Biomolecular archaeology reveals ancient origins of indigenous tobacco smoking in North American Plateau

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Edited by Patricia L. Crown, The University of New Mexico, Albuquerque, NM, and approved September 26, 2018 (received for review August 9, 2018)

Chemical analysis of residues contained in the matrix of stone smoking pipes reveal a substantial direct biomolecular record of ancient tobacco (*Nicotiana*) smoking practices in the North American interior northwest (Plateau), in an area where tobacco was often portrayed as a Euro-American–introduced postcontact trade commodity. Nicotine, a stimulant alkaloid and biomarker for tobacco, was identified via ultra-performance liquid chromatography-mass spectrometry in 8 of 12 analyzed pipes and pipe fragments from five sites in the Columbia River Basin, southeastern Washington State. The specimens date from 1200 cal BP to historic times, confirming the deep time continuity of intoxicant use and indigenous smoking practices in northwestern North America. The results indicate that hunting and gathering communities in the region, including ancestral Nez Perce peoples, established a tobacco smoking complex of wild (indigenous) tobacco well before the main domesticated tobacco (*Nicotiana tabacum*) was introduced by contact-era fur traders and settlers after the 1790s. This is the longest continuous biomolecular record of ancient tobacco smoking from a single region anywhere in the world—initially during an era of pithouse development, through the late precontact equestrian era, and into the historic period. This contradicts some ethnohistorical data indicating that kinnikinnick, or bearberry (*Arctostaphylos uva-ursi*) was the primary precontact smoke plant in the study area. Early use likely involved the management and cultivation of indigenous tobaccos (*Nicotiana quadrivalvis* or *Nicotiana attenuata*), species that are today exceedingly rare in the region and seem to have been abandoned as smoke plants after the entry of trade tobacco.

biomolecular archaeology | tobacco | ancient plant use | North America | indigenous health

Despite being the leading cause of preventable death, nicotine dependence is a worldwide epidemic, and tobacco continues to be exploited by hundreds of millions of people around the world (1). While antitobacco campaigns and global health initiatives have resulted in declines in tobacco use over the last 50 y, use rates remain stubbornly high in many developing nations and among certain populations, for example, American Indians, Native Alaskans, and Canadian First Nations peoples in North America (2). Modern commercial tobacco has a wide range of additives that serve to enhance physiological nicotine delivery and addictiveness, mask environmental cigarette odors, and conceal deleterious symptoms and illnesses associated with smoking (3). Commercial tobacco is also advertised and packaged in such a way as to be attractive to consumers, for example, marketing campaigns that feature American Indian imagery (4–6) (*SI Appendix*, Fig. S1). However, humanity's dance with this powerful plant is much more ancient than the 140 y since the first mass-marketed cigarettes were produced (7). Indeed, the roots of nicotine addiction stretch back many thousands of years, and scholars are still just beginning to understand the deep time history of this ancient plant and its coevolutionary relationship with humans.

It has been hypothesized that tobacco (genus *Nicotiana*) was the first domesticate in the Americas, predating, and possibly laying the foundation for, the farming of maize and other food plants (8). The process of domestication began perhaps 6,000–8,000 y ago in the Andes of South America (8, 9); genetic selection and modification by people ultimately produced species such as *Nicotiana rustica* and *Nicotiana tabacum*, which have larger leaves and higher nicotine content than earlier wild varieties (8). Domesticated tobaccos spread into Mesoamerica and the Caribbean and reached parts of what is now the southeastern and southwestern United States by 2500–3500 cal BP (8, 10, 11). However, they were absent in most of western North America, a vast area inhabited by hunter-gatherers where several different species of indigenous (often referred to as "desert" or "wild") tobaccos are found, including *Nicotiana quadrivalvis*, *Nicotiana attenuata*, and *Nicotiana obtusifolia* (12–14).

By the time of Euro-American contact, many species of tobacco were used by indigenous communities throughout North and South America, and tobacco was esteemed as a plant with great power, with special ritual, medicinal, and ceremonial significance. Rather than being the habitual recreational product it has become today, in traditional contexts tobacco is typically used in limited quantities and by certain community members. Although pipes are some of the most well-known artifacts associated with tobacco, the plant was also smoked with perishable materials (e.g., in cigars and reed "cigarettes") and was ingested by other means (e.g., chewing with lime, used as snuff, by enema) (15–20). In 1492, Taino Arawak Indians introduced tobacco to Columbus in the Bahama Islands during his first encounter with

Significance

While tobacco is one of the most heavily consumed (and abused) plant substances of the modern era, with profound global health consequences, its early use remains poorly understood. Here we report a substantial direct biomolecular record of ancient tobacco smoking by hunter-gatherers of interior northwestern North America. Nicotine-positive samples demonstrate deep time continuity of indigenous tobacco smoking in a place where tobacco has been depicted as being introduced by early Euro-American traders and explorers. The spread of domesticated trade tobacco seems to have overtaken and obscured ancient indigenous tobacco practices. The information—represented here by the longest continuous biomolecular record of tobacco use from a single region—informs programs designed to combat persistent commercial tobacco use rates among modern Tribal communities.

Author contributions: S.T., C.M.S., and D.R.G. designed research; S.T., C.M.S., K.J.B., W.J.D., and D.R.G. performed research; S.T. and D.R.G. contributed new reagents/analytic tools; S.T., K.J.B., and D.R.G. analyzed data; and S.T., C.M.S., K.J.B., W.J.D., and D.R.G. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1813796115/-/DCSupplemental.

Published online October 29, 2018.

the Americas (21), and later European explorers to the Americas were quick to adopt tobacco after recognizing its special properties. By the 1500s, varieties of domesticated *N. tabacum*, selected over *N. rustica* for its "smoother" qualities, were farmed in plantations in the British and American colonies throughout the Caribbean and eastern North America. By the late 1600s, tobacco was introduced to the Old World and soon became a global trade commodity, a mass consumable that—along with another stimulant of a different sort (sugar)—"revolutionized the world and changed the course of history" (ref. 22, p. 1).

Globalization also fundamentally changed the use of tobacco by indigenous peoples, particularly in the west. In a fascinating twist of fate, westward expanding Euro-Americans introduced domesticated trade tobacco (likely *N. tabacum* farmed on eastern plantations) to northern and western indigenous hunting-gathering and fishing communities. Beginning as early as the mid-1600s, explorers, missionaries, and traders soon discovered that tobacco was highly prized by native peoples, especially in places where tobacco was difficult to obtain and hard to grow, such as northwestern North America. Dried trade tobacco—much more potent than any previously available native smoke plant—was conveyed in sizable, easy to transport bundles known as "twists" or "carrots." Its use spread like wildfire as Hudson's Bay Company explorers and fur traders began infiltrating the region in the 1700s. This occurred so rapidly and so early in the historical record that a complete understanding of in situ precontact smoking practices has been obscured. This is particularly true in the interior northwest Plateau and northern and central northwest coast of North America, places where indigenous tobaccos (which tend to thrive in warmer, drier climates) are scarce (9, 14). The only extant indigenous tobacco in the area is *N. attenuata* (coyote tobacco), a small, scrubby species that is sparsely distributed in sandy river bars (23). Where it was available, native peoples preferred *N. quadrivalvus*, although the plant's natural range is limited to well south of the study area, in northern California and southwestern Oregon (9).

In many places, tobacco is presumed to have been introduced after contact (20); in others, there is sporadic mention of native use of indigenous tobacco in explorer and trader journals after the 1790s (23), as well as some ethnographic evidence that native peoples used and sometimes cultivated indigenous tobaccos (24–29). In a dramatic case of anthropogenic range extension, the Haida of Haida Gwaii (also known as the Queen Charlotte Islands), British Columbia, Canada, and the Tlingit of the far northern Pacific Northwest coast into southern Alaska, cultivated and chewed *N. quadrivalvis*, but abandoned the practice after gaining access to trade tobacco, and the absence of human manipulation led to the plant's extirpation from the region (24). It has been suggested that within the interior northwest, *N. quadrivalvus* was likely more widespread in the past due to similar human management practices (8, 13, 23). Unfortunately, historical processes and events have obscured our understanding of in situ indigenous tobacco use in the northwest before the introduction of domesticated trade tobacco.

Identification of Ancient Tobacco Use

Archaeological evidence marking the spread and use of tobacco is challenging. Pipes are found in the archaeological record, although recent syntheses have demonstrated they are quite rare in northwestern North America (30, 31). Charred tobacco seeds are exceedingly small, are likely combusted during the smoking process, and to our knowledge have not been recovered at any sites in northwestern North America. Tobacco use is often equated with the archaeological pipes, but this is not a safe assumption, since as many as 100 plant species representing 55 genera of smoke plants were used in the postcontact era throughout North America (32). In many parts of the northwest, kinnikinnick, or bearberry (*Arctostaphylos uva-ursi*) was the main smoke plant used by indigenous peoples, smoked with tobacco (when available), on its own, or mixed with a suite of other indigenous plants (20). In addition to being smoked, tobacco was commonly used as an offering in religious contexts as well as in daily practice, for example, by casting or sprinkling unburned tobacco leaves or stems or through burning (i.e., smudging) (20, 28, 33).

Chemical identification techniques using gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) allow archaeologists to identify tobacco use through the identification of the biomarker nicotine found in residues extracted from ancient pipes, human hair, ceramics, and dental calculus (10–12, 34–38). At present, the earliest bimolecular evidence of tobacco use/ancient smoking practices is in areas where tobacco was farmed, in eastern North America and South America; much less is known about hunter-gatherers' tobacco use, especially in northwestern North America (13). To date, the only direct biomolecular evidence of tobacco smoking in western North America is from California (12, 13, 39). The only other known attempt to conduct residue analysis on pipes from the Plateau was conducted at the Keatley Creek site in interior British Columbia, but this produced only negative results (ref. 40, p, 71).

For this study, we used extraction and ultra-performance LC-MS (UPLC-MS) protocols designed to improve our ability to identify a suite of smoke plants (e.g., indigenous and domesticated tobacco species, and *A. uva-ursi*) through a program of experimental method development conducted at Washington State University. The methods thus developed were applied to this archaeological case study, which was developed in a collaboration with members of the Nez Perce Tribe and Culture Department staff who requested residue analysis of archaeological pipes from their ancestral sites so that they may better document traditional use of tobacco in the past. The Nez Perce Culture staff are specifically concerned with Tribal programs that speak to the sacred nature of tobacco smoking, that emphasize the use and renewal of indigenous tobaccos (*N. quadrivalvis*, *N. attenuata*) over commercial cigarettes and trade tobacco (*N. tabacum*), and that demonstrate that marijuana was not a traditionally smoked plant (5).

According to an ethnographic synthesis of tobacco use in the west, kinnikinnick (*A. uva-ursi*) was the primary smoke plant used in the area where the pipes were found (ref. 20, map 8), suggesting that tobacco was not smoked here until after the introduction of trade tobacco by Euro-American explorers and traders beginning around the 1790s–1840s. Historic data demonstrate that tobacco was prized by the Nez Perce and that it may have been introduced. According to 1874 testimony from Nez Perce Chief Joseph, in the late 1700s, early French-speaking fur trappers brought many trade items, including tobacco, "which was new to us" (ref. 41, p. 12), and in 1805–1806 the Nez Perce traded horses for guns and tobacco with the explorers Meriwether Lewis and William Clark (ref. 41, p. 12). Within the next 50 y, the use of tobacco was clearly widespread, as attested to by numerous photographs of Nez Perce leaders pictured with large Euro-American–style tobacco pipes dating to as early as the 1850s (Fig. 1).

A recent regional synthesis of archaeological pipes demonstrates that pipes, while rare, are found in increasing numbers after approximately 2500 y ago into the historical period (30). The earliest archaeological pipes found in the mid-Columbia and Snake River region are associated with the Tucannon Phase (2500–500 BC), a period associated with early pithouse development (42). Many of these early pipes are of relatively large size and of a distinct form that contrasts with later precontact pipes (43). Later examples include tubular pipes with a moderately flared bowl and composite pipes consisting of a stone bowl that would have been used with a stem fashioned of some perishable material, such as wood or bone (30). Steatite, also known as soapstone, is a soft stone used in carving throughout northwest North America that appears to have been the preferred material for pipe construction in both the early and later periods of pipe use. However, more common and less easily worked materials, such as basalt and sedimentary rocks, were also used to make pipes throughout the precontact era (30).

Both traditional stone pipes and newer Euro-American historic pipes are found in protohistoric and postcontact dating

ANTHROPOLOGY

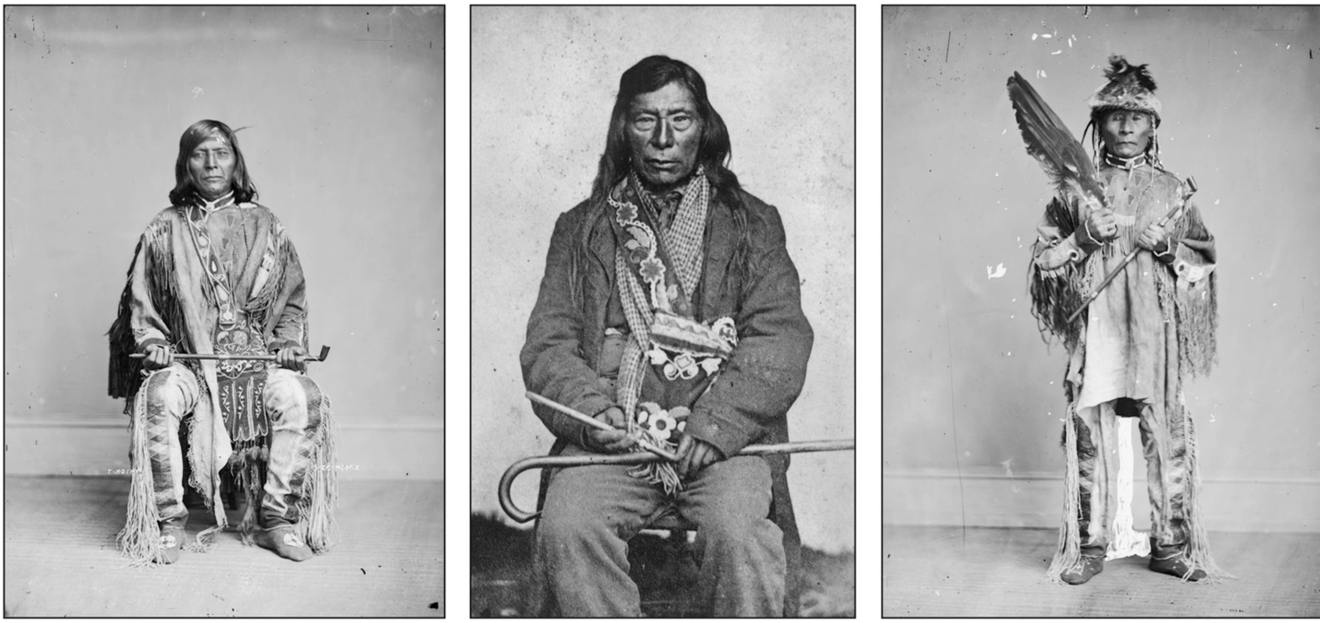

Fig. 1. Iconic images of postcontact Nez Perce (North American Plateau American Indian) with large postcontact-era–style elbow pipes in use after the spread of trade tobacco into the region. Pictured *Left* to *Right*: Chief Tamason (Timothy), 1868 (image courtesy of the National Anthropological Archives, Smithsonian Institution GN 02923A); Chief Lawyer, ca. 1861 (image courtesy of University of Washington Libraries, Special Collections, NA627); Chief Kal-kalshuatash, 1868 (image courtesy of the National Anthropological Archives, Smithsonian Institution GN 02922A).

archaeological sites in the region; colonial-style clay elbow-shaped pipes are found in increasing numbers over time, and by the mid-1800s, large, long-stemmed ceremonial pipes (an iconic style often referred to colloquially as "peace pipes") are common features of early photographs of American Indian leaders (Fig. 1). By this time, trade tobacco (*N. tabacum*) was an entrenched part of native lifeways, and while archaeological data suggest that the practice of smoking has ancient roots, exactly what plant (or plants) people were smoking has remained elusive.

Archaeological Materials

The materials analyzed in this study include 12 pipes and pipe fragments associated with four known and one unknown archaeological sites along the Snake and Columbia Rivers, in Nez Perce ancestral territory, southeastern Washington State (Fig. 2). The analyzed pipes are part of legacy collections associated with salvage excavation of archaeological sites currently inundated due to water reclamation projects in the Lower Granite Dam (Offield Bar, Wexpúsnime, and Silcott) and the McNary Dam (Wallula). Analyzed pipes were selected from archaeological contexts spanning several thousand years (*SI Appendix*, Table S1), from the historical period (AD 1900–1950), and the following Snake River archaeological phases: *Numípu* (AD 1750–1900), *Piqúnin* (AD 1300–1750), Harder (500 BC–AD 1300), and Tucannon (2500–500 BC). The tested samples include specimens from the region that date to every major archaeological phase where they have been identified; to our knowledge, archaeological pipes have not been found from earlier Windust and Cascade contexts dating to before 2500 BC.

Extraction and UPLC-MS Methods

Residue Extraction. The artifacts were submersed in clean glass beakers by acetonitrile:2-propanol:water (3:2:2, vol:vol:vol) and then sonicated for 10 min. The pipes were removed from the solvent and air-dried on blotting paper on the laboratory bench. The artifact extracts were then frozen at −80 °C and lyophilized for 3 d. The dried extracts were resuspended with 0.60 mL of 0.10% formic acid/water:acetonitrile (1:1, vol:vol) by vortexing, and then centrifuged at $10,000 \times g$ for 10 min at 4 °C. An amount of 0.50 mL from each sample was placed into amber colored autosampler vials for analysis by UPLC-MS. A solvent blank was prepared following the same extraction method as for the artifacts, but with no artifact present in the beaker.

Analysis of Target Metabolites. All solvents used for extraction and analyses were of UPLC-MS grade, and the arbutin and nicotine standards were purchased from Sigma-Aldrich. UPLC was conducted with a Waters ACQUITY UPLC system with photodiode array detection ranging from 210 to 400 nm. For this, 1 μL of sample was injected through a 2.0- μL sample loop using the partial loop injection mode onto a Waters ACQUITY UPLC HSS T3 column, 1.8 μm, 2.1 × 100 mm, and eluted at 0.32 mL·min^{-1} with 0.10% formic acid in water (A) and 0.10% formic acid in methanol:acetonitrile (2:3) (B) as solvents. The elution gradient was as follows: the initial conditions were 97% A:3% B at time 0, followed by a linear gradient to 15% A:85% B at 12.00 min, then a gradient to 3% A:97% B at 12.10 min, maintained at 3%

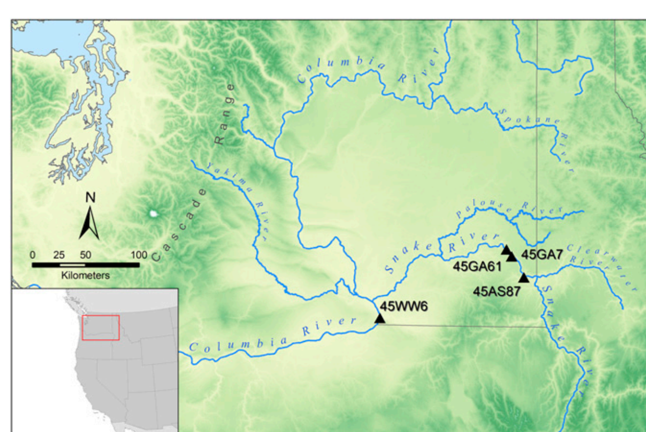

Fig. 2. Location of sites with pipes sampled in the study. Topographic and hydrologic data from the US Geologic Survey.

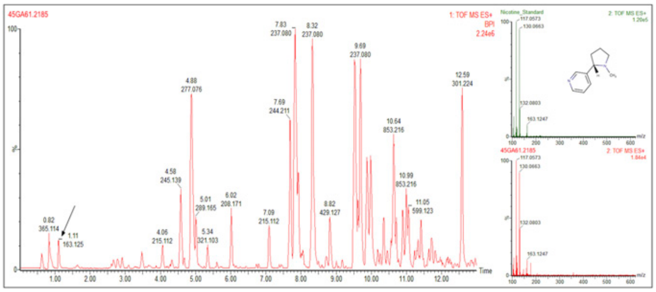

Fig. 3. UPLC-MS chromatogram of specimen 45GA61.2185 showing the presence of nicotine (black arrow) and MS^E spectra comparing a nicotine standard with the peak identified as nicotine in 45GA61.2185.

A:97% B until 14.00 min, returned to the initial conditions of 97% A:3% B at 14.10 min, and then held until 16.00 min to reequilibrate the column. The total analysis time per sample was 16.00 min. The autosampler chamber and column temperatures were 8 °C and 35 °C, respectively.

A Waters Synapt G2-S high-definition quadrupole time-of-flight MS with lockspray ionization was operated in the ESI-positive mode and resolution mass mode using MS^E with a high collision energy (ramp 15–40 V) for fragmentation. The scan range was 100–1,200 *m/z*, with a scan time of 0.3 s. The capillary voltage, sampling cone voltage, and source offset voltage were 3.0 kV, 60 V, and 60 V, respectively. The source temperature was 100 °C, with a cone gas (nitrogen) flow rate of 50 $l \cdot h^{-1}$. The desolvation temperature was 250 °C, with a desolvation gas (nitrogen) flow rate of 900 $l \cdot h^{-1}$. The nebulizer gas (nitrogen) flow was 6.0 bar. The lock mass compound was leucine encephalin (reference mass, 556.2771 *m/z* $[M-H]^+$). Under these UPLC-MS conditions, the analytical standards, arbutin (295.08 $[M+2Na]^+$ *m/z*) and nicotine (163.12 $[M+H]^+$ *m/z*), eluted at 1.75 min and 1.08 min, respectively.

Results and Discussion

The biomarker nicotine was identified via UPLC-MS (Fig. 3) in 8 of the 12 analyzed pipes from five archaeological sites in the Columbia River Basin, which date to a period spanning 1,300 or more years (Table 1). Nicotine-positive specimens include complete tubular pipes to very small pipe stem and bowl fragments fashioned of steatite, sandstone, basalt, and historic Bakelite plastic (Fig. 4). As expected, a Euro-American–style pipestem dating to the early 1900s and thus in use well after the spread of trade tobacco was found to contain nicotine (Fig. 4*H*; specimen 45AS87.1401). Nicotine was also found in a precontact-style steatite pipestem that was in use during protohistoric *Numípu* (AD 1750–1900) times (Fig. 4*F*; 45WW6.73). Less expected were the positive results associated with numerous artifacts dating to before Euro-American contact (Fig. 4 *A*–*E*), including nicotine-positive samples from deposits associated with *Piqúnin* (AD 1300–1750), and Harder (500 BC–AD 1300) phases (*SI Appendix*, Table S2). One specimen (45GA7.776; Fig. 4*A*) was recovered in earlier Tucannon Phase deposits (2500–500 BC). Although radiocarbon dating of associated materials from 45GA7 indicates the site deposits are mixed, this specimen likely dates to the Tucannon Phase, as it is stylistically similar to well-dated Tucannon Phase pipes at another site in Washington State (45DO172) (30, 43).

In addition to nicotine, we searched for arbutin, a compound associated with kinnikinnick (*A. uva-ursi*). Although its presence was expected, arbutin was not identified in any of the samples (Table 1). The lack of arbutin suggests that kinnikinnick was not smoked in any of these pipes, although there are two alternative interpretations: (*i*) the plant was smoked in these pipes but could not be identified in detectible amounts through our process, or (*ii*) that our sample size was insufficient to capture this form of smoking.

Conclusion

Tobacco has clear negative health consequences worldwide, yet we still have much to learn about the evolution of the human–tobacco relationship. The attractiveness of psychoactive plants to humans is clearly related to their physiological effects when ingested. Tobacco's powerful pull on humans is due to its intoxicating properties and addictive nicotine content, which is the result of thousands of years of coevolution with people. Historical processes have obscured our understanding of early tobacco use by indigenous peoples of western North America. One of the greatest puzzles in northwestern North American archaeology is the timeline and trajectory of indigenous tobacco consumption by Plateau hunter-gatherers before the introduction of domesticated trade tobacco (*N. tabacum*) (13, 24). This study establishes a pattern of ongoing tobacco smoking in this region from at least 1,200 y ago through the historical period. These results conflict with ethnohistoric descriptions of smoking practices on the Columbia Plateau; the sites represented in this study are located securely within a region previously portrayed as an area where only kinnikinnick was smoked and tobacco was a completely introduced trade commodity (20). Thus, it appears that when the famous leader Chief Joseph stated that

Table 1. Archaeological pipe sample results summary

Site/specimen ID	Description	Archaeological phase*	Nicotine, +/–	Arbutin, +/–
Offield Bar (45GA7)				
45GA7.776	Complete basalt tubular pipe, ovular in cross-section	Tucannon/Harder	+	–
Wexpúsnime (45GA61)				
45GA61.12400	Sandstone pipe bowl fragment	Harder	+	–
45GA61.2828	Sandstone pipe fragment	Harder	+	–
45GA61.2185	Sandstone pipe bowl fragment	Harder/*Piqúnin*	+	–
45GA61.2187	Steatite pipe bowl fragment	Harder/*Piqúnin*	–	–
45GA61.2456	Sandstone pipe fragment, likely bowl	Harder/*Piqúnin*	+	–
45GA61.4593	Steatite pipe fragment	Harder/*Piqúnin*	–	–
45GA61.5249	Steatite pipe fragment	Harder/*Piqúnin*	–	–
45GA61.11902	Sandstone bowl fragment	Harder/*Piqúnin*	–	–
Walulla (45WW6)				
45WW6.73	Steatite pipe stem/mouthpiece fragment	*Numípu*	+	–
Silcott (45AS87)				
45AS87.1401	Historic Bakelite pipe stem	Historic	+	–
Columbia River site				
45UNUN	Complete tubular sandstone pipe	Unknown	+	–

*Established archaeological phases for the Snake River Region (44); see also *SI Appendix*, Table S2.

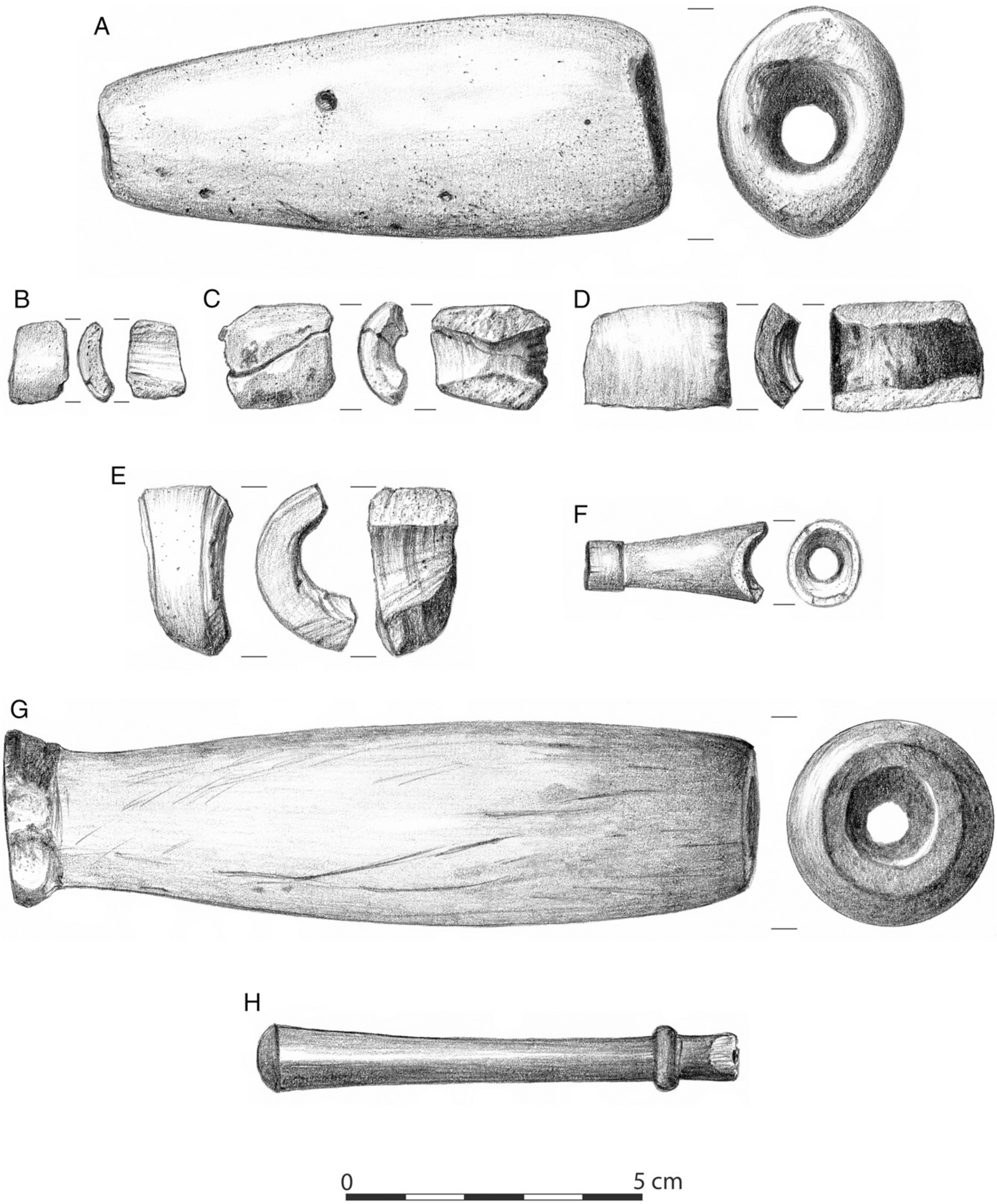

Fig. 4. Nicotine-positive archaeological pipes and pipe fragments analyzed in the study. (*A*) Complete tubular pipe from Offield Bar, specimen 45GA7.776. (*B*) Pipe bowl fragment from Wexpúsnime, 45GA61.12400. (*C*) Pipe fragment from Wexpúsnime, 45GA61.2828. (*D*) Pipe bowl fragment from Wexpúsnime, 45GA61.2185. (*E*) Pipe bowl fragment from Wexpúsnime, 45GA61.2456. (*F*) Pipe stem from Walulla (45WW6.73). (*G*) Tubular pipe from unknown Columbia River site, 45UNUN. (*H*) Historic pipe stem from Silcott, 45AS87.1401. Pipe data are provided in Table 1. Image courtesy of Tammara Norton (artist).

French traders introduced tobacco to the Nez Perce in the early 1800s (41), he was specifically referring to trade tobacco.

Commercially grown species of tobacco have replaced indigenous tobacco and use of other smoke plants among many native peoples, a process that began several hundred years ago during earliest days of European colonization of the Americas. The shift from traditional smoking of indigenous tobaccos and other smoke plants to commercial tobacco products has had significant deleterious effects on tribal culture and health (5, 45, 46). Increased awareness of the history of tobacco and its role in the process of colonialization is critical to future smoking abatement programs, as is distinguishing between commercial manufactured tobacco and tobacco used in ceremonial and other traditional manners (often distinguished as "sacred tobacco" by native peoples) (4, 47). Indeed, few realize the extent to which domesticated strains of commercial tobacco have replaced indigenous species of tobacco and other smoke plants. In many cases, commercial tobacco has become the tobacco used for ceremonial purposes, "replacing the many *Nicotiana* species or other botanicals originally used by Indians who had a religious smoking tradition" (ref. 4, p, 133; see also refs. 47 and 48). This information can be helpful to Tribal efforts toward educating youth about the dangers of commercial tobacco and the sacredness of indigenous tobaccos. Rejuvenation of additive-free indigenous tobaccos (e.g., *N. quadrivalvus*, *N. obtusifolia*) used in traditional contexts can be part of this process, including sacred use practices in daily or ceremonial contexts (e.g., tobacco smudging, tobacco used as gifts and as offerings) (13, 33, 47, 49, 50). Future studies that include a larger suite of smoke plants may shed new light on the exact species and mixtures that were being smoked in these archaeological pipes. Such research will continue to provide insight into the great time depth of tobacco's use by humans—and the coevolutionary processes that ultimately led to the rise and spread of this powerful and intoxicating plant.

ACKNOWLEDGMENTS. We thank Josiah Pinkham and Nakia Williamson (Nez Perce Tribe) for their insights, support, and encouragement, as well as Mary Collins and Marsha Quinlan (Washington State University), Jackie Cooke and Guy Moura (Confederated Tribes of the Colville Reservation), Jack Nisbet, and David Rice. We also thank Leah Bonstead, Alice Roberts, and the US Army Corps of Engineers Walla Walla District for supporting the research and curation of legacy collections. This research is based on work supported by the National Science Foundation under Grant BCS-1419506.

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