Human Lice in Paleoentomology and Paleomicrobiology

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ABSTRACT Lice are a classic example of cospeciation. Human lice confirm this cospeciation with lice specialized in hominids which differ from those of gorillas and chimpanzees. Head lice and body lice seem to belong to closely related species with different ecotypes and a different geographical distribution which may reflect population movements. Paleo-entomology allows us in some cases to trace the migrations of archaic human populations. The analysis of lice found on mummies in Egypt and South America has clarified a certain number of these migrations, also the study of lice and the diseases they transmit has shed a new light on the epidemics of the past.

Lice (Insecta, Phthiraptera) are permanent obligate parasites of birds and mammals. Approximately 4,900 species of lice are recorded and distributed into four suborders: chewing or biting lice, including Rhynchophthirina, Ischnocera, and Amblycera, and sucking lice, Anoplura ([1]).

The recent discovery of two fossils, with estimated ages of 44 million years ([2]) and 100 million years ([3]), provides an indication about the origin of lice. These insects even survived the mass extinctions of species 65 million years ago, corresponding to the Cretaceous–Paleogene (K–Pg) boundary ([4]).

Among the 15 families of lice included in the suborder Anoplura, two are found in humans ([5]). The family Pediculidae contains the genus *Pediculus*, which is shared by humans and chimpanzees, and the family Pthiridae contains the genus *Pthirus*, which is shared by humans and gorillas ([6]). With the use of molecular clock analysis, a divergence time of 11.5 million years ago was found for the *Pthirus*–*Pediculus* split, and a divergence time of 5.6 million years ago was found for the split between *Pediculus schaeffi*, found in chimpanzees, and the human louse *Pediculus humanus* ([7], [8]). The age of the most recent common ancestor of the two *Pediculus* species studied matches the age predicted by the host divergence (approximately 6 million years), whereas the age of the ancestor of *Pthirus* does not. The two species of *Pthirus* (*Pthirus gorillae* and *Pthirus pubis*) last shared an ancestor approximately 3 million to 4 million years ago, which is considerably later than the divergence between their hosts (gorillas and humans, respectively) at approximately 7 million years ago. This would be the result of host switching from archaic gorillas to archaic hominids at roughly 3 million years ago via direct contact between them ([9]).
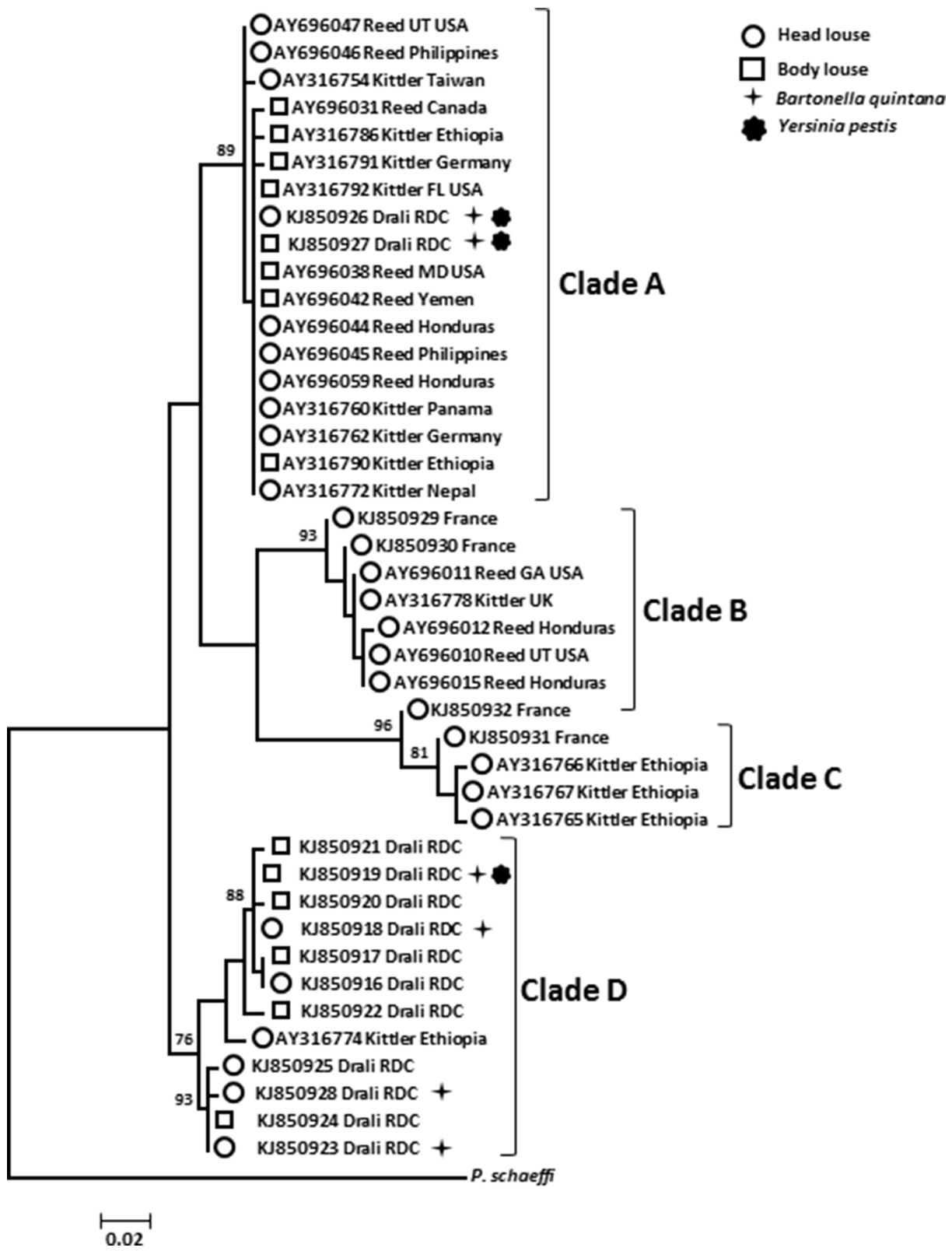
Population expansion in human lice is coincident with the out-of-Africa expansion of humans (100,000 years ago) ([54]). Molecular clock analysis of head and body lice indicates that body lice originated 72,000 ± 42,000 years ago, which correlates with the expansion of humans out of Africa and the wearing of clothing for protection from the colder climatic conditions ([10]).

MODERN HUMAN LICE

P. humanus humanus, the body louse, and *P. humanus capitis*, the head louse, are two louse ecotypes, each of which occupies an ecological niche in its host. The head louse lives and breeds in the hair of the head, whereas the body louse lives in clothing, where it lays its eggs in the seams and folds (11).

Pediculosis due to the body louse affects exclusively precarious populations, such as the homeless, prisoners, and war refugees (12). In contrast, head lice preferentially infest schoolchildren, with hundreds of millions of cases reported each year worldwide, regardless of hygienic conditions (13).

For some time, various comparative studies were unable to differentiate between these two types of lice. As recently as 1919, Nuttall designated body lice and head lice as two ecotypes representing extreme variations of the same species because they could not be distinguished in all essential points of structure (14). However, the advent of molecular biology allowed the use of several genetic markers in the analysis of lice in an attempt to find answers to the various remaining questions.

The analysis of mitochondrial genes allowed the classification of *P. humanus* into three different clades, designated clades A, B, and C. Only clade A comprises both head and body lice and is distributed worldwide (7). Clade B contains head lice found in the Americas, western Europe, Australia, and North Africa, whereas clade C contains head lice found in Nepal, Ethiopia, and Senegal (15). The study of lice recovered from pre-Columbian mummies made it possible to determine that clades A and B were present before the arrival of European settlers in the Americas (16, 17), reinforcing the assessment that lice belonging to clade B were of American origin and were subsequently introduced into the Old World through the return of European settlers (15). A fourth mitochondrial clade, clade D, is found in Africa, in the Democratic Republic of the Congo. Similar to clade A, clade D contains both body and head lice (Fig. 1).

Regarding nuclear genes, the phylogenetic analysis of 18S rRNA sequences showed that human lice can be classified into those from sub-Saharan Africa and those from other regions (18). Conversely, analyses targeting elongation factor-1α and RNA polymerase II genes showed that there is more diversity in African than in non-African lice, and more diversity in head lice than in body lice (10).

Two studies based on the use of intergenic spacers have revealed existing associations between the geographical sources and genotypic distributions of lice (19, 20). It has also been suggested that body louse populations may emerge from local head louse populations under poor hygienic conditions (20).

After two centuries of debate about the ecological and genotypic status of head and body lice, the comparison of their transcriptional profiles showed that both ecotypes have the same number of genes, with the exception of one gene that is missing in the head louse (PHUM540560) (21). Subsequently, analysis of a portion of this gene in 142 head and body lice belonging to mitochondrial clade A, collected from mono-infested hosts from 13 countries on five continents, showed that the PHUM540560 gene is present in both ecotypes. However, 22 polymorphisms were characterized between the sequences of body and head lice, allowing the development of a valuable tool using multiplex reverse transcription-PCR (RT-PCR) to differentiate quickly between the two ecotypes (22). Now, it is possible to differentiate between head and body lice, including those collected from the head and clothing of dually infested individuals (23).

To date, only the body louse is considered to be the vector of three dangerous diseases that have ravaged entire populations throughout history (24): epidemic typhus, trench fever, and relapsing fever, which are caused by *Rickettsia prowazekii*, *Bartonella quintana*, and *Borrelia recurrentis*, respectively (25). Body lice are also suspected in the transmission of *Yersinia pestis*, the agent of plague (26–28).

In recent years, the DNA of *B. quintana* was detected in head lice belonging to clade A (12, 28–30) and clade C (31, 32), whereas the DNA of *B. recurrentis* was found in head lice belonging to clade C (33) and the DNA of *Y. pestis* was detected in head lice of clade A and clade D (Fig. 1).

FIGURE 1 Maximum likelihood (ML) phylogram of the cytochrome *b* mitochondrial gene. ML bootstrapping supporting values greater than 75 are located above the nodes. Mitochondrial clade memberships are indicated to the right of each tree. GenBank accession numbers, manuscript lead author, and locality are indicated for each louse specimen. Localities are abbreviated as follows: Florida, FL; Georgia, GA; Maryland, MD; Democratic Republic of the Congo, RDC; United Kingdom, UK; Utah, UT.

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HUMAN LICE FROM ARCHAEOLOGICAL EXCAVATIONS AND HUMAN BODY REMAINS

The three human lice (i.e., head, body, and pubic lice), together with the two follicle mites, *Demodex folliculorum* and *Demodex brevis*, are most likely the oldest permanent ectoparasites of humans. Lice are mentioned in the Bible as the third plague visited on the Egyptians when Pharaoh denied the request of Moses to let the Israelites go (Exodus 8:16). In the 16th century BC, an Egyptian text known as the Papyrus Ebers described a remedy for lice prepared from date flour.
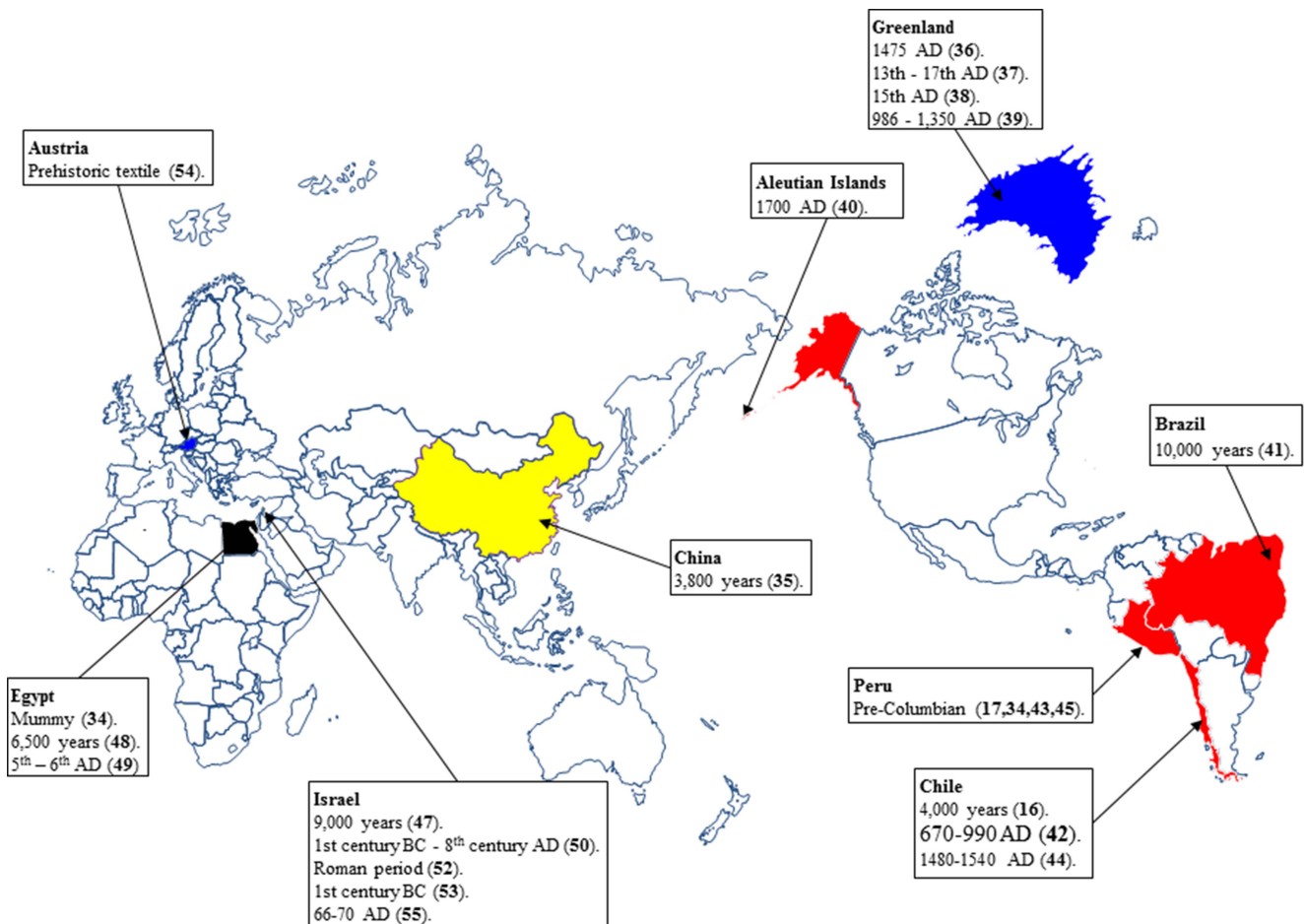
In recent decades, ancient lice and nits have been recovered in excavations and human body remains from different archaeological sites around the world (Fig. 2). The first ancient lice were isolated from Egyptian mummies in 1924 by Ewing (34). Later, head lice and eggs were found on mummies in China (35), Greenland (36–39), the Aleutian Islands (40), and South America (16, 17, 34, 41–46).

Head louse eggs were recovered on hair from human remains found in Brazil and were carbon-dated to approximately 10,000 years (41); in addition, 9,000-year-old louse eggs were found on hair samples from an individual who lived in Nahal Hemar Cave near the Dead Sea in Israel (47).

Head louse combs, very similar to modern louse combs, were already being used for delousing in Egypt in Pharaonic times (approximately 6,500 years ago) (48). Seven head lice were recovered from the debris found among the fine teeth of a wooden comb excavated in Antinoë, Egypt, and were dated to between the 5th and 6th centuries AD (49).

Head lice and their eggs were found in 12 of 24 hair combs recovered from archaeological excavations in the Judean and Negev Deserts of Israel, including from Masada and Qumran (50, 51). More recently, the head and apical part of one of the legs of a head louse were found in a wooden louse comb from the Roman period

FIGURE 2 Map showing major areas of ancient lice recovery worldwide. The numbers in parentheses refer to references.

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excavated in the "Cave of the Pool," close to the En Gedi Oasis near the Dead Sea (52). A head louse egg dating to the 1st century BC was also found in a louse comb excavated in the Christmas Cave in the Qidron Valley near the Dead Sea and Qumran (53).

Most of the combs found in archaeological excavations are made of wood, although some are made from bones and ivory. Most of the combs have two sides: one side to open hair knots and another to remove lice and eggs. It can be assumed that these handmade combs were very effective in mechanically removing lice and eggs because they were not smooth or flexible; however, they were therefore more painful to use.

Body lice eggs were found in a prehistoric textile from Hallstaetter / Salzberg in Austria (54) and from deposits of farmers in Viking Greenland, dated to 986 to 1350 AD (39).

The remains of a body louse were also found in one of the rooms (the Casemate of the Scrolls) at the fortress of Masada. Originally constructed during the last decade of King Herod's reign, the casemate room was converted into a dwelling unit during the first Jewish revolt against the Romans. Following the conquest of Masada, the room was used by Roman soldiers as a dumping area. The context of the textiles associated with the louse and their nature clearly suggest an origin at the time of the rebellion (55).

Ancient specimens of pubic lice were found from a 2,000-year-old South American mummy (56), in human remains (mid-1st or 2nd century AD) from the Roman era in Britain (57), from post-medieval periods in Iceland, and in 18th century London (58–60).

MOLECULAR ANALYSIS OF ANCIENT LICE

Since the 1950s, paleoparasitological studies have contributed significantly to the understanding of parasite evolution and ecology (61). Because lice are obligatory parasites that complete all stages of their development in hosts, they are excellent markers of the evolution of humans (7). Ancient lice found in mummies and buried human remains are particularly useful in providing valuable information for tracking parasite and host migrations over time. Although studies based on molecular analyses of ancient lice are not numerous, it is clear that the results obtained are amazing.

In 2008, by analyzing two mitochondrial genes (*cytb* and *cox1*) in 1,000-year-old head lice collected from a Chiribaya mummy in Peru, Raoult et al. showed that the most prevalent and well-distributed mitochondrial clade of human lice – namely, clade A – had a pre-Columbian presence in the Americas (17). By analyzing a partial sequence of the mitochondrial cytochrome *b* gene in two operculated nits collected from a 4,000-year-old Chilean mummy, Boutellis et al. in 2013 showed that head lice belonging to clade A and clade B, which predated the arrival of European settlers in the Americas, can live in sympatry (16). In 2015, Drali et al. developed a tool based on real-time PCR to test ancient head louse eggs recovered in Israel and dating from the Chalcolithic period (fourth millennium BC) and the early Islamic period (650 to 810 AD) (22). The phylogenetic analyses of these head louse egg sequences showed that they most likely were associated with people originating in West Africa because they belonged to the mitochondrial subclade specific to that region (Fig. 3).

MOLECULAR ANALYSIS OF ANCIENT LICE ASSOCIATED WITH BACTERIA

Proving the presence of louse-borne pathogens in ancient lice can help to determine the cause of death when a mass grave is discovered. The most broadly used approach for achieving this is the detection and characterization of DNA (62). Host-associated microbial DNA can persist for 20,000 years (63), and bacterial DNA preserved in permafrost specimens has been dated to up to 1 million years (64).

Thus, in 2006, Raoult et al. concluded that the louse-borne infectious diseases that affected approximately one-third of Napoleon's soldiers buried in Vilnius, Lithuania, might have been a major cause of mortality during the French retreat from Russia (65). DNA from *B. quintana*, the agent of trench fever, was detected by PCR and sequenced in three of five body lice segments identified morphologically and molecularly by targeting a portion of the mitochondrial reduced nicotinamide adenine dinucleotide (NADH) dehydrogenase subunit 4 (*ND4*) gene. Similarly, DNA of *B. quintana* was found in the dental pulp of seven of 35 soldier remains, and the DNA of *R. prowazekii*, the agent of typhus, was found in another three soldiers (65).

In other studies, pathogens transmitted by lice have been identified successfully in the dental pulp of human remains from other sites and different periods. However, the presence of lice at archaeological sites from which human remains were extracted has not been reported. Because it is known that human infestation by lice was almost continuous during past periods (24), one might suspect that the absence of these parasites at archaeological sites is due to their degradation. For example, in 1998, Drancourt al. detected *Y. pestis* DNA in the dental pulp of a human who died 400 years ago, at a time when
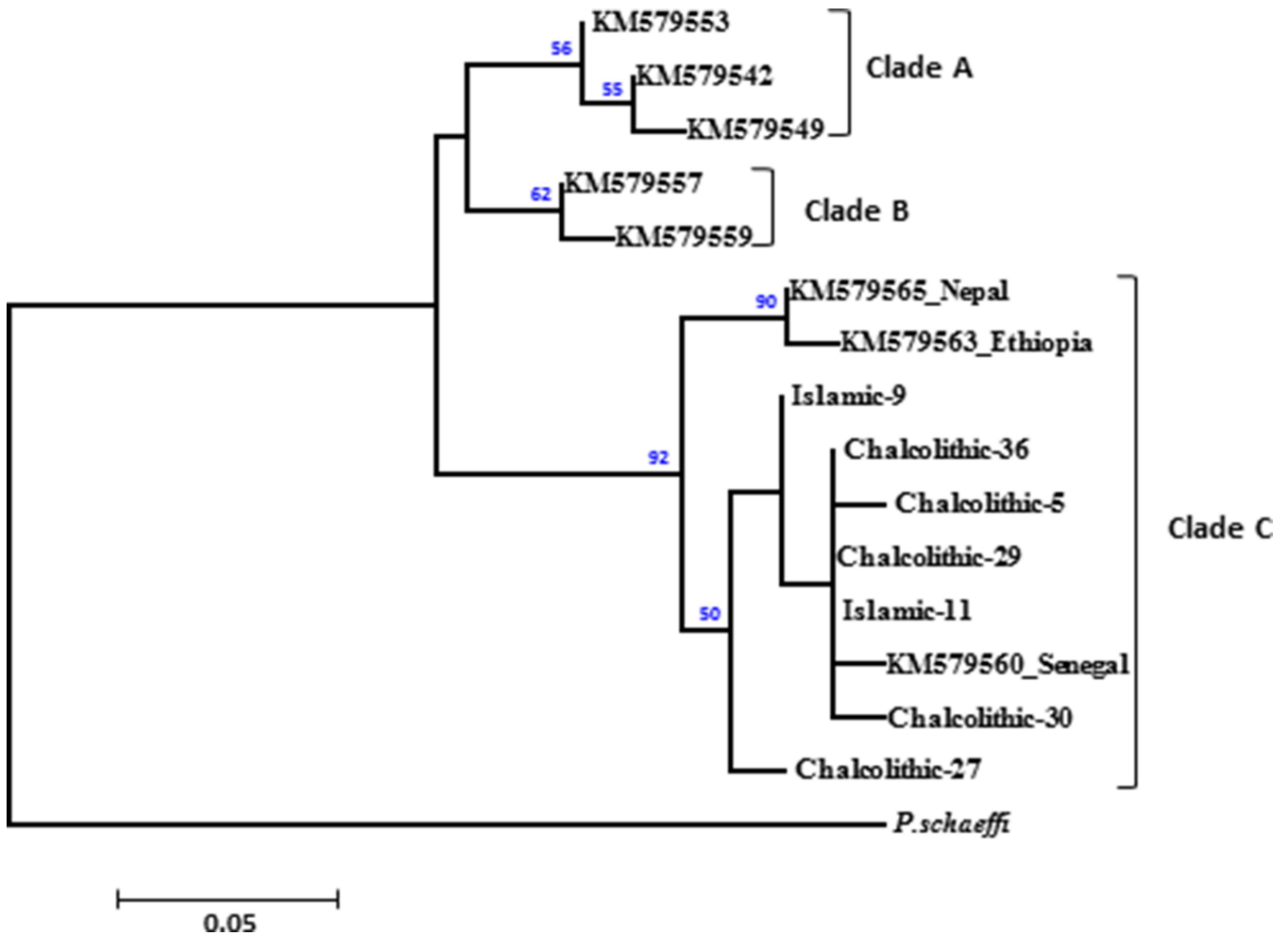
FIGURE 3 Maximum-likelihood (ML) phylogram of the cytochrome *b* mitochondrial gene. ML bootstrapping supporting values greater than 50 are located above the nodes. Mitochondrial clade memberships are indicated to the right of each tree.

the plague was raging in France ([66](#)). In 2005, the DNA of *B. quintana* was found in the dental pulp of a person who died 4,000 years ago ([67](#)). DNA of *B. quintana* and *R. prowazekii* was also detected in ancient remnants of bodies from graves in Douai, France. This result supports that typhus and trench fever were involved in the decimation of the besiegers of Douai (1710 to 1712) during the War of the Spanish Succession ([68](#)).

CONCLUSION

The study of ancient parasites provides an excellent opportunity to access additional information in time and space, facilitating the understanding of parasite–host relationships. Identifying only the present configurations of genetic diversity or geographical distribution is not sufficient for understanding all the events that have marked

this association. The main objective of paleoparasitology is to forge links between the past and present.

Thus, as we have observed through the study of ancient lice, it is possible to trace the migratory movement of *Homo sapiens* through the centuries, and it is also possible to learn more about the circulation of pathogens transmitted by lice. Lice have proved to be an exceptional record that allows the construction of credible scenarios, so that what occurred in the past can be understood upon the discovery of mass graves.

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