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Chemical evidence of prehistoric passive tobacco consumption by a human perinate (early Formative Period, South-Central Andes)

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ABSTRACT

Consumption of psychoactive substances is a long-standing tradition among indigenous peoples of the Americas. Archaeologically, consumption of tobacco has been shown through analysis of archaeological smoking pipe residues, mummy hair and dental calculus. Analysis of the hair of a perinate recovered from a rock shelter in Northern Chile and dated ca. 2400 yrs BP showed the presence of nicotine and its main metabolic product, cotinine. Quantitative segmental hair analysis showed that nicotine was taken up from the mother via the placenta and not through breast milk and that the death of the perinate occurred soon before or at the time of delivery. The analysis also indicated that the mother consumed high quantities of tobacco, suggesting she was a "tobacco shamaness" who might have experienced a miscarriage, since tobacco consumption by pregnant women is strongly associated to spontaneous abortions and perinatal death. Thus, at the dawn of pastoralism and agriculture in the highlands of the Atacama Desert, female individuals were already intensively consuming this psychoactive plant, transmitting its signals, and perhaps its lethal effects, to a child during gestation. This is the first evidence of simultaneous presence of nicotine and cotinine in pre-Hispanic bioanthropological remains from the Americas, thus constituting unequivocal direct evidence of tobacco consumption, and it refers to the currently earliest and youngest passive tobacco consumer in the Americas.

1. Introduction

The consumption of psychoactive substances is a widespread and long-standing tradition among indigenous peoples of the Americas, as indicated by many ethnohistorical and ethnographic accounts (Dobkin de Rios, 1984; Furst, 1976; Russell and Rahman, 2016; Wilbert, 1987), and also by the presence in the archaeological record of consumption paraphernalia such as smoking pipes, snuffing trays and enema tubes, as well as various types of objects associated to the processing and storing of the substances (Torres, 1995; Wilbert, 1987; Winter 2000). Although the finding of these objects in the archaeological record is taken to constitute indirect evidence of use of psychoactive substances, it does not inform about the nature of such substances. Instead, archaeobotanical and chemical analysis of residues in consumption paraphernalia, and also analysis of bioanthropological remains, may provide direct evidence of the nature of the substances consumed. Moreover, the finding of psychoactive compounds in bioanthropological remains not only demonstrates unequivocally their consumption even in the absence of paraphernalia, but also identify the individual consumers and, eventually, the intensity of consumption by those individuals, leading to a type of information that is not revealed by residue analysis of paraphernalia. Such information can generate a better understanding of the social dimensions of consuming practices of psychoactive compounds in prehistorical times.

Archaeobotanical and chemical analyses of pipe residues from archaeological sites in the South-Central Andes, particularly the *circumpuna* area (Fig. 1), have shown consumption of tobacco, *Nicotiana* spp. (nicotine) and cebil, *Anadenanthera colubrina* var. *cebil* (*N,N*-dimethyltryptamines) in Northwestern Argentina (NWA) (Andreoni et al., 2012; Aschero and Yacobaccio, 1994; Bugliani et al., 2010; Capparelli et al., 2006; Fernández Distel, 1980; Lema et al., 2015; Martin Silva et al., 2016; Pochettino et al., 1999; Rosso and Spano, 2005/2006) and of tobacco (nicotine) in the coast and in the Salar de Atacama area of Northern Chile (Carrasco et al., 2015; Gili et al., 2017). The smoking

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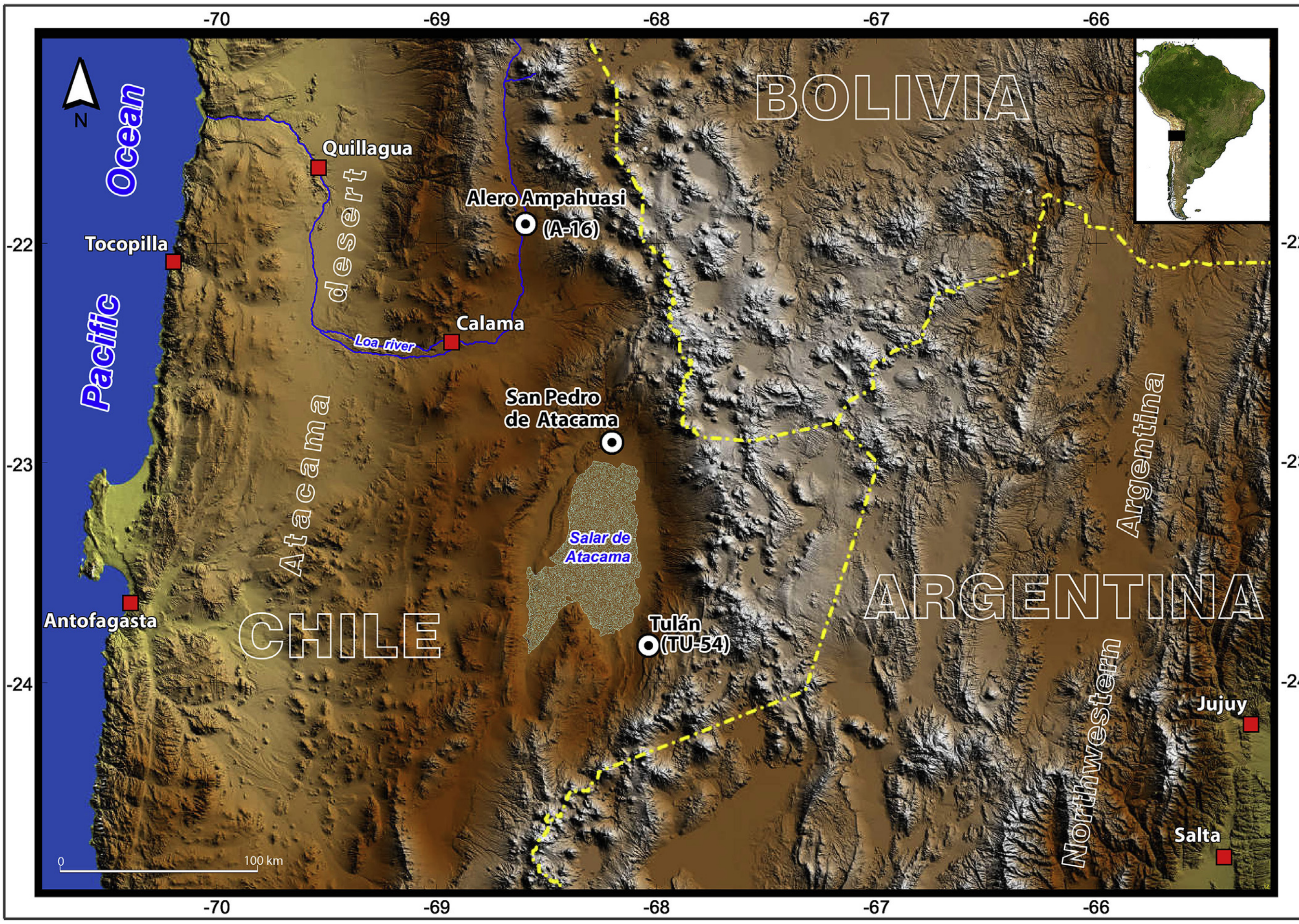

Fig. 1. Map of the *circumpuna* area of the South-Central Andes.

complexes experienced different developments on both sides of the Andes. In NWA, smoking pipes appear in the archaeological record as early as 2130 BCE (Fernández Distel, 1980). In the rich archaeological area of San Pedro de Atacama (SPA) in the Salar de Atacama, smoking pipes appear mainly during the Late Formative Period (400 BCE – 400 CE), although a pipe fragment is reported in the Tulan site Tu-54 dated to the Early Formative Period (1400–400 BCE) (Núñez et al., 2006). Some of the pipes found in SPA bear a close stylistic resemblance to those found in NWA and, in fact, they are thought to have been brought from there (Llagostera, 2001, 2015; Tarragó. 1989; Torres, 1999; Westfall. 1993–1994) at the same time as other ritual objects such as some ceramic vessels and the valves of terrestrial mollusks (Núñez et al., 2006). The changes in ritual activities that these objects brought to SPA and neighboring areas followed the deep changes that the local communities had been experiencing in the sociopolitical and economic spheres: the hunter-gatherer millenarian tradition had led to the first pastoralist societies of the area, and stable long-distance trade and interchange networks with communities who lived in the eastern slopes of the Andes had been established (Agüero, 2005; Llagostera, 1996; Núñez et al., 2007).

The ritual objects brought from the eastern slopes of the Andes are not the sole expression of ceremonial practices in the Atacama Basin during the Early Formative Period. In the southern part of the basin, a ceremonial center was erected in Tu-54 during this period which functioned as a point of social convergence and ritual practices (Núñez et al., 2017). Multiple burials of children and newborns were found at the bottom of the main structure, many of them associated to rich offerings such as gold icons, stone vessels, necklaces and terrestrial mollusks from the eastern slopes of the Andes (Núñez et al., 2017). Moreover, a fragment of the first pipe reported in the area was found inside the deposits of the site (Núñez et al., 2006). Undoubtedly, the emergence of these ritual practices during this period, among them smoking practices, was not casual and it may be suggested that they played an important role in the reproduction of the new sociopolitical order (cf. Gallardo and DeSouza, 2008).

Nevertheless, we still know little about the onset of smoking practices in the western slopes of South-Central Andes, particularly in relation to the substances being consumed and the agents of these consumption practices. Although the multiple-infant burials found at the Tu-54 site could be a good target to assess this, no individuals with preserved hair have been reported. Thus, it has not been possible to assess smoking practices during this period through the chemical signals in the remains of these infants.

During an archaeological rescuing effort in a rock shelter in the Upper Loa River, situated 100 km north of the Atacama Basin, the almost complete skeleton of a perinate was found in a context with rich offerings dated to 500 BCE. The burial resembles those of Tu-54 and is an expression of the burial practices peculiar to the Early Formative period in the area. The skull of the perinate showed well preserved portions of scalp with hair. Since this date appears to coincide with the onset of consumption of psychoactive substances in Northern Chile, this individual provides an opportunity to explore direct evidences of such consumption in the regional prehistory, and also to indirectly address the consumption of these substances by a pre-Hispanic woman.

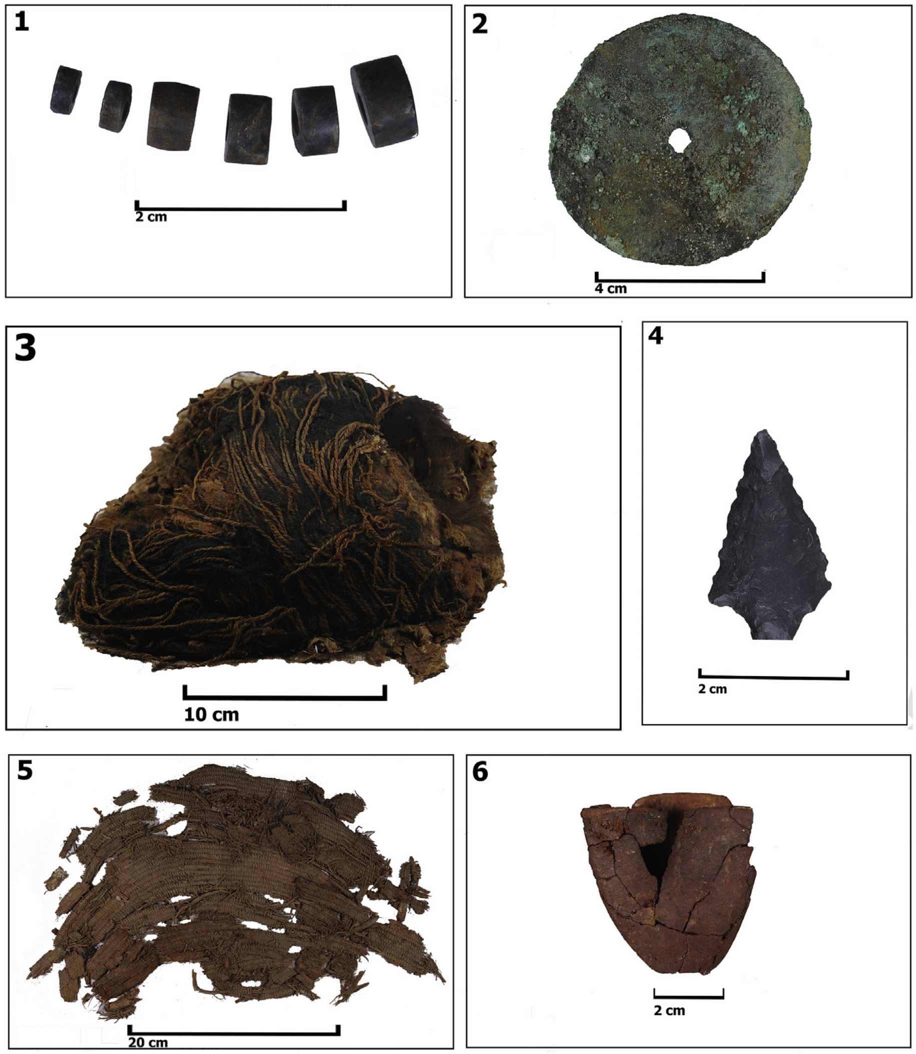

Fig. 2. Objects associated to the perinate burial at Alero Ampahuasi – A16. 1. Lithic beads; 2. Copper metal pendant; 3. Wool turban (dated by ^{14}C); 4. Lithic projectile point; 5. Fragments from spiral-technique basket (dated by ^{14}C); 6. Pumpkin container.

2. Materials and methods

2.1. The perinate

The perinate burial was found during the excavation of a rockshelter located in the Loa River upper basin (Alero Ampahuasi, site A16), in northern Chile, at 3100 m.a.s.l., on the western slopes of the Andes range. The burial was arranged at about 70 cm under the surface, within a roughly cylindrical pit about 40-cm in diameter and 40-cm in height. Clothing included a tunic, a kilt, and a turban of wool yarn that covered the infant's skull. The offerings associated with the child included two mollusk shells (*Scurria viridula* and *Argopecten purpuratus*) from the Pacific Ocean, two spiral-technique baskets, a pumpkin (*Lagenaria siceraria*) container, the remains of a bag, a lithic projectile point, a copper metal pendant, and six small lithic beads (Fig. 2).

The infant showed a high degree of preservation of its anatomical units, allowing the identification of 98.5% of the bone pieces. Based on the development and eruption of dental pieces and metric measurements such as the long diaphyseal length of long bones and other measurements of short and irregular bones, the age at death of the infant was estimated between 38 weeks of gestation and 2 months of extrauterine life (De Souza et al., 2018).

Samples for ^{14}C dating were extracted from two of the offerings associated to the perinate (De Souza et al., 2018): one from the vegetable fiber of a basket placed under the infant's body, and the other from the wool yarn of the turban that covered the skull. The plant sample corresponds to the terrestrial rush *Cortaderia speciosa* (syn. *Cortaderia atacamensis*), while the wool sample corresponds to *llama* fibers (*Lama glama*). Both objects were in contextual association with the perinate; hence, it may be assumed they were incorporated to the burial along with the body of the child. The date determined for the textile was 2.450 ± 30 BP (*2.700 – 2.*340 cal *BP*) and for the basket 2.410 ± 30 BP (*2.680 – 2.*330 cal *BP*). The dates obtained from both objects agreed closely and according to the Ward and Wilson test (Ward and Wilson, 1978) they can be combined (T = 0.373, 5% 3.841, A_{comb} = 109.7%) to yield a combined date of *2.480 - 2.*340 cal *BP*. The chronology of the burial can thus be assigned to the end of the Early Formative Period (3350 - 2350 BP).

2.2. Sample preparation

The analysis of nicotine in hair using gas chromatography with mass spectrometric detection was first described in the seminal work by Zahlsen and Nilsen (1994); the use of the method in the analysis of nicotine in archaeological smoking pipe residues was pioneered by Rafferty (2002, 2006). The present hair analysis was based on the method described by Chetiyanukornkul et al. (2004) which uses liquid chromatography and mass spectrometry. Hair strands were severed close to the scalp from the occipital region of the skull and measured under the stereoscopic loupe (mean ± SD: 3.2 ± 0.6 cm; range: 1.8–4.5 cm). To determine more precisely the age of the perinate at the time of death, segmental hair analysis was performed since it can inform about time course of exposure to exogenous molecules and their metabolites (Pragst et al., 1998). Contamination of samples by exogenous nicotine can be a major issue in the analysis of nicotine in archaeological materials (see discussions in: Musshoff et al., 2009). In this work, nitrile gloves were used during manipulation of hair in the laboratory and all equipment and glassware were washed with neutral pH detergent, distilled water and alcohol before use. Hair strands between 2.6 and 3.8-cm long were cut into basal and apical halves. Since hair grows at a rate of approximately 1 cm per month (Pragst, 2006), the two sets of hair strands should reflect dynamic nicotine exposure during approximately the last three months of life of the perinate. Individual hair strands were further minced with scissors into *ca*. 1-mm segments. Samples (ca. 4 mg) were washed three times for 10 min with 3 mL dichloromethane and evaporated to dryness under a nitrogen flow. Internal standards were added to the dry extracts: 10 ng of tetradeuterated nicotine (20 µL at 500 ng/mL) and 5 ng of trideuterated cotinine (100 µL at 50 ng/mL). After addition of 400 µL of 2.5 M NaOH, the solution was sonicated for 2 h and extracted three times with 400 µL of dichloromethane. The combined organic phases were mixed with 500 µL of 25 mM HCl in methanol, evaporated to dryness under a nitrogen flow, redissolved in 1000 µL mobile phase, and transferred to a sample vial. A 10 µL aliquot of the solution was then injected into an ultraperformance liquid chromatograph coupled with a high resolution mass spectrometer (UPLC-HRMS).

2.3. UPLC-HRMS analysis

The UPLC platform consisted of an LPG-3400RS pump, a TCC-3000RS column oven, and a WPS-3000TRS autosampler (all from Thermo Scientific, Bremen, Germany). The analysis was performed on an Acquity UPLC HSS T3 1.8 µm (2.1 × 100 mm, Waters, Milford, MA, USA) analytical column. The auto sampler was maintained at 4 °C and the column at 35 °C. Isocratic elution was employed using water-acetonitrile (40/60% v/v) as the mobile phase at a flow rate of 0.5 mL/min. Mass spectrometry was performed with an Orbitrap Exactive Plus mass spectrometer (Thermo Scientific) equipped with an electrospray ionization (ESI) interphase. Data handling used the Xcalibur 3.1 software (Thermo Scientific). Capillary temperature was 300 °C, auxiliary gas heater temperature 200 °C, S-lens RF level 50, spray voltage 6.0 kV, sheath gas flow rate 35 (arbitrary units), auxiliary gas flow rate 5 (arbitrary units) and cone sweep gas flow rate 1 (arbitrary units). Given the record of use of psychoactive substances in the South-Central Andes, the following alkaloids were used in the study: (±)-nicotine, (−)-cotinine, *N,N*-dimethyltryptamine and 5-methoxy-*N,N*-dimethyltryptamine obtained from Sigma (Sigma-Aldrich Co., St Louis, MO, USA), and 5-hydroxy-*N,N*-dimethyltryptamine obtained from Cerilliant Corp. (Austin, Texas, USA). (±)-d_4-nicotine and (±)-d_3-cotinine were used as internal standards. Solvents were analytical chromatographic grade from Merck (Darmstadt, Germany).

2.4. Initial analysis

High resolution mass spectra were obtained for all alkaloids to determine the mass of the molecular ions to be used for quantitative analysis. A chromatographic run was then performed with a mixture of the five alkaloid standards and deuterated derivatives to determine their retention times and finally, an extract of the perinate's hair was injected. Since no tryptaminic alkaloids were found, further analyses were aimed at quantitating only nicotine and cotinine in hair.

2.5. Calibration and validation procedures

Twelve nicotine and 12 cotinine standard solutions were prepared with final concentrations between 2 and 70 ng/mL and between 1 and 15 ng/mL, respectively; they contained 10 ng of d_4-nicotine and 5 ng of d_3-cotinine in a final volume of 1000 µL mobile phase. The ratio of the peak area of nicotine to the peak area of d_4-nicotine and the ratio of the peak area of cotinine to the peak area of d_3-cotinine were plotted against the concentration of nicotine and cotinine, respectively.

Precision and accuracy of the method was determined by analyzing as above four 4-mg portions of non-smoker's hair which were spiked with 8 ng nicotine, 50 ng nicotine, 1 ng cotinine or 12 ng cotinine, respectively. Analyses were carried out with three replicates. Concentrations were determined from the peak area ratio of the analytes to their respective deuterated internal standard. Precision was determined as the relative standard deviation and accuracy as [(concentration extrapolated from the calibration line)/(spiked concentration)] × 100. The limit of detection of the method was defined as the concentration of alkaloid which produced a signal-to-noise ratio of 3 and the limit of quantitation as three times the limit of detection.

3. Results

The high resolution mass spectra in positive ion mode showed protonated molecular ions $[M + H]^+$ at *m/z* 163.123, 177.102, 167.146 and 180.123 for nicotine, cotinine, d_4-nicotine and d_3-cotinine, respectively. Nicotine and cotinine could also be distinguished by their retention times (Fig. 3). Linear calibration lines were generated for pure nicotine and cotinine (regression lines: $Y = 0.0195 + 0.0136*X$, $R^2 = 0.999$ and $Y = 0.288 + 0.121*X$, $R^2 = 0.999$, respectively, where Y is the ratio of the peak area of the analyte to its respective deuterated internal standard and X is the set concentration of the analyte in ng/mL). Points for non-smoker's hair spiked with the alkaloids fell within the 95% confidence limits of both lines, showing the absence of interference of the hair matrix (Fig. 4). Precision varied between 1.13 and 4.18%, and accuracy between 98.1 and 116.4%. The limits of detection for nicotine and cotinine were 0.28 and 0.04 ng/mg hair, respectively, and the limits of quantitation 0.84 and 0.12 ng/mg hair, respectively.

Both nicotine and cotinine were absent from washing solutions. Authentic chromatograms of mummy hair are presented in Fig. 5. Mean concentrations of nicotine and cotinine in the basal and apical sections of hair strands are shown in Fig. 6. Two-way ANOVA showed significant differences between alkaloid concentrations ($F_{1,26} = 70.977$, $P < 0.001$), non-significant differences between hair segments ($F_{1,26} = 1.319$, $P = 0.261$) and no interaction between both factors ($F_{1,26} = 0.0317$, $P = 0.860$).

4. Discussion

Previous quantitative analysis of pre-Hispanic mummy hair showed presence of nicotine and absence of cotinine (Musshoff et al., 2009). In the present study, cotinine was found in all samples analyzed along with nicotine. Since cotinine is the main metabolic product of nicotine (Benowitz et al., 2009; El-Khoury and Wang, 2014), its presence in the hair is indicative of nicotine metabolization and rejects the possibility that it arises from contamination of the samples. Because it is unlikely that nicotine reached the perinate's hair through active consumption, the mother must have supplied it through the placenta and/or through breast milk (Jacqz-Aigrain et al., 2002; Joya et al., 2015; Köhler et al., 2007; Napierala et al., 2016).

The lack of differences both in nicotine and cotinine concentrations between basal and apical sections of the perinate's hair strands suggests similar uptake of tobacco-derived chemicals during the two periods of hair growth examined. Since nicotine concentration in breast milk is higher than in serum (Luck and Nau, 1984), it seems likely that the perinate did not experience the transition from *in utero* to breast

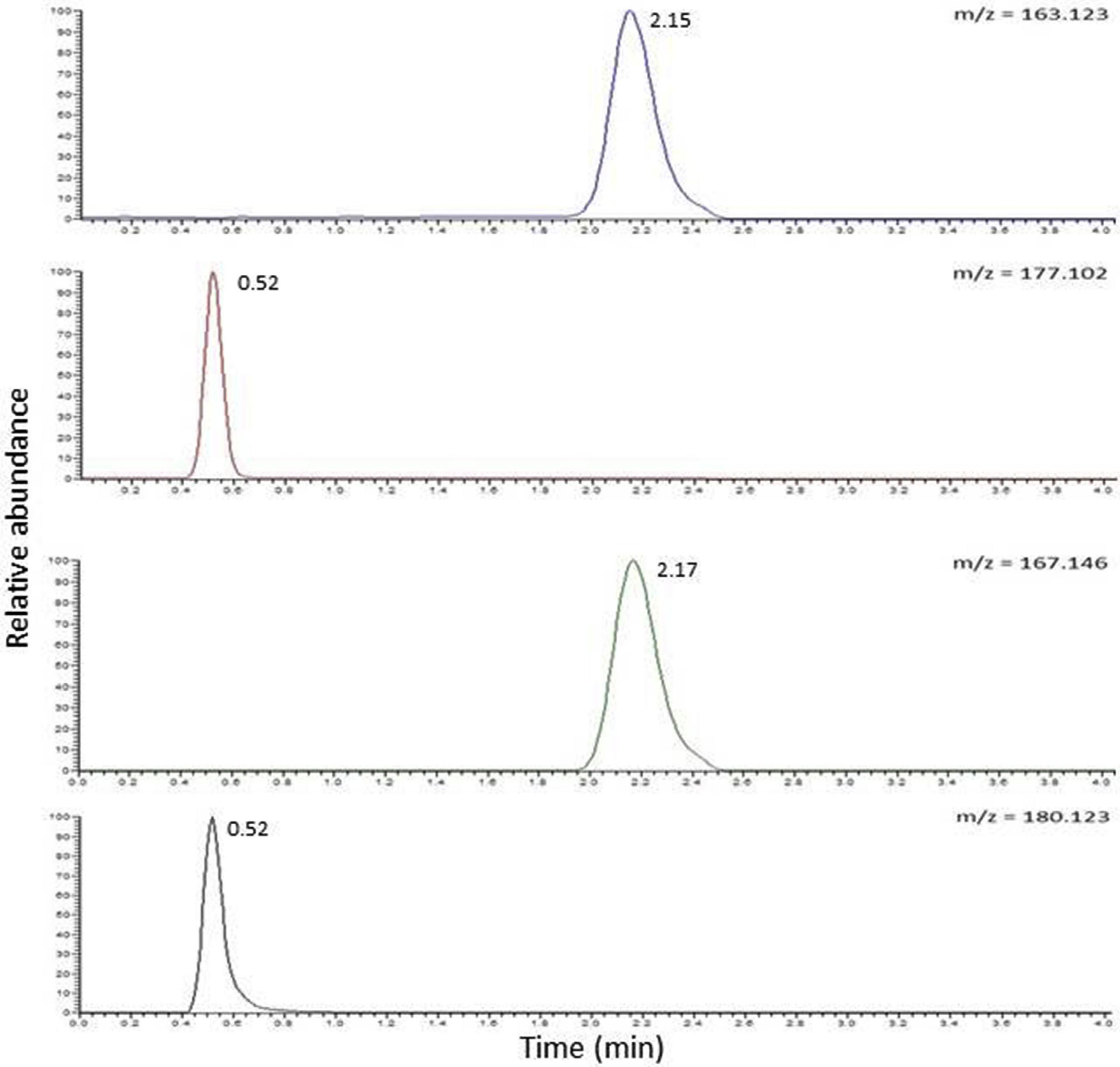

Fig. 3. Analysis of alkaloids in the hair of the perinate. UPLC-HRMS traces of pure alkaloids showing molecular masses monitored. From top to bottom: Nicotine, 50 ng/mL; cotinine, 10 ng/mL; d_4-nicotine, 10 ng/mL; and d_3-cotinine, 5 ng/mL.

feeding, implying that the perinate died soon before or during delivery.

Several studies have reported mean nicotine and cotinine hair concentrations in contemporary smoking mother-neonate pairs [mean value of ng Nic/mg hair: 4.32–6.37 (Jacqz-Aigrain et al., 2002), 2.4 (Klein and Koren, 1999), 2.4 (Eliopoulos et al., 1994), 0.15–11.80 (Kintz et al., 1993); mean value of ng cotinine/mg hair: 0.62–1.61 (Jacqz-Aigrain et al., 2002), 2.8 (Klein and Koren, 1999), 2.8 (Eliopoulos et al., 1994)]. Concentration of nicotine in the hair of the perinate (9.7 ng/mg hair) was within the range reported for contemporary hair. Concentration of cotinine in the hair of the perinate (4.3 ng/mg hair) was higher than the values reported in contemporary hair analysis. Significant positive linear correlations have been reported between alkaloid concentrations in the hair of contemporary neonates and their mothers. Moreover, further analysis of the data reported by Jacqz-Aigrain et al. (2002) showed linearity between the mean number of cigarettes per day smoked by the mother and the cotinine concentration in the neonate's hair. Extrapolation from this line to the cotinine concentration found in the perinate suggests a consumption by the mother of the equivalent to more than 70 cigarettes per day. This high level of tobacco consumption by the perinate's mother might have led to a misscarriage since tobacco consumption by pregnant women is strongly associated to spontaneous abortions and perinatal deaths (Aliyu et al., 2007; Bell and Lau, 1995; Cui et al., 2016; DiFranza and Lew, 1995).

Ethnographic accounts reveal that during rituals South American shamans may consume huge quantities of tobacco. For example, Wilbert (1987) cites the smoking of five 90-cm long cigars while chewing tobacco in one case and over 100 cigarettes in another, during the course of one session. Moreover, ethnographic research in South America has shown differences in the use of tobacco between men and

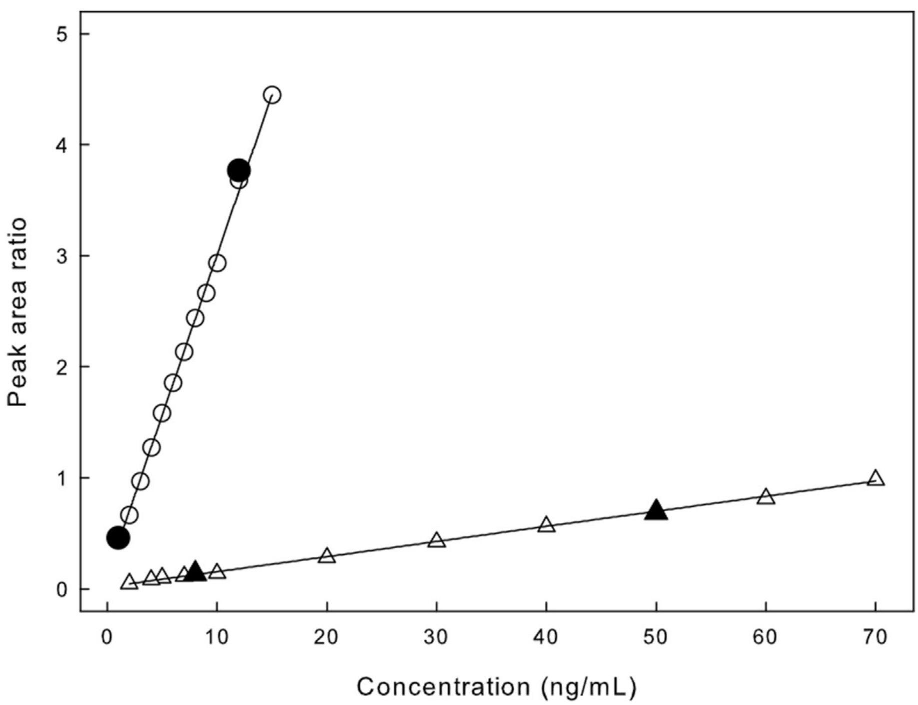

Fig. 4. Calibration lines for pure alkaloids: the ratio of the peak area of nicotine to the peak area of d_4-nicotine and the ratio of the peak area of cotinine to the peak area of d_3-cotinine were plotted against the concentration of nicotine and cotinine, respectively. Nicotine (open triangles) and cotinine (open circles), with points for hair spiked with nicotine (filled triangles) and cotinine (filled circles).

women, particularly regarding the amounts consumed, the techniques and the artifacts employed, and also the social contexts (e.g.: ritual or secular) where consumed by each sex (Wilbert, 1987). Thus, while in some societies tobacco consumption is restricted exclusively to men (for example, it is a taboo for women among the Witotoan people of the Peruvian Andes; Wilbert, 1987: 96), in others women may even act as “tobacco shamanesses” *sensu* Wilbert (1987), for example, among the Jivaro and Cuna groups of South America (Wilbert, 1987: 155).

Although the shamanic role of women in pre-Hispanic times is not well known, it is possible that the mother of the perinate was involved in ritual practices leading to large consumption of tobacco and hence to a large accumulation of cotinine. Alternatively, a mode of consumption of tobacco by the mother different from smoking may be sought. Ethnohistorical accounts show that tobacco has been used in a variety of ways (Charlton, 2004; Wilbert, 1987) and such ways may lead to differential cotinine accumulation (Benowitz, 1992; Jarvis, 1992). For example, chewing tobacco leads to higher presence of cotinine in the consumer than smoking tobacco (Çok and Öztürk, 2000), and the air-to-skin-to-blood pathway, as may occur through the use of cataplasms to treat ulcerated abscesses, fistulas, and sores (Charlton, 2004), may lead to higher incorporation of nicotine than inhalation (Bekö et al., 2017). These results point to the possible use of cotinine and nicotine concentrations as lines of evidence to identify different modes of tobacco consumption in the past, including some ways that are not always associated with durable archaeological artifacts, such as chewing or licking.

Overall, the present study offers new perspectives in the differentiation of consumption by gender within archaeological contexts, opens the possibility of comparing amounts consumed and suggesting possible techniques of administration, and also refers to particular situations (i.e.: pregnancy) where tobacco would have been consumed in prehistory by a specific gender. Future similar studies with individuals of different sexes and ages will likely open an unsuspected field of knowledge about the particularities of these practices in the past, offering long-term diachronic perspectives on tobacco consumption that ethnographic records are not able to reveal.

Although the consumption of local *Nicotiana* species cannot be ruled out, several lines of arguments point to the eastern Andean slopes as the origin of the tobacco consumed by the perinate's mother: i) during the Early Formative Period, the local archaeological record begins to show goods that come through long-distance exchange with the eastern Andean valleys, such as the terrestrial mollusk *Megalobulimus oblongus* (syn. *Strophocheilus oblongus*) from the Yungas and, suggestively, smoking pipes that have earlier records in those valleys (Fernández Distel, 1980; Núñez et al., 2006), ii) *N. sylvestris*, an endemic species from Northwestern Argentina (Goodspeed, 1954), stands out as the *Nicotiana* species with highest nicotine concentration (Saitoh et al., 1985) and hence would be an efficient provider of the alkaloid, and iii) the use of *Nicotiana* spp. has been reported in Northwestern Argentina until ethnographic times (Pérez Gollán and Gordillo, 1993).

The chemical evidences presented in this study, together with other recent ones from the Atacama Desert that indicate nicotine consumption in pre-Hispanic times (Carrasco et al., 2015; Echeverría and Niemeyer, 2013; Gili et al., 2017), confer a new visibility to tobacco as a psychoactive plant of wide usage in the region. This opens a new cultural scenario in relation to the consumption of psychoactive plants in the area: while *Anadenanthera* spp. has been considered previously as the major psychotropic element used in pre-Hispanic times (Torres and Repke, 2006), the data now available suggests that tobacco was another main psychoactive substance during the prehistory of the region. Moreover, it may well be that tryptamines and nicotine were consumed together, a situation that has been reported in ethnographic studies in South America (Torres and Repke, 2006; Wilbert, 1987). The absence of tryptamines in the hair of the perinate and of other individuals analyzed from the western Andes may be due to metabolic pathways that degrade them at a relatively high rate (Barker et al., 1981; Echeverría and Niemeyer, 2013: Yu et al., 2004). It would be desirable to analyze pre-Hispanic bioanthropological material with highly sensitive chemical tools, perhaps with emphasis on specific metabolization products of plant-derived tryptamines.

The evidence of an important consumption of tobacco by a pregnant woman of the Formative Period of the region, suggests that ritual practices with this plant were already deeply inserted in the social life at this time. Moreover, this evidence is contemporary with the first manipulation of crops in the area (McRostie, 2014), showing that, as in other parts of the continent (e.g. Carmody et al., 2018), psychoactive plants are important in the social life of pre-agricultural societies in the American continent and their use might precede the cultivation of food

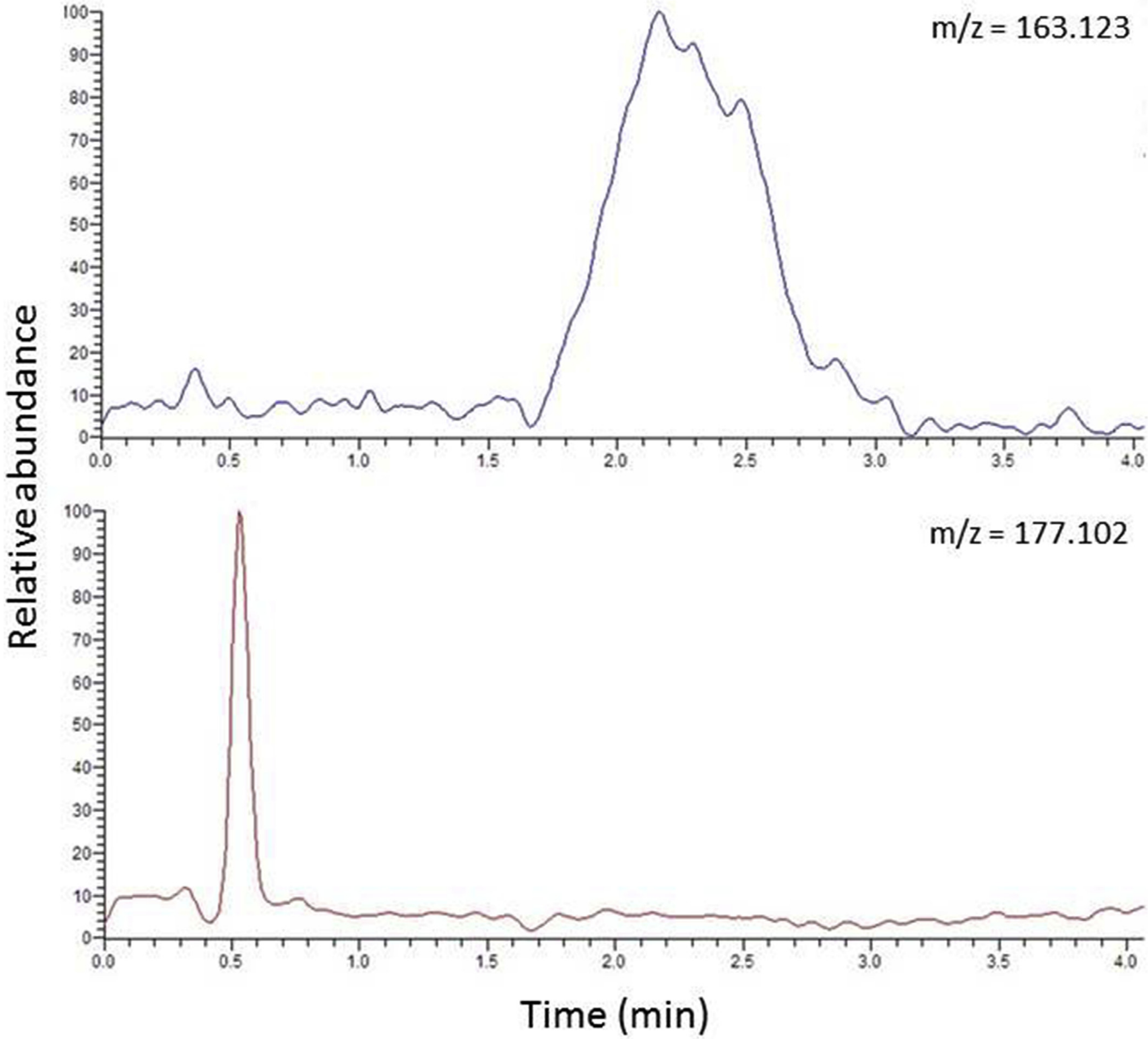

Fig. 5. UPLC-HRMS traces of a sample of hair from the perinate: Above: nicotine, 7.40 ng/mg hair; Below: cotinine, 3.14 ng/mg hair.

plants.

5. Conclusions

Chemical analysis of the hair of a pre-Hispanic perinate revealed the hitherto earliest and youngest passive tobacco consumer in the archaeological record of the continent. Although this individual possibly post-dates by several hundreds of years or even millennia the first uses of tobacco in South America, possibly in contexts other than psychotropic (Etkin, 1988) and even without well-defined associated paraphernalia, this finding demonstrates that close to 2400 years BP, at the dawn of pastoralism in the highlands of the Atacama Desert, female individuals were already intensively consuming this psychoactive plant, transmitting its signals, and perhaps its lethal effects, to a child during gestation.

The detection of tobacco in the archaeological record is a central issue to reach an understanding of the adoption and uses of this psychoactive plant in the past because the sole presence of smoking pipes is not sufficient to demonstrate tobacco consumption, given the wide variety of other possible plants that may have been smoked (Tushingham et al., 2013). The chemical analyses of human hair, such as that reported in this study, or of other bioanthropological remains such as dental calculus (Eerkens et al., 2018) open especially favorable perspectives to identify consumption in the absence of associated paraphernalia and also to quantify intensity of use and to detect forms of consumption different from smoking. At the same time, chemical analysis of hair allows the identification of singular social agents of these consumptions in the past, such as pregnant women, and also passive victims, such as fetuses and new-born children.

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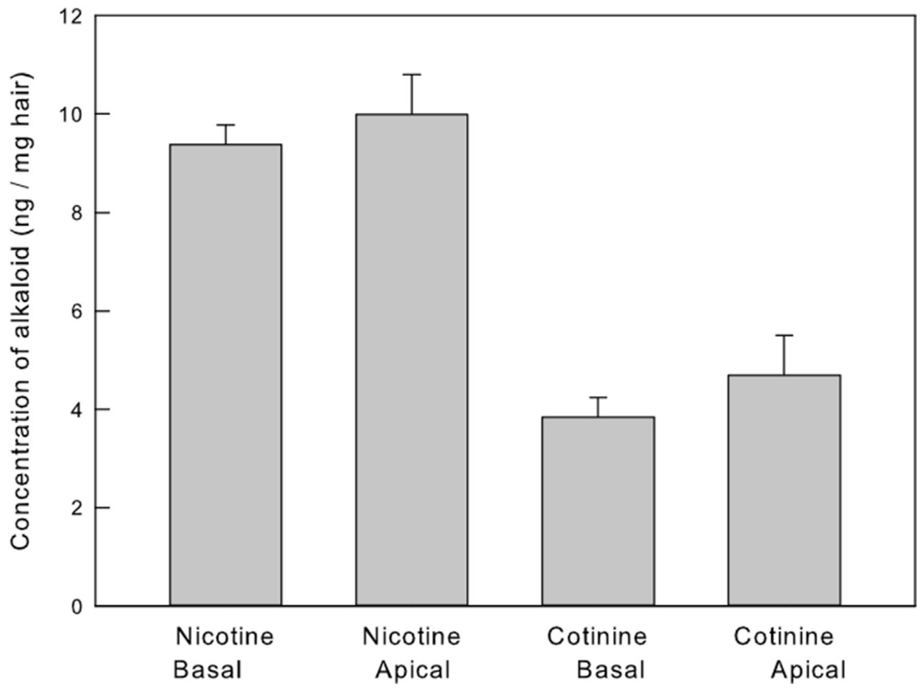

Fig. 6. Concentrations of nicotine and cotinine found in the perinates's hair. Apical and basal segments refer to the halves in which each hair strand was cut.

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