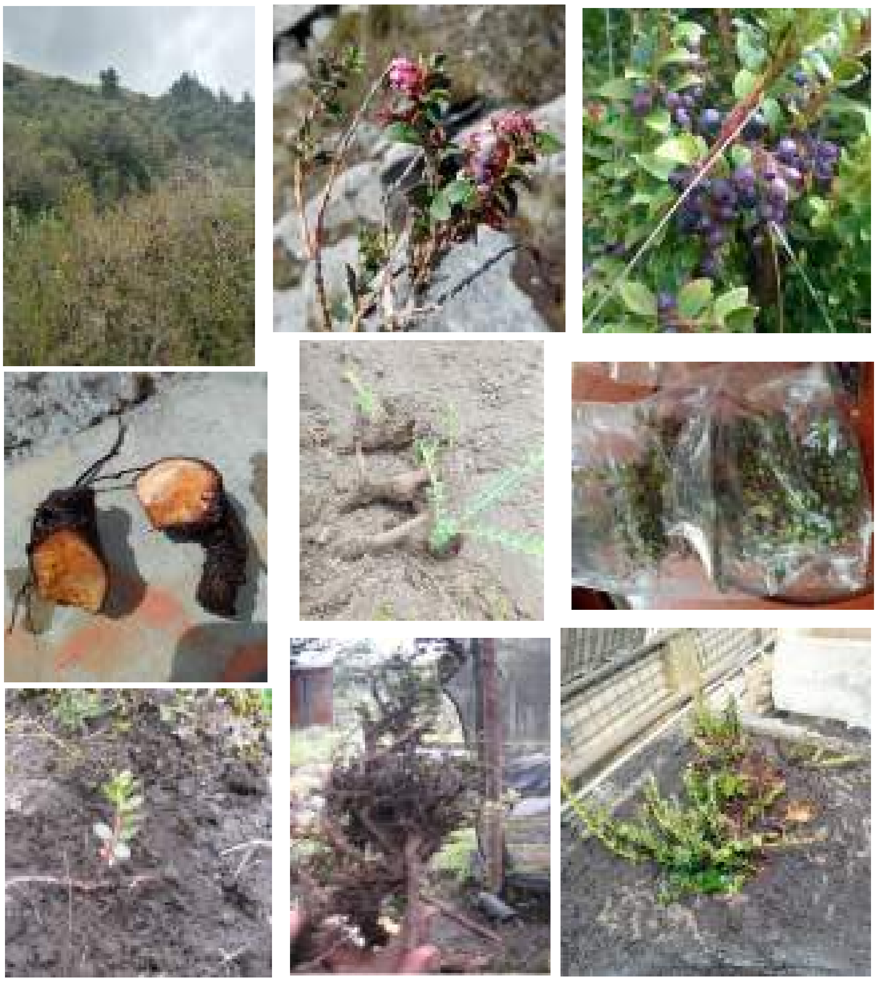
- Debido a sus bajos niveles de sodio, las bayas de mortiño podrían recomendarse para dietas bajas en sodio.

- Los principales compuestos polifenoles en las bayas de mortiño son los ácidos hidroxicinámicos, flavonoles y antocianinas.

- Tres antocianinas: petunidina, peonidina y pelargonidina se identificaron y cuantificaron, por primera vez, en las bayas de mortiño.

- La altitud y la etapa de desarrollo del fruto afectan significativamente a la calidad de las bayas de mortiño.

7. INVESTIGACIONES FUTURAS



INVESTIGACIONES FUTURAS

En base a los resultados obtenidos durante el desarrollo de esta Tesis Doctoral, surgen nuevos escenarios que pueden ser estudiados con el fin de profundizar más en el conocimiento del mortiño, lo que indudablemente ayudará a mejorar la sostenibilidad y preservación de este rico recurso natural y, ofrecerá nuevas oportunidades económicas y sostenibles para los agricultores del páramo del volcán Chimborazo en Ecuador.

Estos son algunos escenarios que pueden ser de especial interés:

- Realizar estudios de adaptación de cultivares a diferentes condiciones ambientales.

- Estudiar métodos de propagación utilizando semillas y rizomas de mortiño que podrían aumentar la disponibilidad de especies tropicales prometedoras, generarando valiosa información para la conservación y protección del ecosistema del páramo del volcán Chimborazo en Ecuador.

- Desarrollar colecciones de germoplasma para la programación de prácticas agronómicas y establecimiento de programas de mejora comercial del mortiño.

- Realizar campañas de concienciación para que se conserven las poblaciones naturales de mortiño como patrimonio del páramo ecuatoriano. La fragmentación continua que experimentan las poblaciones de mortiño debido a procesos antropogénicos como la deforestación, la reconversión de tierras productivas y la sobreexplotación, ponen en peligro la superviviencia de la especie.

- Profundizar más en el conocimiento de las propiedades funcionales de las bayas de mortiño, lo que contribuirá no solo a mejorar las ventas en el mercado del mortiño, sino a desarrollar nuevos productos y usos de las mismas.

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