

A historical and ethno-botanical overview of *Ilex guayusa* Loes. (Aquifoliaceae) with notes on its distribution.

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Abstract

Guayusa (*Ilex guayusa*, Loes.) is a holly found in the Upper Amazon basin of Colombia, Ecuador and Peru. Indigenous and mestizo societies throughout this region traditionally consume an infusion of guayusa brewed from its leaves. Guayusa has been found to have high concentrations of caffeine and other xanthines, plus many other compounds that are considered of medicinal value. Because of this, guayusa is rapidly emerging as an economically important species in the region. Yet, very little has been published since the formal description of guayusa as a species more than a century ago. Here we report detailed ethnographic accounts on how guayusa is used by different societies across its range, summarize archaeological and historical evidence of its use, present a map of its current distribution based on recent field surveys, and provide data on seed morphology. The evidence summarized here suggests that guayusa should be treated as a domesticated species rather than a wild plant. We hypothesize that humans are the primary drivers of current guayusa distribution and highlight the urgent need for studies to better understand the origin and domestication process of this important species.

Introduction

Guayusa (*Ilex guayusa*, Loes.; pronounced ‘why-you-sah’) is a South American tree belonging to the only extant genus of the family Aquifoliaceae (Judd et al. 1999). The cosmopolitan genus *Ilex* contains 600 species, of which c.300 species can be found in the Neotropics (Loizeau and Barreira 2007). In Ecuador thirty-two species are recognized (Loizeau and Barreira 1999). The high diversity currently recognized for this genus could be due to the difficulty of discriminating *Ilex* species based on their morphology. Members of the *Ilex* genus exhibit low inter-specific floral, palynological, and anatomical variation as well as a tendency for hybridization (Brizicky 1964; Manen 2004). Loizeau et al. (2005) hypothesize that speciation in *Ilex* occurred mainly through vicariant events due to drastic reductions in temperature and humidity during the Quaternary, as well as major volcanic and tectonic events, such as the Andes uplift. Once the climatic conditions improved, some of the previously isolated entities resumed out-crossing, resulting in the taxonomic puzzle we see today. Incongruence between phylogenies inferred from plastid and nuclear DNA seem to support this view (Manen et al. 2010).

Guayusa is distributed on the western Amazon basin from Colombia to Peru. Available literature and museum collections have registered guayusa from 200 to 2600 m.a.s.l. (Rios et al. 2007). The core of this wide distribution range is the Napo River basin in Amazonian Ecuador, spreading towards southern Colombia and northern-central Peru. Nearly all guayusa records have been found to occur in current or abandoned cultivation sites. Specimens of guayusa have also been recorded in Sumaco-Napo Galeras National Park and Jatun Sacha Reserve, both located in Napo province in Ecuador, however it is likely that those areas were subjected to human influence prior to their designation as protected areas.

Although the species was formally described in 1901 (Loesener, 1901), very little has been published since. Guayusa flowers were described nearly 80 years after its tentative designation as a species (Shemluck 1979), highlighting a notorious scarcity of flowering material and raising doubts about its taxonomic status. A typical guayusa individual can grow to an average of 10 m height and presents a multitude of stems originating from the base that together complete a diameter of 50-80 cm at breast height (Figure 1). When mature and if undisturbed, individuals of guayusa can reach a height of approximately 25 m and a stem diameter of 23-50 cm at breast height (Figure 2). Although mature trees of guayusa are a rare sighting, there is anecdotal evidence of ancient guayusa plantations both in Colombia and Ecuador (Schultes 1979).

Guayusa is a dioecious species, presenting pistillate and staminate flowers. The inconspicuous flowers of guayusa are small, axillary and forming thyrus. The corolla is white, glabrous and is composed of four petals. The guayusa fruit is a drupe of 6-7 mm in diameter, green when immature and dark red when ripe (Loizeau and Barreira 2007). Guayusa fruit morphology suggests suitability for bird dispersal, and flower characteristics indicate pollination by hymenopterans or possibly wind, both of which are consistent with observations of other *Ilex* species (Judd et al., 1999; Zomlefer 1994). Nonetheless, these speculations are yet to be confirmed. Despite the presence of seeds, guayusa is only known to reproduce asexually by human planting of the leafless hardwood stem cuttings extracted from the base of a stock plant.

Like several other species within the genus, guayusa leaves have been found to contain alkaloids (caffeine and theobromine), and other compounds such as phenols, tannins, reductive sugars, steroids, terpenes, flavonoids, and quinones (Radice and Vidari 2005). The rich phytochemical arsenal of guayusa is currently generating interest in the global market, adding to the list of economically important species of this genus such as *Ilex paraguariensis* and *Ilex kudingcha* (Hao 2013). Notably long before modern recognition, the perceived and actual health benefits of guayusa were greatly appreciated by different human societies along its distribution range. In Ecuador, there is evidence of its use among at least six Amazonian ethnic groups: Kichwa, Cofán, Secoya, Záparo, Shuar and Achuar (Kvist & Holm-Nielsen 1987; de la Torre et al. 2008), one in the Andean region: highland Kichwa; and one on the coast: Tsa' chi (de la Torre et al. 2008); as well as mestizos¹ throughout the country. Guayusa is also known to be used by the Inga and Kamsá in Colombia, as well as the Awajún in Peru.

In this paper we offer detailed ethnographic accounts of the use of this plant by various human societies across its range. We present a map of its known distribution in South America, enumerate synonymies of the vernacular name, and present data on seed morphology. Finally, the role of humans in the domestication and distribution of guayusa is discussed.

¹ Individuals of the dominant ethnic group in Latin America who self-identify as having mixed Spanish and indigenous heritage.

Materials and Methods

Specimen Collection

A total of 161 samples of guayusa were collected in Ecuador, and forty samples of *Ilex guayusa* were collected in Peru between August 2012 and October 2013. On each visit we asked local residents to inform us of the presence of *Ilex guayusa* trees. Once an individual was positively identified, consent from the plant owner was requested to collect 20 mature and immature leaves and 5 to 20 leafless hardwood or semi-hardwood stem cuttings from each stock plant. The collected plant material was stored in polythene bags and clearly labeled. Whenever possible, the cuttings were delivered to the Fundación Runa's nursery within 24 hours of collection. Otherwise the cuttings along with the leaf samples were kept in a cooler fitted with ice and under the shade for the duration of the trip. Upon arrival to Fundación Runa's headquarters in Archidona, Napo, leaves were stored in a fridge at 4° C until they were submitted to the Plant Biotechnology and Analytical Chemistry laboratories at the Universidad San Francisco, in Quito. Samples from Peru were taken in the same method and kept at the offices of Asociación Civil Ríos Nete in Tarapoto, Peru.

The geographic coordinates where each specimen was collected were recorded in decimal degrees, with a WGS84 datum using a Garmin 62s GPS unit. We requested that the owner of the plant estimate the tree's age and took notes on the cultivation conditions (e.g. irradiation conditions).

Geographic coordinates were used to create a map of the probable natural range of *I. guayusa* using ArcMap software. The WWF ecoregions dataset was used to overlay with the geographic coordinates for the samples collected. The delineation of WWF ecozones is informed by a variety of biophysical factors, allowing us to infer a map indicating the environmental characteristics of the regions in which *I. guayusa* has been found.

Field Interviews

Interviews were conducted in Napo, Ecuador (June and July, 2010, and October 2011-January 2012), Bogotá, Pasto, and Valle de Sibundoy, Colombia (August 2013), and northern Peru (September-October of 2013). Twenty-three interviews were conducted with indigenous Kichwa farmers in the communities of Rukullakta, Yawari, Awayaku, San José, Mondayaku, Lushianta, Alta Shicama, Nueva Esperanza, and Kindi Urku. In Colombia, interviews were conducted with two vendors at the market in Pasto, capital of Nariño Department, owners and shopkeepers at four natural medicine and handicraft stores in the Valle de Sibundoy, one conservation worker in the town of Colón, and one indigenous Inga healer in the town of Santiago. In Peru, interviews were conducted with 40 landowners who have guayusa on their properties in the departments of Amazonas, Cajamarca, and Piura.

Literature Review

A literature review of scholarship on the archaeology, distribution and history of guayusa was conducted. This consisted of examination of primary reports of archaeological discoveries of guayusa, as well as primary and secondary documentation of the ethnobotany of guayusa.

Additional records of guayusa distribution were accessed at the Ecuadorian National Herbarium (QCNE) and the TROPICOS database of the Missouri Botanical Gardens (MO).

Supporting literature on the ethnobotany of guayusa was retrieved from the libraries of the Catholic University Herbarium (QCA) and the Central University ‘Alfredo Paredes’ Herbarium (QAP) in Quito.

Seed morphology

A batch of guayusa fruit was delivered to Runa’s nursery on September 2013. Drupes were still green and were left to dry in the shade for approximately one week. Drupes were weighed to the nearest gram. Subsequently, seeds from each drupe were carefully extracted using soft pressure from a wood roller. The number of seeds per drupe was counted. The length and width of each seed was measured to the nearest mm using a caliper.

Results

Guayusa distribution

In Ecuador, guayusa was found in the provinces of Sucumbios, Orellana, Napo, Pastaza, Morona-Santiago and Zamora Chinchipe. Guayusa was also found as a cultivar in Vilcabamba valley in Loja Province (Figure 3). A revision of museum specimens and available literature revealed that guayusa is also present in the provinces of Pichincha (Ríos 1991) and Tungurahua (Tropicos.org 2013). In Colombia, guayusa was found in the departments of Nariño and Putumayo. In the northern Amazonian region of Peru, guayusa was found in the departments of Amazonas, Cajamarca, and Piura. None of the specimens exhibited flowers or fruits during the collection period.

In Ecuador, three ethnic groups have different vernacular names to refer to *Ilex guayusa* (Table 1). The usage of the vernacular name may also refer to different species with similar usages, of which two are within the genus *Ilex* (Table 2). Some Amazonian Kichwa groups use the vernacular name *waysa* in combination with a series of other terms (Table 3). It is unclear whether they use these terms to refer to varieties of *Ilex guayusa* or to species with similar properties. In Peru, guayusa is also a common name given to a variety of medicinal plants in the Piperaceae and Gesneriaceae families, most notably *Piper callosum*. The Shipibo of the Ucayali River make a tea-like infusion of dried and ground leaves of *Piper callosum*, which is also sold at the markets of Iquitos.

Archaeology and history of guayusa

Archaeological data and historical records suggest guayusa has been present in the greater Andes-Amazon region since at least 500 C.E. In 1970, archaeologist Henry Wassén found a collection housed at the Bolivian National Archaeological Museum that contained bundled guayusa leaves dated to about 500 A.D. The items came from a multi-family tomb in a town called Niño Korin in Bautista Saavedra province and were thought to belong to a ‘medicine man’ of the Callawayá (Tiahuanacoid) society (Wassén 1972).

Guayusa also appears in numerous reports throughout the colonial era. Father Juan Lorenzo Lucero noted in 1682 that Jivaroan groups consumed *Banisteriopsis caapi* (a hallucinogenic vine known by the vernacular names *yagé* and *ayawaska*), ‘guañusa’ and tobacco, all ‘invented by the devil,’ in infusions (Schultes 1979). Despite this missionary’s association of guayusa with what he understood as dangerous consciousness-altering substances such as

yagé, the Jesuits acknowledged guayusa's medicinal properties and themselves used the species as a medicine in the Río Marañón region of Peru (Patiño 1968; Schultes 1979). For example, in the 18th century, Father José Berrutieta, the head priest at Santa Rosa mission in Colombia, noted multiple beneficial health qualities of guayusa drink, including its use as a remedy for venereal diseases, 'cleansing the blood,' improving digestion and appetite, and strengthening the body. He also observed that women drank guayusa with honey to increase fertility (Schultes 1979).

The Jesuits even transported guayusa leaves from their missions and sold them as medicine in Quito, at a price of five leaves for half a *real*, usually marketing them as a cure for sexually-transmitted diseases (Schultes 1979). Interestingly, guayusa use appears to have declined significantly after the Jesuits were expelled in 1766 (Patiño 1968)

British botanist-explorer Richard Spruce also makes extensive reference to guayusa in his writings. Spruce found guayusa among indigenous peoples near the 'ancient site' of Antombós, near what is now the town of Baños, Ecuador, in 1857 (Schultes 1979). During his trip, Spruce used guayusa as a substitute for coffee (Schultes 1979). Spruce also explained the common practice of daily cleansing with guayusa during the morning hours. The Ecuadorian geographer Miguel Villavicencio even described people using feathers to provoke vomiting after drinking large amounts of guayusa (1858:373-4). Spruce also suggested the possibility of marketing guayusa in Europe (Schultes 1979).

In addition to acknowledging the medicinal and commercial value of guayusa, historical records mention various aspects of guayusa's cultivation and storage. Father Serra planted guayusa using cuttings, just as indigenous people do today (Schultes 1979). Father Berrutieta stored guayusa leaves as bundles tied together by strings, a practice still common in Ecuador today (Schultes 1979). Spruce also noted that guayusa trees seemed to be cultivated near settlements, and 'small clumps of it in the forest on the ascent of the Cordillera indicate deserted Indian sites' (quoted in Schultes 1979).

Ethnographic uses of guayusa²

Ecuador

Amazonian Kichwa³

The Amazonian Kichwa people grow guayusa in secondary forests, swiddens, and homegardens. A recent study of plant use among Kichwa from the Sumaco region found guayusa to be the most commonly mentioned plant species due to its ritual uses and medicinal properties (Innerhofer and Bernhardt 2011). Most Kichwa people have at least a few *ruku yuraguna*, or old trees, near their homes for easy access to prepare daily infusions, which families drink together during the early morning hours. People sometimes take cuttings from their *ruku yuraguna* and plant them in their *chakras* (family gardens used to grow manioc, plantains, fruit trees, hardwood trees, medicinal plants, and many other species).

Kichwa people harvest guayusa leaves in the morning or evening and place them in pots of boiling water, which they cook over wood coals, sometimes for multiple hours. Generally, it

² A brief summary of guayusa uses by different ethnic groups can be found on Table 4.

³ A more extensive discussion of the role of guayusa in Kichwa culture is available in Jarrett, Shiguango, and Salazar 2013.

is considered the responsibility of women, especially daughters-in-law, to wake up early to heat the guayusa tea and serve gourds full of the drink to all family members and any visitors. Among Kichwa people, guayusa drinking plays a central role in daily sociality, helping to promote forms of ‘conviviality’ (*convivencia* in Spanish), such as informality, high affectivity, and close relationships among kin, that are highly valued among many Amazonian peoples (Overing and Passes 2000). While drinking guayusa, some Kichwa adults will weave fishing nets and traps and shoulder bags, play music (hide drums, bamboo flutes, hollowed turtle shells, and mouth bows), and tell stories. Some elder men and women will also interpret dreams, and some Kichwa people say guayusa helps them to dream. During guayusa time, elders also give advice to young people and carry out ‘advising rituals,’ or traditional punishments, such as putting capsicum pepper juice in children’s eyes and shaking a nettle branch over their bodies. These rituals are meant to reorient young people’s lives and strengthen their bodies.

During festival times, Kichwa people often organize guayusa-themed events. For example, the last evening dance of a community *fiesta* is often referred to as the “Gran Guayusazo Bailable” (Great Danceable Guayusa Fest). For many *fiestas*, the popular beauty queen contests include an award for the second- or third-place contender, who is often given the title of “Guayusa Warmi” (Guayusa Woman). Finally, during one of the last mornings of *fiestas*, the newly elected beauty queen walks with a group of friends, family, and local political authorities, from house to house serving guayusa tea to community members. Often the beauty queen’s group is accompanied by Kichwa musicians, whose songs announce the group’s arrival before sunrise.

Kichwa people recognize a variety of useful qualities of guayusa leaves. They use the high caffeine content to wake up during the early morning hours and to obtain the energy needed for work in the forest, gardens, or in town throughout the day. Some refer to the plant as the ‘Night Watchman’ as it helps hunters to stay awake while they wait for game. The Kichwa use guayusa as a stomach tonic, diuretic, and flu remedy (usually with some combination of ginger, lime juice, *chuchuwasu*,⁴ and sugar cane liquor); to calm body aches; to increase fertility and libido; to cleanse the mouth; and to avoid insect and snakebites (many Kichwa people will spit small amounts of the tea on their arms and legs as a ‘repellent’). Many Kichwa also use guayusa to rinse their mouths and wipe guayusa water on their arms, legs and face, which some suggest helps to keep skin from aging.

Some Kichwa people also bathe their children in warm guayusa water and prepare vapor baths using guayusa. Finally, some Kichwa people wash their dogs’ faces with guayusa tea, which they believe helps dogs to dream and become good hunters (Kohn 2007).

Shuar and Achuar

Guayusa is found in the gardens of the Shuar and Achuar (formerly known as Jívaros) in a very similar fashion to the Kichwas, as a planted tree that is consumed in an informal morning setting for its medicinal/energetic properties (Descola 1992:127-8).

The Achuar drink guayusa daily in the early morning hours similar to the Kichwa, but the Achuar vomit guayusa after their daily intake. According to Descola, for the Achuar, ‘it is unseemly for a man to start the day with a full stomach and the *wayus* helps him to cleanse

⁴ A thick, dark red tonic made from the bark of the *Maytenus krukovii* tree.

his bowels' (1992:127). The emetic effect of guayusa is learned and does not appear to be due to emetic compounds found in guayusa itself (Lewis et al. 1991). When an Achuar couple is married, they plant a guayusa tree, and from there they build their house (Jaime Vargas, personal interview, April 2013). For the Shuar, drinking guayusa is not a daily affair. Guayusa is seen as a medicinal plant that has healing and particularly cleansing properties. As a Shuar woman said in an interview, "Yes, guayusa is a good plant. But we have many other plants as well" (Nartichu Catani, personal interview, September 2013).

Sociologist and religious scholar Rafael Karsten explains that guayusa plays an important role in Jivaroan rituals. For example, during the 'Jivaro Tobacco Ceremony of the Women,' women are required to cleanse their mouths by drinking and spitting out guayusa tea, and during the 'Victory Festival and the Tsantsa Feast' the warrior and his wife and daughter must wash their mouths out before eating the ceremonial meal (cited in Schultes 1972).

Mestizos

After Jesuits were expelled from the country in 1765, mestizo and white populations of highland Ecuador continued using guayusa infusions as an additive to spirits. This usage became widespread, especially during official and religious festivals throughout the late 20th century. In present times, guayusa mixtures with alcohol have almost disappeared from Quito, but they continue to be served in provinces of highland Ecuador such as Loja and Chimborazo, as well as in the Amazonian provinces. Guayusa leaves are sold in the form of tight necklaces in markets in the main Andean and Amazonian cities, or by vendors stretched along the main highways that lead to the Amazon. The infusion is said to increase fertility, and it is given to pregnant women to increase their energy.

In Amazonian provinces mestizo immigrants, also known as *colonos*, have the custom of brewing guayusa, leaving it to cool, and mixing it with lemon juice and unrefined sugar. The drink is served cold during lunchtime. This custom is particularly widespread in the capital cities of Napo, Pastaza and Morona Santiago provinces.

Highland Kichwa and Tsa'chi

Indigenous people in the highlands have adopted the use of guayusa as a medicinal beverage to treat several ailments but also as an additive to spirits. In Azuay and Loja, the infusion is used to treat stomach ailments and to stop diarrhea in children. In Chimborazo, it is used to treat arthritis and to reduce fever. In Imbabura, it is used to reduce the effects of alcohol consumption, stress and to treat kidney problems (de la Torre et al. 2008).

The use of guayusa amongst *Tsa'chi* on the coast of Ecuador is largely related to treating pain. The leaves are used to prepare steam baths, and serve to treat menstrual cramps and relieve pain after an abortion has occurred (de la Torre et al. 2008).

Cofán and Secoya

Although the Cofán and Secoya are familiar with guayusa, its use appears not to be as prevalent. Cofán people use guayusa predominantly as an additive to spirits during festivals while Secoya are reported to use guayusa tea to treat body pains (de la Torre et al. 2008).

Colombia

As of August 2013, guayusa is available for purchase in the public herb market in the city of Pasto, capital of Nariño Department, which lies in the Andean highlands less than eighty kilometers from Colombia's border with Ecuador. According to vendors, the leaves come from the Valle de Sibundoy and other parts of Putumayo Department.

In Pasto, guayusa is most often consumed in *peñas*—downtown bars where young people meet to drink and listen to Andean music. *Peñas* serve *hervidos*, hot cocktails made with sugar cane liquor, guayusa, and various mixtures of fruits and spices, such as passion fruit, lime, *lulu* (called *naranjilla* in Ecuador: *Solanum* spp.), and cinnamon. At least one *peña* in Pasto obtains guayusa leaves from Tulcán, a highland town on the Ecuadorian side of the border.

In the town of Sibundoy, guayusa was found for sale in a bottled sarsaparilla tonic called 'The Bristol of Putumayo'.⁵ The drink claims to eliminate pimples and blackheads, 'cleanse the blood,' treat illnesses of the liver, kidney and stomach, reduce pain from arthritis, 'nourish the brain,' and 'cure the womb and ovaries.' The ingredients in the tonic include: sarsaparilla, Chinese root (*raíz china*, possibly *Boerhaavia coccinea*), *cuazia*, boldo (*Peumus boldus*), *gualanday* (of the *Jacaranda* genus), *riobardo*, and *granizillo*. The leaves for the tonic were obtained from the Lower Putumayo region, south of the town of Mocoa. Guayusa is also reported to exist in the Valle de Sibundoy, at altitudes of up to 3,000 meters. One Inga healer in the town of Santiago claims to have guayusa on her land five kilometers from town, the leaves of which she uses to facilitate post-partum uterine healing.

Peru

While guayusa is enjoyed in this region as a stimulating tea, most people report using guayusa for its medicinal qualities. It is known to 'clean blood' (to remove excess sugars from the blood), a remedy used to treat diabetes. It is also commonly used to cleanse the vagina after giving birth in the hope that it stops bleeding. Some people report making an alcoholic beverage with guayusa similar to what has been reported for the Ecuadorian and Colombian highlands.

The Awajún people of northern Peru, related to the Shuar and Achuar of Ecuador, use guayusa similarly to their relatives north of the border. Most of the *Ilex guayusa* found in Peru is said to come either from Ecuador or from the land of the Awajún (near the border with Ecuador) (Fernando Rubio, Personal communication). Myths related to guayusa have passed from the Awajún to mestizo cultures. Reportedly, the town of Hualango in the department of Amazonas was founded around a mythical guayusa tree whose inhabitants drank the guayusa water that was naturally made from the spring that existed below it (Various authors, 2012).

Seed morphology

Drupes examined contained four seeds most frequently, but the number of seeds ranged from four to six. Total fruit weight was 22,55 g while total seed weight was 9,78 g (n = 1201). The mean length of each seed was 5,12 mm ($\pm 0,72$; 3—8 mm), the mean width was 3,02 mm (\pm

⁵ Original text in Spanish: "Preventivo orgánico en general puede usarse con eficiencia en las enfermedades del Hígado, Riñones, y Estómago. Obra en enfermedades reumáticas, alimenta el cerebro, cura la matriz y ovarios, limpia la sangre, cura los forúnculos de la piel."

0,63; 1—6 mm), and the average weight was 0,008 g. It is undetermined if all the drupes came from the same individual.

Discussion

The current known distribution of *I. guayusa* in South America is primarily known through the existence of a variety of geo-referenced botanical samples obtained from the Tropicos Botanical Database (Tropicos 2013). While many of these specimens were gathered during field botanical surveys, some appear to have been bought at local markets. It should be noted that there is a clustering of samples within the Ecuadorian Amazon, with other clusters found in the Northern Amazon/Andes interface of Peru. Outlying samples have been found as far north as Venezuela and as far south as the Madidi area of Bolivia. With regard to some of the outliers in these samples, there is some reason for skepticism as to whether the specimens had been correctly identified. As pointed out in the introduction, due to high intra-specific leaf morphology and highly conserved anatomy between species, it is reasonable to think that some of the outlying samples were misidentified. If *I. guayusa* is indeed present in Southern Peru and Bolivia it would likely have been identified in more of the numerous botanical surveys that have taken place there.

Defining *I. guayusa*'s distribution in Ecuador is complicated by the fact that samples have only been found in areas known to be cultivated by humans. Even so, some general limits on the natural range of the plant can be inferred from the known characteristics of the plant. Firstly, based on the distribution of samples, it can be reasonably inferred that *I. guayusa* can be found in humid, forested areas. This seems to be the basic environmental requirements for the entire genus (Loizeau 2005). Secondly, there is no evidence from either the botanical record or from interviews with local farmers about *I. guayusa* being present in the Chocó region or in the Eastern Amazon despite these areas meeting the basic ecological requirements for the species. This points to the Andes presenting a possible dispersal barrier. The Amazonian floodplain could also be acting as a natural dispersal barrier. Available information shows a lack of presence of *I. guayusa* in the Eastern Amazon. The known sample points thus portray *I. guayusa* as native to the Andean piedmont and the upper Amazonian terra firme forests. If we look at the extensive distribution range of *I. guayusa*, it is possible such a wide distribution has been driven by the migration and exchange of human populations across its actual range.

Historical and archaeological evidence support the status of *I. guayusa* as a cultivar. First, the age of the guayusa leaves found in the Tiahuanacoid tomb indicates that this species has been used for at least 1,500 years, before the rise of the Inca Empire and long before the arrival of the Spanish. Second, the existence of guayusa leaves in a region far south of any contemporary botanical finds suggests that the tree likely was cultivated throughout a much larger range in the past, and that this range has potentially shrunk over the last millennium and a half (Schultes 1972). Third, the presence of guayusa at an altitude above the tree's known altitudinal range suggests some form of trade or exchange of the leaves between lowland and highland regions (Schultes 1972). Fourth, since implements such as snuff trays, inhalation tubes, and mortar and pestles were found with the guayusa leaf bundle, it has been cautiously suggested that guayusa might have also been consumed as a snuff or enema. The possibility of non-oral consumption of guayusa is significant insofar as no plant containing caffeine has been known to be consumed other than as a beverage (Schultes 1972)

Historical accounts also reinforce the important role of humans in guayusa management. As Schultes (1979) writes, ‘I find no evidence in the literature to suggest its occurrence in an undoubtedly wild state. All references indicate that guayusa, when not planted, grows as an escape or vestige of former plantings around abandoned human habitation sites.’ Indeed, there are no confirmed discoveries of ‘wild’ guayusa in the literature. Still, evolutionary processes need to be considered as partially responsible for guayusa current distribution. Although rare, vegetative propagation has evolved as a reproductive strategy in some tropical tree species, most of which naturally occur at low densities. It is well known that *I. guayusa* has a notable capacity for adventitious growth, which suggests co-evolution with human dispersers has allowed it to colonize such an extensive range.

Analytical treatment of guayusa as a managed species fits with a growing literature on the important role of human societies in promoting species diversity in the Amazonian region. For instance, archaeological discoveries of dark, nutrient rich anthropogenic soils (Heckenberger et al 2008) and remnants of monumental earthworks (Erickson 2010), as well as evidence of semi-domesticated species, such as peach palm (*Bactris gasipaes*) (Heckenberger and Neves 2009; Rival 1996), suggest a much more extensive utilization of the environment by native peoples, and significantly larger, more stable settlements in the region than previously thought.

Although the wealth of historical and geographical evidence seems to suggest that *I. guayusa* is a cultivated species, there are important pieces of information that need to be determined before we can reach a solid conclusion on the matter. The apparent absence of guayusa individuals obtained through sexual reproduction is one example. Embryos on guayusa seeds could be immature and therefore require long germination periods, as is the case with other *Ilex* species (Mroginski et al. 2011). The absence of specific pollinators might also be affecting the onset of fertile seeds in *I. guayusa*. Guayusa flower morphology suggests pollination is carried by hymenopterans. It has also been demonstrated that selective logging affects the behavior of pollinators with low mobilities, such as small bees (Ghazoul & McLeish, 2001), which could be the case in extensively cultivated areas, such as Napo Province in Ecuador. A decrease of natural pollinators, or a change in their behavior, will effectively reduce the chances of out-crossing for dioecous species occurring at low densities. Consequently, it is possible that the reported absence of seed germination in *I. guayusa* is due to the difficulty of pollination in disturbed areas. Here, we have reported for the first time anatomical information of *I. guayusa* seeds, in hopes that this might lead to more research on the reproductive ecology of this species.

Shemluck (1979) noted that guayusa was long thought to be a variety of *Ilex paraguariensis*. This idea was discarded shortly after flowering material of *Ilex guayusa* was described. Despite *I. guayusa* having been granted full recognition as a species, its relationships with close relatives have never been clearly established. *Ilex inundata* for instance, is a morphologically similar and sympatric species that appears to have analogous usages to guayusa as reported here. Phylogenetic studies of Ecuadorian *Ilex* are crucial to shed light on the possible status of guayusa as a cultivar and will provide important information for the management of guayusa in the context of modern cultivation.

Ilex guayusa is found throughout a wide range of the Andean foothills and Amazonian lowlands, from southern Colombia to northern Peru. A combination of botanical, historical, and ethnographic data suggests that guayusa has a long history of human management and cultivation. While there is considerable diversity of cultural practices and medicinal uses

associated with guayusa among different ethnic groups, there also seems to be extensive diffusion of these traditions across ethnic lines. While the close relationship of human societies with this species seems to suggest a domesticated status, we believe a wealth of research needs to be undertaken to allow us to further explore this topic. Meanwhile, *I. guayusa*'s medicinal properties will continue to position it as a valued species of the Andean-Amazonian interface.

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Table 1. List of vernacular names given to *Ilex guayusa* Loes. by different indigenous groups in Amazonian Ecuador.

Term	Language
Waysa	Kichwa
Wais/wayus	Shuar/Achuar
ëšë tara	Secoya

Table 2. Species with similar uses also sometimes referred to as guayusa

Species name	Reference
<i>Ilex inundata</i>	de la Torre et al., 2008
<i>Brumfelsa chiricaspi</i>	Vacas-Cruz et al., 2012
<i>Brumfelsa grandiflora</i>	Vacas-Cruz et al., 2012
<i>Pterocarpus rohrii</i>	Cerón, et al., 2012
<i>Tapura jurana</i>	Cerón, et al., 2012
<i>Drypetes amazonica</i>	Cerón, et al., 2012
<i>Dendropanax caucanus</i>	Cerón, et al., 2012
<i>Piper</i> sp.	Kvist & Holm-Nielsen, 1987
<i>Piper callosum</i>	this publication

Table 3. Uses of the vernacular Kichwa name waysa in various common combinations, possibly referring to species other than *Ilex guayusa*

Kichwa terms	Approximate Spanish equivalent
sacha waysa	forest guayusa
chiri waysa	cold guayusa
urku waysa	guayusa from the mountain
jatun waysa	big guayusa
waysa yura	guayusa tree
waysa kaspi	guayusa branch
waysa panga	guayusa leaf

Table 4. Uses of guayusa by region and ethnic group.

Country	Ethnic group	Uses	Reference	
Ecuador	(Colonial period)	treatment for venereal diseases	Schultes 1979	
	(Colonial period)	emetic	Schultes 1979	
	(Colonial period)	stimulant	Schultes 1979	
	Kichwa	stimulant		
		stomach tonic		
		diuretic		
		cold/flu remedy (combined with other substances)		
		pain reliever		
		fertility and libido enhancer		
		mouth cleanser		
		insect and snake bite prevention		
		dream enhancer/hunting aid (for humans and dogs)		
		emetic		
	Shuar and Achuar	mouth cleanser		
		fertility enhancer and stimulant for pregnant women		
Mestizo	stomach tonic			
Highland Kichwa	treatment for diarrhea			
	treatment for arthritis			
	fever reducer			
	treatment for kidney problems			
	pain reliever			
Tsa'chi	treatment for menstrual cramps			
	pain reliever for women after termination of pregnancy			
	pain reliever			
Colombia	Secoya	aid for post-partum uterine healing		
	Inga	stimulant		
Peru	blood cleanser' (removes excess sugars from blood)			
	treatment for venereal diseases		Schultes 1979	
	fertility enhancer		Schultes 1979	
Bolivia	(Pre-colonial period)	snuff?	Schultes 1979	
	(Pre-colonial period)	enema?	Schultes 1979	

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Figure 1. Map of guayusa samples collected in Ecuador and Peru with the major WWF ecoregions

