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Review

Apes, lice and prehistory

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Abstract

Although most epidemic human infectious diseases are caused by recently introduced pathogens, cospeciation of parasite and host is commonplace for endemic infections. Occasional host infidelity, however, provides the endemic parasite with an opportunity to survive the potential extinction of its host. Such infidelity may account for the survival of certain types of human lice, and it is currently exemplified by viruses such as HIV.

Hans Zinsser's *Rats, Lice and History* [1] is a classic in microbiology. Written in 1934 and subtitled *The biography of a bacillus*, it tells the tale of that dreaded disease typhus, its reservoir in rats and its transmission among humans by lice. Here, I discuss how we may in the course of prehistory have acquired the lice, and how other infections may, like the typhus bacillus, come to be shared by us and the animal species with which we are in close contact. It is a tale of infidelity that I shall begin with the recent research on lice of David Reed and colleagues [2,3] and of Mark Stoneking's group [4] who, on the basis of phylogenetic analysis, have speculated that we may have acquired a clade of head lice from another hominid species and pubic lice from gorillas; they have also suggested that lice might help determine the date when humans adopted clothing. I shall examine this unfolding story in the context of what we know about microbial infections, and will look at the promiscuity of viruses through the lens of modern molecular technology; and I will add my own speculation on why naked apes have pubic hair.

Lice are small, wingless insects that cannot live independently from their hosts (Figure 1). They are frequent parasites of birds and mammals, each host species having its own type of louse. Humans harbor three kinds of these ectoparasites: head lice, body lice and pubic lice. For much of human history and prehistory blood-sucking lice have been so prevalent that they became part of our everyday language. We speak about feeling lousy and admonish our friends for nit-picking. Nits are the eggs of lice, expertly cemented onto hair shafts, as many parents know from painstakingly combing them out of their children's hair. Grooming among monkeys and apes is not only a means of social bonding but also a useful way of controlling nits and lice (Figure 2).

Family heirlooms and new acquisitions

Writing on infections of humankind, Tony McMichael and I [5] have called those that cospeciated with their hosts 'family heirlooms' and those that crossed over from other hosts in recent evolutionary time 'new acquisitions'. The new acquisitions were initially derived from zoonotic infections but have flourished as self-sustaining infections in the human population. They have diverged from their progenitors in the original host: for example, measles is now distinct from rindepest. The majority of zoonoses, however, remain in their animal reservoirs and, so far as their sojourn in humans goes - even with limited human-to-human transfer (as with Ebola or severe acute respiratory

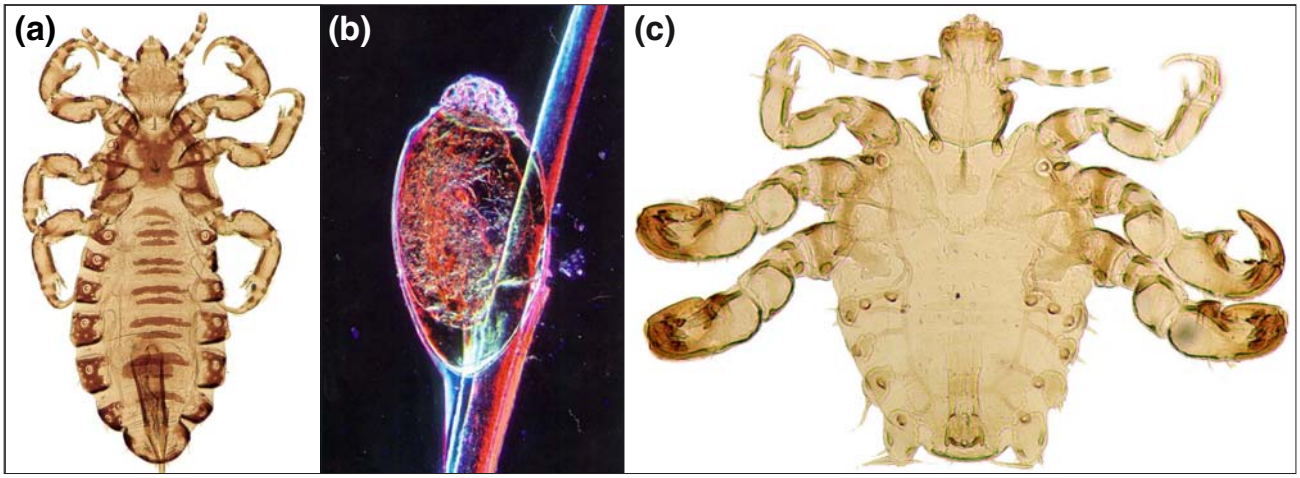

Figure 1
Human lice. **(a)** Head louse (*Pediculus humanus*). **(b)** Nit (egg) of head louse. With permission from www.headlice.org. **(c)** Pubic louse or 'crab' (*Pthirus pubis*). (a) and (c) are by Vince Smith and are reproduced with permission.

syndrome (SARS)) - we can regard them as 'temporary exhibits'.

Human DNA might show 98% similarity to that of chimps, but we share less than 50% of our microbes and parasites with them. Ashford [6] argues that the great apes became more specialized forest dwellers at the same time that early hominids explored the savannah, and that human gut parasites resemble those of omnivorous baboons more than those of chimps because humans, like baboons but unlike chimps, are omnivorous. Further opportunities for horizontal crossover of microbes and parasites from animals to humans arose when humans spread out of Africa. When we domesticated ruminants, and animals such as dogs, cats and rats 'domesticated' us for the rich pickings around human habitation, we acquired many infections from our new neighbors [5,7]. Thus a shared habitat, rather than a shared ancestry, is important for the acquisition of many infections.

Most human pandemic infections were acquired horizontally very recently on the evolutionary timescale, even though diseases such as typhus, measles and smallpox first occurred in prehistoric times. These new acquisitions originate from evolutionarily distant host species. The influenza pandemic of 1918-1919 came from birds, and the 1968 influenza strain could be an avian-porcine recombinant. Today's novel viral infections are more likely to originate from exotic species than from animals that were domesticated long ago [5]. The market for 'bushmeat' has led to Ebola virus outbreaks in Africa from butchering primates, and to the SARS outbreak in China from eating small carnivores, such as civet cats. The primary reservoirs of the Ebola filovirus, the SARS coronavirus and the Nipah paramyxovirus, however, seem to be in fruit bats (flying foxes).

Lice and nits have been found in textiles, hair and combs excavated from archaeological sites [1,8]. Given that the closest relative of the human head and body louse, *Pediculus humanus*, is *P. schaeffi*, which infests chimpanzees, one might assume that human and chimp lice have cospeciated with their hosts as family heirlooms ever since they diverged from a common ancestor. This requires, however, that the divergence among hosts and parasites approximates to the same timescale. After all, the closest relative to human immunodeficiency virus type 1 (HIV-1) is the simian immunodeficiency virus of chimpanzees (SIVcpz), but it would be facile to suggest that HIV-1 co-evolved with humans, because molecular clock estimates place the most recent common ancestor of the pandemic form of HIV-1 at 75-100 years ago [9,10], and this is likely to be close to the time of the species crossover event. HIV-1 has invaded humans at least three times (groups M, N and O), and such is the power of modern forensic DNA virology that the precise location in Cameroon has been mapped for the chimpanzees carrying the SIVcpz most closely related to group M [11,12].

Origins of head and body lice

Using nuclear and mitochondrial DNA markers, Reed *et al.* [2] estimated the divergence of chimp and human *Pediculus* lice at 5.5 million years ago (MYA) and provided evidence of cospeciation with their hosts. A recent revision to 4.1 MYA for the most recent common ancestor of chimps and humans [13] may require a similar adjustment of the

Figure 2
Nit-picking is an ancient habit, as seen in **(a)** apes (photograph by Eric C Matthews, reproduced with permission) and in humans as shown in **(b)** a painting by Jan Siberechts, *Cour de ferme*, 1662. Musée des Beaux-Arts, Brussels, Belgium.

louse molecular clock. What is more remarkable, however, is that Reed *et al.* [2] found that human lice split into two quite distinct clades, A and B, about 1.18 MYA. There is a worldwide clade (which includes both head and body lice) and a New World clade (exclusively head lice). So how can humans harbor two clades of louse that diverged from each other over one million years ago, when that separation is tenfold older than the emergence of *Homo sapiens*?

The answer, Reed *et al.* [2] suggested, is that the separation took place around the time of divergence of the ancestors of modern humans from *Homo erectus*. These two hominid lineages then co-existed for about one million years until the demise of *H. erectus*. When modern humans radiated across Asia they might have had contact with *H. erectus*, just as in more recent millennia *H. sapiens* met *H. neanderthalensis* in Europe, as dramatized by the Nobel laureate William Golding [14]. There is no evidence that different human species interbred, but they may well have exchanged ectoparasites. Thus, the New World clade of head louse may have crossed horizontally from *H. erectus* to *H. sapiens* within the last 100,000 years.

Zinsser [1] noted that the hair of ancient Peruvian mummies and the scalps of pre-Columbian Native Americans contained nits or lice. Recent DNA analysis of lice from similar remains indicates that they belong to the worldwide clade A, so this clade must have been present in pre-Columbian American populations [15]. A third clade of head lice has been delineated in Ethiopia and Nepal and this clade, C, diverged from clades A and B about 2 MYA [16]. If Reed *et al.* [2] were correct to postulate that clade B came from *H. erectus*, one must wonder in which hominid population might clade C lice have maintained their separate identity.

Lice and clothing

Head and body lice used to be designated *Pediculus capitis* and *P. corporis* but they are now known to belong to the same species, *P. humanus* [16,17]. Fifty years ago Levene and Dobzhansky [18] showed that head lice could be trained or adapted to become the rather larger body lice by attaching them to the body in small pill boxes. As we celebrate the 150th anniversary of Darwin's *Origin of Species* we might recall that it was Theodosius Dobzhansky, an eminent evolutionary biologist and a Russian Orthodox Christian, who in 1973 famously challenged creationists by declaring that "Nothing in biology makes sense except in the light of evolution." His research on lice was no exception.

Kittler *et al.* [4] initially reckoned that body lice diverged from head lice approximately 70,000 years ago, but they

later increased this estimate to 107,000 years ago by correcting an error concerning the original outlier sequence. They postulated that this date of about 100,000 years ago coincided with or followed soon after the origin of clothing, because the naked human body is an inhospitable place for lice to breed. Head lice feed on the scalp and breed in hair, whereas body lice feed on the skin but breed in clothing.

More extensive phylogenetic analyses [16,17] indicate that body lice evolved from head lice several times within the worldwide clade A, as they are found in many branches of the cladisitic tree. Multiple derivations of body lice from head lice had already been considered by Zinsser [1], and it makes good sense if one considers that clothing was not a single invention. Wearing animal pelts fur-side next to the skin would have provided a suitable place for lice to breed before fabrics were developed with the inventions of spinning and weaving.

In 17th and 18th century Europe, most of the aristocracy and gentry shaved their hair and wore wigs. Had this custom arisen to protect them from lice as Zinsser [1] suggests? Not according to Samuel Pepys' diary, as he complained more than once about his wig being infested: "Thence to my barbers, to have my periwig cleared of its nits." I wonder if they were head or body lice - is a wig hair or clothing?

Lice and nudity

Why naked apes are naked and when we 'lost' our hair has long been disputed, as discussed by Desmond Morris in *The Naked Ape* [19]. Rantala [20] suggested that nakedness could have had a selective advantage to rid the body of lice and other ectoparasites, a view also championed by Pagel and Bodmer [21], who added that being seen to be free of lice would be a fitness indicator and a good mating strategy. I am rather drawn to the theory first postulated by Alister Hardy [22] that humans evolved through an aquatic stage, although most anthropologists disparage this hypothesis. Yet Ashford [6] points out that several parasites specific to humans, such as three *Schistosoma* species, depend for their transmission on our entering water, which again distinguishes us from the great apes.

Pubic lice

Pubic lice are commonly called crabs because of their appearance (Figure 1c). They belong to a different genus, *Pthirus* (a misspelling of *Phthirus* dating back to Linnaeus), from head and body lice. On the basis of morphology, human *Pthirus pubis* is closely related to the gorilla louse, *Pthirus gorillae*. In a recent paper David Reed [3] chose a punning Miltonian title, "Pair of lice lost or parasites regained", because it poses the conundrum of whether all the great apes had variants of both *Pediculus* and *Pthirus* and lost one or the other, or whether humans gained *Pthirus* in addition to *Pediculus*.

Molecular phylogeny indicates that human pubic lice diverged from gorilla lice as recently as 3.3 MYA [3], whereas the chimp and human host lineage split from the gorilla lineage at least 7 MYA (Figure 3). Thus, it seems clear that humans acquired pubic lice horizontally, possibly at the time of the *Pthirus* species' split and probably directly from gorillas. Because they were already adapted to the coarse body hair of the gorilla, crabs would have found a suitable niche in human pubic hair. Indeed, the diameter of the hair is most likely the key rather than the pubic region, because pediatric infestation of *P. pubis* is well documented: the crab is then found on the eyelashes of the infant.

Origin of pubic hair

Reed *et al.* [3] suggest that the most recent common ancestor of the genera *Pediculus* and *Pthirus* was about 12.5 MYA, which is earlier than the estimated divergence of gorillas and the chimpanzee-human lineage. So was there duplication and separation of lice in the African anthropoid ape lineage, where they could have occupied separate ecological niches, rather as human head lice and pubic lice do today? Although this is an intriguing hypothesis, I was having difficulty envisioning a clear separation of habitats between the groin and other parts of our ancient common ancestor. My 'eureka moment' came, appropriately enough, in the shower: although naked apes have pubic hair, surely our hairy cousins don't?

How could I test my hypothesis? I knew that there was a stuffed chimpanzee in the Grant Zoological Museum at University College London and I called in on the way to my laboratory. Alas, he was a juvenile, which left the question open. A brisk walk across Regent's Park to inspect the adult gorillas in their splendid new pavilion at London Zoo strengthened my suspicion, and this was later confirmed by a visit to the chimpanzees at Whipsnade Zoo north of London. Indeed, as I noted previously [23], all the species of apes, Old World monkeys and New World monkeys seem to be less hairy in the pubic region than elsewhere; fur is present but it is short and fine.

Why do adult humans sport a thick bush of wiry pubic hair, uniquely among primates? It must surely be because we are otherwise naked. It probably serves both a visual and an odorous function, because hair aids the distribution of apocrine scent secretion, like our less visually stunning

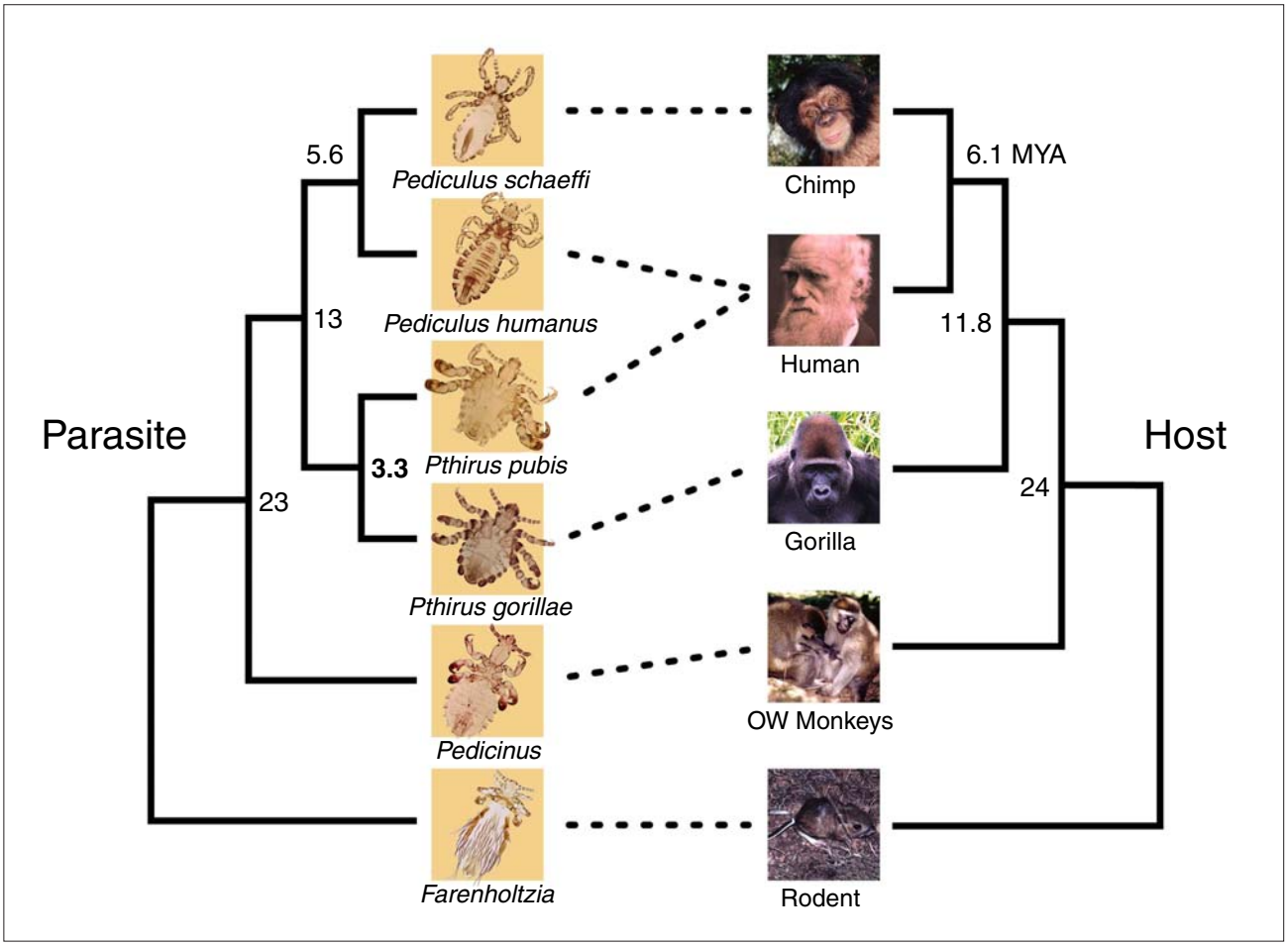

Figure 3
Host and louse phylogenies. Dotted lines indicate which lice parasitize which host. MYA, million years ago; OW, Old World. Adapted from Reed *et al.* [3].

axillary hair. Unlike a beard, pubic hair is not sexually dimorphic, yet it is a feature of sexual maturation. No wonder that pubic lice are said to be the most contagious of all sexually acquired infections. Which came first, nakedness or pubic hair? I would postulate that the development of pubic hair was a consequence of the visible nakedness elsewhere on the body. Perhaps the acquisition of *P. pubis* 3.3 MYA provides the clue to when hominids developed thick pubic hair, rather as the evolution of body lice is thought to be broadly contemporaneous to the development of clothing.

It is noteworthy that the prevalence of infestation by pubic lice seems to be decreasing among women and men who remove their pubic hair using 'bikini wax', rather as the Renaissance painters discreetly depilated classical female nudes. A study from The General Infirmary, Leeds, UK, records the increasing predilection among attendants at a clinic for sexually transmitted infections to undertake extensive pubic hair waxing known as the 'Brazilian', leaving only a thin strip of hair. Armstrong and Wilson [24] noted a significant fall in the incidence of pubic lice among patients, although gonorrhea and chlamydia increased over the same period. Thus, there may be a health benefit to this emerging sexual lifestyle, and this finding would also lend support to the notion that nakedness protects humans from ectoparasites.

Humans as repositories of ancient infections

Given that humans are *nouveaux riches* regarding our collection of infections [5], can we infer further examples of infections that could have come from other hominid species or from more distantly related primates? The infections for which we have the most accurate evolutionary record are the endogenous retroviruses that have invaded host DNA. Some 8% of the human genome represents

'fossil' integrated proviruses. The human lineage accumulated several thousand retroviral genomes after the split between New World and Old World primates, but only a handful since we diverged from chimpanzees.

Parasites that have tightly cospeciated with their hosts are, of course, in grave danger of extinction when that host becomes an endangered species. But the 'smart' parasite would gain a whole new lease of evolutionary opportunity if it engaged in occasional 'infidelity', analogous to mutation in DNA replication. A DNA lineage that does not mutate would have an extraordinarily slow rate of evolution, whereas a super-mutator without repair would provide little opportunity for natural selection before the genotype changed further. Thus, a low mutation rate within broad fidelity of DNA replication allows for both inheritance and evolution. Likewise, total cospeciation dooms the parasite to the fate of the host, whereas the ability to move horizontally to closely related hosts would provide flexibility. Parasite jumping between related hosts might occur more frequently than realized because it might easily be overlooked.

By this reckoning, the New World clade of head lice formerly faithful to the *H. erectus* lineage [2] adopted modern humans in the nick of time. HIV-1 might well be another successful case of jumping off a sinking ship, because its former host is not likely to survive for many generations longer in the wild. Now that HIV-1 has adopted a new host species, it is enjoying a most successful adaptive radiation and has already colonized around 60 million humans (25 million of whom have died from AIDS). Such crossover events, however, are relatively rare, and only one of the three ape-to-human transfers of HIV-1 has taken off to cause the AIDS pandemic [11]. It pays the host to place barriers known as restriction factors in the path of potential pathogens. If a species barrier is not recognized by the new invader or is successfully circumvented, the infection can be more virulent in the new host.

Regarding the intestinal parasites and ectoparasites that specialize in human infestation, Ashford [6] pointed out that we not only house two kinds of closely related lice (he meant *Pediculus* head and body lice), but also two species of *Cimex* bedbugs, two of *Demodex* mites and two of *Taenia* tapeworms. He therefore asked if we were once two separate populations that rejoined after a long separation, but Ashford did not have DNA sequences and molecular clock estimates available to him. Can we now view this phenomenon in the same way as the lice, as one of the pair of parasites cospeciating with *H. sapiens* and the other jumping from non-ancestral archaic humans to the modern human lineage? It would be intriguing to conduct similar molecular phylogenies of Ashford's other pairs.

Ashford [6] finished by stating: "Over to the microbiologists: What do the bacteria, viruses and fungi tell us?" There are ample examples both of cospeciation and horizontal transmission. The malaria parasite *Plasmodium falciparum* exemplifies a complex cospeciation between the parasite, its human host and its mosquito vector. *Anopheles gambiae* has also coevolved to be a specialist feeder on humans; by contrast, the other human malaria parasite, *P. vivax*, has an origin in South East Asian monkeys and is transmitted by the more promiscuous *Culex* species. Might *vivax* malaria have first adapted to *H. erectus* as an intermediate between monkeys and humans?

As a virologist, I have felt stimulated [23] to take up Ashford's challenge to consider pairs of related human viruses. HIV-1 and HIV-2 are very recent 20th century arrivals, from chimpanzees and sooty mangabey monkeys, respectively. With human T-cell lymphotropic viruses types 1 and 2 (HTLV-1 and HTLV-2), there have been multiple introductions of HTLV-1 from African and Asian monkeys and apes [25]. The provenance of HTLV-2 is puzzling, as its reservoir is in indigenous American populations and in African pygmies, which are as far apart in *H. sapiens* as one can find. Humans have five distinct polyoma viruses and multiple genital papilloma virus types. The different clades of hepatitis C virus (HCV) have deep roots but there are no known animal relatives to provide an anchor or time calibration. It would be fascinating to learn whether archaic humans harbored HCV variants, but as HCV has an RNA genome there is not much hope of gaining direct evidence by sequence amplification from ancient specimens.

All members of the herpesvirus family are thought to have strictly cospeciated with their hosts, though I have my doubts. The closest relatives to human herpes simplex virus (an α herpesvirus), cytomegalovirus (β) and Epstein-Barr virus (γ), seem to be those in the chimpanzee. Several simian species have a pair of distinct rhadinoviruses, whereas so far humans are only known to harbor one, Kaposi's sarcoma herpesvirus. On the other hand, humans have two herpes simplex viruses (HSVs), types 1 and 2. Phylogenetic analysis [26] indicates that HSV-1 and HSV-2 are further apart from each other than HSV-1 is from its chimp ortholog [27]. So where did HSV-2 come from? From its estimated age of divergence from the chimp-human HSV-1 lineage, I would place a bet on horizontal transmission from gorillas or possibly from orang-utans [23].

Is it a coincidence that three human parasites acquired horizontally from great apes, namely pubic lice, HIV-1 and speculatively HSV-2, are sexually transmitted? Now, before one conjures up a King Kong scenario, it should be noted that predators can pick up parasites from their prey. The

close contact involved in human ancestors butchering gorillas could have enabled *Pthirus* to jump hosts, rather as bushmeat slaughter practices probably led SIVcpz and other retroviruses to invade humans from chimpanzees in modern times [11,25].

Humans as a source of infections

With the advent of globalization, previously isolated human populations lost nine-tenths of their number to the infections introduced by intrepid migrants and invaders [7]. Hernan Cortes conquered the mighty Aztec empire thanks to smallpox and measles, which the invaders inadvertently introduced to the disease-naive New World peoples [5]. The subsequent population crash was severe; so few indigenous people survived that the lucrative African slave trade was established in order to provide labor in the plantations. This pattern of export of Old World diseases was repeated in South America, Australia and Oceania. As Charles Darwin remarked in his diary on the voyage of the Beagle, "Wherever the European has trod death seems to pursue the Aboriginal."

It is plausible, then, that modern humans could have transmitted lethal infectious diseases to archaic human species. *H. erectus* might have given us a clade of head lice, but *H. sapiens* may have conveyed more deadly infections to *H. erectus* and later to *H. neanderthalensis*. Hard evidence is lacking, and prehistoric legends seldom tell the tale from the point of view of the vanquished, although Golding did through the eyes of the Neanderthals [14]. To paraphrase Darwin, wherever modern humans trod during the Pleistocene era, death seemed to pursue archaic humans. There is also a danger today that the surviving great apes may be subjected to a *coup de grace* from human infections transmitted through jungle safaris and ecotourism.

Cross-species virulence is well known. Myxamotosis resident in American cotton-tail rabbits devastated the European rabbit, and the disappearance of red squirrels in Britain wherever American gray squirrels occur is probably due to a pox virus rather than direct competition for habitat. Similarly, the α-herpesviruses that have cospeciated with Indian and African elephants cause nothing more severe than cold sores in their natural host but each seems to be lethal to the other when the two species are unnaturally housed together in zoos [28]. SIVcpz has little effect on chimpanzees but HIV-1 causes AIDS in humans. Thus, it might pay the host to carry a fairly harmless parasite if that infection is lethal to the host's competitors.

Epilog

Body lice, and occasionally head and pubic lice, transmit bacterial diseases: typhus (*Rickettsia prowazekii*) [29], trench fever (*Bartonella quintana*) and relapsing fever (*Borellia recurrentis*). The lice themselves succumb to typhus infection but pass the *Rickettsia* in their feces, which the human then scratches into the skin. Typhus is known as 'war fever' and 'jail fever' because it appears in conditions where lice thrive. At the end of *Rats, Lice and History* Zinsser [1] wrote "Typhus is not dead. It will live on for centuries and will break into the open whenever human stupidity and brutality give it a chance." Sadly, Zinsser's words were prophetic. Within a few years, typhus became the major slayer in the Nazi concentration camps; typhus broke out among Rwandan refugees in Burundi in 1994, in Bosnia in 1995 and most recently in Goma.

Human brutality is another feature shared with chimpanzees that has survived in the human lineage [30]. It is too bad that we are not closer to the pygmy chimp (bonobo), which evolved a means of conflict resolution between troupes through alpha females engaging in lesbian sex. But that is another story.

Acknowledgements

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Research article

Pair of lice lost or parasites regained: the evolutionary history of anthropoid primate lice

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Abstract

Background: The parasitic sucking lice of primates are known to have undergone at least 25 million years of coevolution with their hosts. For example, chimpanzee lice and human head/body lice last shared a common ancestor roughly six million years ago, a divergence that is contemporaneous with their hosts. In an assemblage where lice are often highly host specific, humans host two different genera of lice, one that is shared with chimpanzees and another that is shared with gorillas. In this study, we reconstruct the evolutionary history of primate lice and infer the historical events that explain the current distribution of these lice on their primate hosts.

Results: Phylogenetic and cophylogenetic analyses suggest that the louse genera *Pediculus* and *Pthirus* are each monophyletic, and are sister taxa to one another. The age of the most recent common ancestor of the two *Pediculus* species studied matches the age predicted by host divergence (ca. 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 a common ancestor ca. 3–4 million years ago, which is considerably younger than the divergence between their hosts (gorillas and humans, respectively), of approximately 7 million years ago.

Conclusion: Reconciliation analysis determines that there are two alternative explanations that account for the current distribution of anthropoid primate lice. The more parsimonious of the two solutions suggests that a *Pthirus* species switched from gorillas to humans. This analysis assumes that the divergence between *Pediculus* and *Pthirus* was contemporaneous with the split (i.e., a node of cospeciation) between gorillas and the lineage leading to chimpanzees and humans. Divergence date estimates, however, show that the nodes in the host and parasite trees are not contemporaneous. Rather, the shared coevolutionary history of the anthropoid primates and their lice contains a mixture of evolutionary events including cospeciation, parasite duplication, parasite extinction, and host switching. Based on these data, the coevolutionary history of primates and their lice has been anything but parsimonious.

Background

Sucking lice (Phthiraptera: Anoplura) are permanent and obligate ectoparasites of eutherian mammals. These highly specialized blood-sucking insects live in close association with their hosts and complete their entire life cycle on the host [1]. Anoplurans have modified mouthparts for feeding on host blood and because mammalian blood differs widely among species in terms of its suitability for louse nutrition [2], sucking lice can be highly host specific [1,3]. Host specificity could also be reinforced by interactions with the host's immune system. High host specificity can arise from a long history of cospeciation between hosts and their parasites. Cospeciation is speciation (or cladogenesis) in a parasite lineage as a result of, or at the same time as, host cladogenesis [4]. The current distribution of parasites on host taxa (the host-parasite associations) can be the result of cospeciation or various historical events [5] such as host switching, sorting events (extinction and lineage sorting), duplication events (parasites speciating on a single host lineage), and failure of the parasite to speciate when the host speciates ('missing the boat'; [6]). These historical processes can be detected by comparing the phylogenies of hosts and their parasites using methodologies such as reconciliation analysis [7]. Previous cophylogenetic studies of lice (both sucking lice and chewing lice) have documented each of these historical events in various combinations (for a review, see [8]).

Humans (*Homo sapiens*) are parasitized by two genera of sucking lice: one shared with chimpanzees (*Pan* spp.) and the other shared with gorillas (*Gorilla gorilla*). Human head and body lice, as well as chimpanzee lice, are members of the genus *Pediculus* (*Pediculus humanus* and *Pediculus schaeffi*, respectively). There is no *Pediculus* species known to parasitize gorillas. Human pubic lice and gorilla lice belong to the genus *Pthirus* (*Pthirus pubis* and *Pthirus gorillae*, respectively), and no *Pthirus* species is known to parasitize chimpanzees. *Pediculus* and *Pthirus* are sister taxa based on morphology and molecular data (Figure 1), and primate lice are known to have cospeciated with their hosts for at least 25 million years [9]. The curious distribution of these two genera raises an interesting question regarding the evolutionary history of primate lice. Why do humans retain both genera, but chimpanzees and gorillas have only one genus each?

Given what is already known about the coevolutionary history of the lice and their hosts, we can speculate that there are two mutually exclusive explanations that can account for the current distribution of *Pediculus* and *Pthirus* (Figure 2). The most parsimonious explanation (i.e., the explanation requiring the fewest number of steps) predicts perfect cospeciation between the primates and lice with the addition of a single host switch. In this scenario, the divergence between *Pediculus humanus* and *Pediculus schaeffi* occurred at the same time as the split between their hosts, humans and chimpanzees, ca. 6 million years ago (MYA; [10]), and the split between *Pediculus* and *Pthirus* occurred contemporaneously with the split between gorillas and the lineage leading to chimpanzees and humans (ca. 7 MYA; Figure 2A; [10]). These events were followed some time later by one host switch of a *Pthirus* species from gorillas to humans (Figure 2A). Host switching among lice is common in many groups of birds and mammals [11-13]. This 'recent host switch' hypothesis requires one evolutionary step and predicts that the divergence between *Pthirus pubis* and *Pthirus gorillae* is more recent than the chimpanzee/human split (Figure 2A). How we might have acquired our pubic lice from gorillas is not immediately apparent, however it would be interesting to know whether the switch was very recent (say less than 100,000 years old) or whether it was considerably older.

The alternative hypothesis involves an ancient louse duplication event that occurred on the ancestor of gorillas, chimpanzees, and humans, which would have created the lineages leading to the two extant genera, *Pediculus* and *Pthirus* (Figure 2B). In this case, the timing of the divergence between *Pthirus pubis* and *Pthirus gorillae* would correspond to that of their hosts (ca. 7 MYA; [10]). In this scenario, humans would have retained both genera, but chimpanzees would have lost a *Pthirus* species and gorillas would have lost a *Pediculus* species to extinction (Figure 2B). Such parasite duplications and extinctions are common in the lice of birds and mammals (e.g., [14]). This parasite extinction or 'pair of lice lost' model is less parsimonious than the 'recent host switch' hypothesis listed above because it requires at least three evolutionary steps (a duplication of the parasite on a primate common ancestor as well as the extinction of one *Pediculus* and one *Pthirus* lineage, Figure 2B). Although the 'recent host switch' hypothesis is more parsimonious, it is not necessarily more likely than the 'pair of lice lost' hypothesis. Each of the historical events (host switch, duplication, and extinction) has some probability of occurrence, the quantification of which is beyond the scope of this paper. There are additional hypotheses that can be postulated that have more evolutionary steps than the two presented above, however there are no other hypotheses that have an equal number of steps or fewer steps. For instance, one might assume that the current distribution of lice resulted from a host switch of *Pthirus* from humans to gorillas (the opposite direction of the switch in the 'recent host switch' model). However, this evolutionary scenario would require at least five evolutionary steps.

The two hypotheses of parasite distributions ('pair of lice lost' and 'recent host switch') are based on the premise of maximizing the number of cospeciation events and mini-

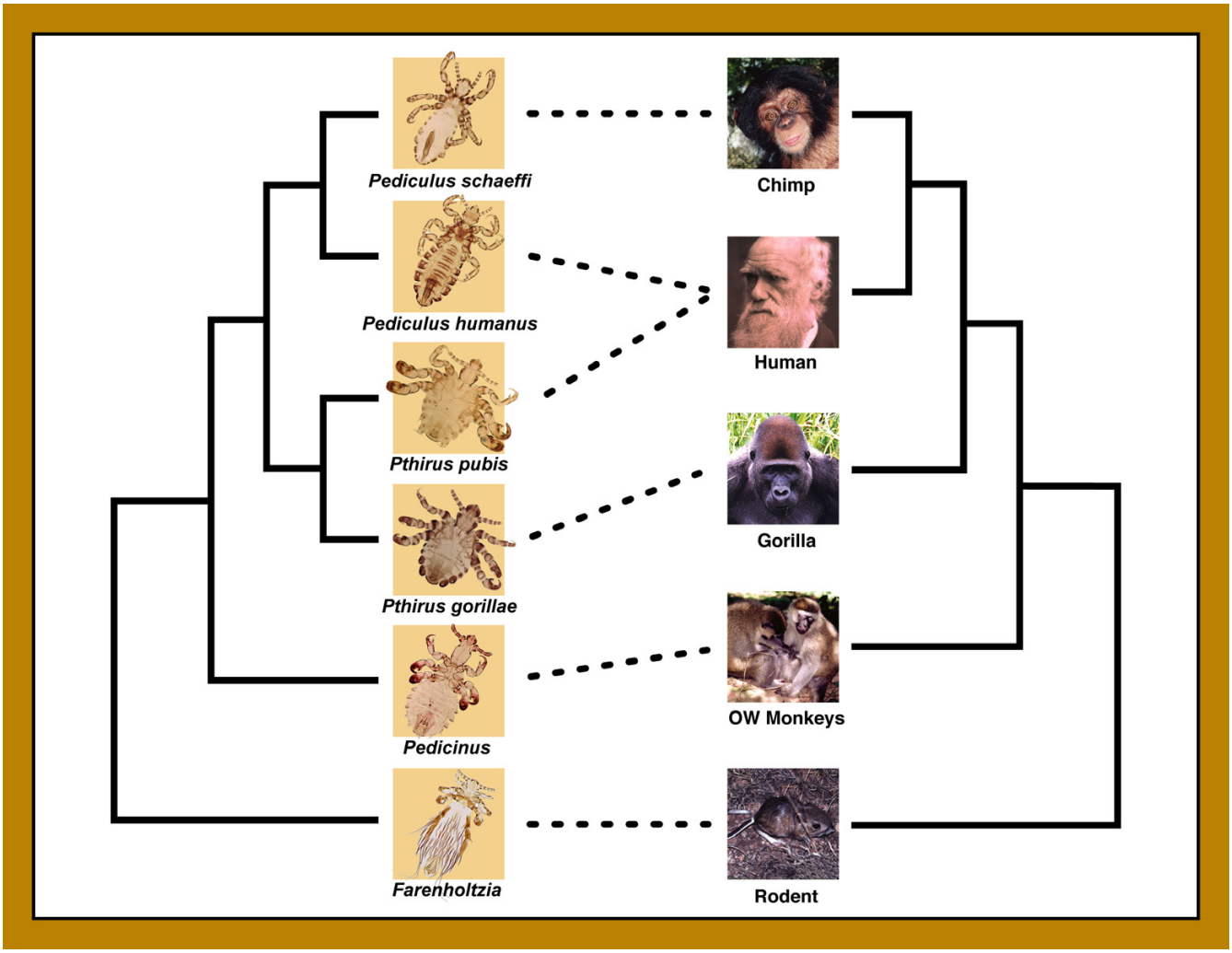

Figure 1
Phylogenetic trees for primate lice and their vertebrate hosts redrawn from Reed *et al.* [9]. Trees are shown as cladograms with no branch length information, and are based on molecular and morphological data. Dashed lines between trees represent host-parasite associations. Humans are unique in being parasitized by two genera (*Pediculus* and *Pthirus*). Photo credits: J. W. Demastes, T. Choe, and V. Smith.

mizing the number of events that deviate from cospeciation, which are typical of analyses that attempt to reconcile host and parasite associations that are based on the concept of evolutionary parsimony (reconciliation analysis as implemented in TreeMap; [7]). However, parsimony-based reconciliation analyses do not take into account branch lengths and divergence times – information that is essential to distinguish between the 'recent host switch' and 'pair of lice lost' hypotheses. In the presence of significant cospeciation we can use the timing of speciation events to differentiate among alternative hypotheses of host-parasite associations [15].

We have performed phylogenetic and cophylogenetic analyses of two genes, the mitochondrial cytochrome *c* oxidase subunit I (Cox1) gene and nuclear gene elongation factor 1 alpha (EF-1α) gene, to determine the shared evolutionary history of primate lice and their hosts. We also investigate the use of standard phylogenetic methods for reconstructing coevolutionary histories when standard cophylogenetic methods (e.g., reconciliation analysis) cannot always find the solution that best fits the observed data.

Results

Phylogenetic and cophylogenetic analyses

The partition homogeneity test determined that the Cox1 and EF-1α genes did not differ significantly ($p = 0.94$), therefore a combined analysis was performed in addition to analyses based on single genes. The best-fit model for

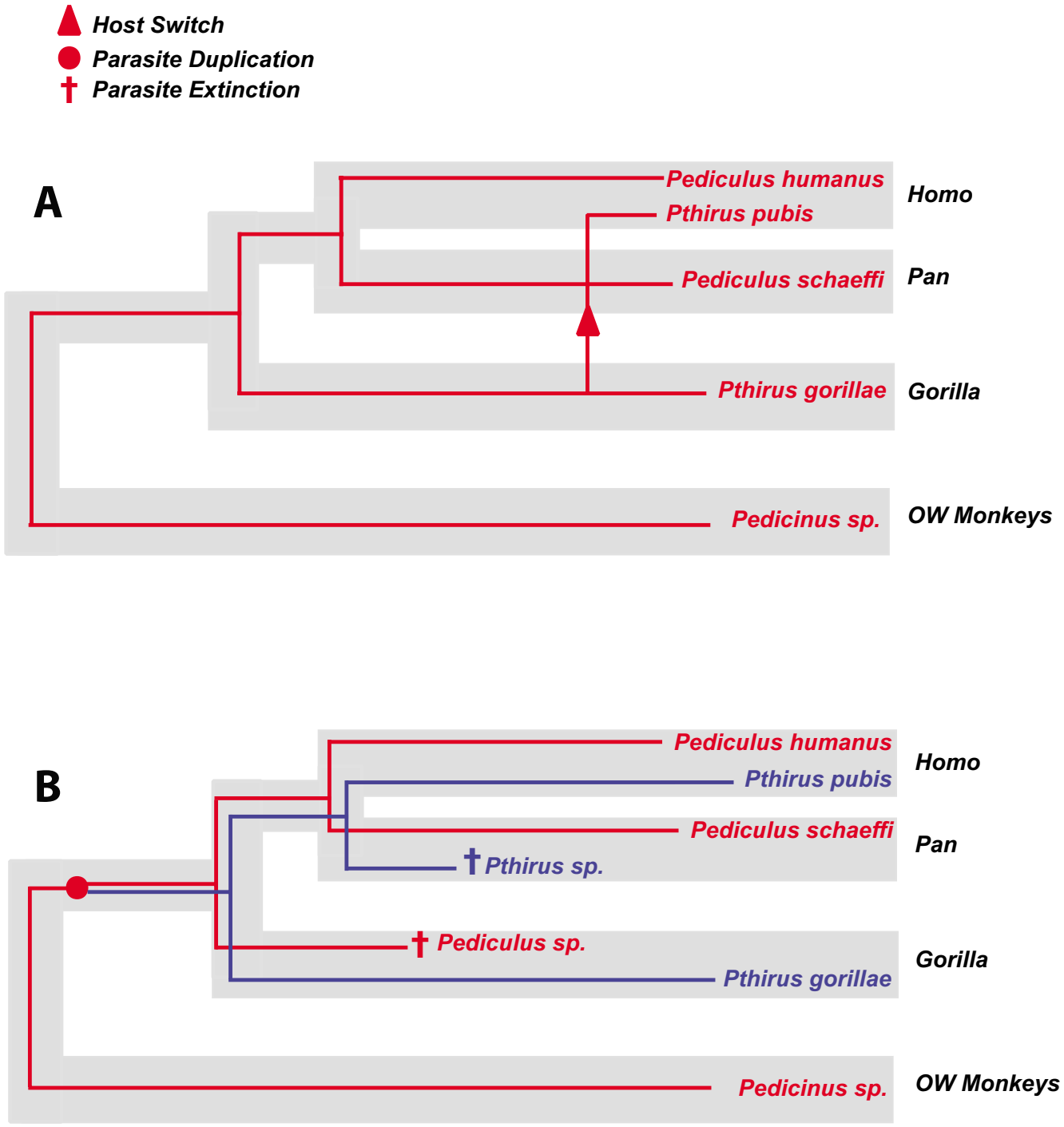

Figure 2
Cophylogenetic reconstructions with the host phylogeny for humans, chimpanzees, gorillas, and Old World monkeys indicated by thick grey lines, and the louse phylogeny indicated by thin red and blue lines. (A) Reconstruction showing perfect cospeciation between with hosts and parasites with the exception of a single host switch of *Pthirus sp*. from gorillas to humans (marked by an arrow). (B) Cophylogenetic reconstruction showing an ancient duplication creating two evolutionarily distinct lineages (*Pediculus* and *Pthirus*), each having cospeciated with gorillas, chimps, and humans with two extinction events (marked with daggers). The reconciliation shown in panel A requires one evolutionary step (the host switch), whereas reconciliation B requires three steps (one duplication and two extinctions).

each individual gene and the combined-gene analysis of lice was a transversion model (TrN+I+Γ model) that permitted two nucleotide substitution rates for transversions, one rate for transitions, unequal base frequencies, a rate heterogeneity parameter (G), and a parameter for invariant sites (I). Similarly, the best-fit model for the Cox1 gene from the primate host taxa was also TrN+I+Γ. Maximum likelihood (ML) analysis of the combined Cox1 and EF-1α dataset (as well as individual gene datasets) from lice produced a single, well-supported phylogeny in agreement with results from previous analyses (Figure 3; [9]). When the same ML analysis was performed enforcing a molecular clock, the resulting tree topology did not change and the resulting tree score was not significantly different than the unconstrained score ($p = 0.7625$). The ML analysis of the host Cox1 data also resulted in a single phylogeny in agreement with known relationships among these primate taxa (Figure 3).

Cophylogenetic analyses using TreeMap [7] produced two reconciliations of host and parasite phylogenies in agreement with the 'recent host switch' and 'pair of lice lost' hypotheses presented in Figure 2. The reconciliation concordant with the 'recent host switch' hypothesis (Figure 2A) included five cospeciation events and one host switch for a total cost of 1.0. The reconstruction concordant with the 'pair of lice lost' hypothesis (Figure 2B) was less parsimonious. While this reconstruction included five cospeciation events, there was also a single duplication event and two losses resulting in a total cost of 3.0. Both reconciliations show significantly greater similarity between the host and parasite trees than would be expected based on chance alone (i.e., both reconciliations show significant cospeciation, $p < 0.05$).

Divergence date estimation

Divergence date estimates differed little between r8s and multidivtime analyses and between individual and combined genes (Table 1). For convenience we will refer to divergence date estimates based on the combined gene tree used in *multidivtime* (Table 1 and Figure 3). Mean divergence date estimates for the split between the chimpanzee and human head/body lice (*Pediculus schaeffi* and *Pediculus humanus*, respectively) averaged 6.39 MYA. The divergence date estimates for the gorilla and human pubic lice (*Pthirus gorillae* and *Pthirus pubis*, respectively) averaged 3.32 MYA and are noticeably more recent than the split between the two *Pediculus* species. The estimated divergence date for the most recent common ancestor (MRCA) of the two genera, *Pthirus* and *Pediculus*, was estimated to be 12.95 MYA (Table 1 and Figure 3), noticeably older than the MRCA of chimpanzees, humans, and gorillas.

Discussion

Reconciliation analysis using TreeMap corroborates earlier reports of significant cospeciation between primate lice and their hosts [9]. These cophylogenetic analyses also result in two reconciliations of the host and parasite phylogenies where the most parsimonious reconstruction favors the 'recent host switch' hypothesis (Figure 2A). However, divergence date estimates conflict with the results of the reconciliation analysis because the 'recent host switch' hypothesis predicts that the divergence of *Pediculus* and *Pthirus* would be roughly contemporaneous with the split between gorillas and the lineage leading to humans and chimpanzees. Our estimates of the MRCA of *Pediculus* and *Pthirus* dates to roughly 13 MYA, not remotely consistent with the MRCA of humans, chimpanzees, and gorillas (ca. 7 MYA; [10]). Given the much older age of our MRCA of *Pediculus* and *Pthirus*, it is more appropriate, although less parsimonious, to assume that the origin of the two genera was the result of a parasite duplication event rather than a cospeciation event ('pair of lice lost' hypothesis; Figure 2B). It is curious that the estimate of the MRCA of *Pthirus* and *Pediculus* (13 MYA) is contemporaneous with the divergence of Orangutans from other apes [10], however this is possibly coincidental. Lice do not parasitize orangutans; therefore, reconstructing their role in the evolutionary history of primate sucking lice will be difficult.

The 'pair of lice lost' hypothesis is also unsatisfactory when compared to divergence date estimates. For the 'pair of lice lost' hypothesis to be correct we must assume that divergence between *Pthirus pubis* and *Pthirus gorillae* is roughly contemporaneous with the split of gorilla from the lineage leading to chimpanzees and humans (i.e., ca. 7 MYA). Our divergence date estimates of roughly 3–4 MYA (Table 1) is much younger than the host divergence of 7 MYA [10] and is even younger than the divergence between chimpanzees and humans (ca. 6 MYA).

The estimates of divergence dates argue for a more complex evolutionary history than estimated by reconciliation analysis. While reconciliation analysis serves to find the most parsimonious reconstruction of host and parasite evolutionary history by maximizing cospeciation events and minimizing the cost of the reconstruction, it can only identify possible scenarios describing the evolutionary history between associated taxa. Incorporating branch length data in other analyses is necessary to determine which scenario best fits the observed data. For example, post-hoc Mantel tests are commonly used to look for overall correlation in host and parasite data sets [16]. However, we have too few taxa to perform such an analysis, and we have instead relied upon ad-hoc phylogenetic tests to determine whether certain nodes of cospeciation were contemporaneous.

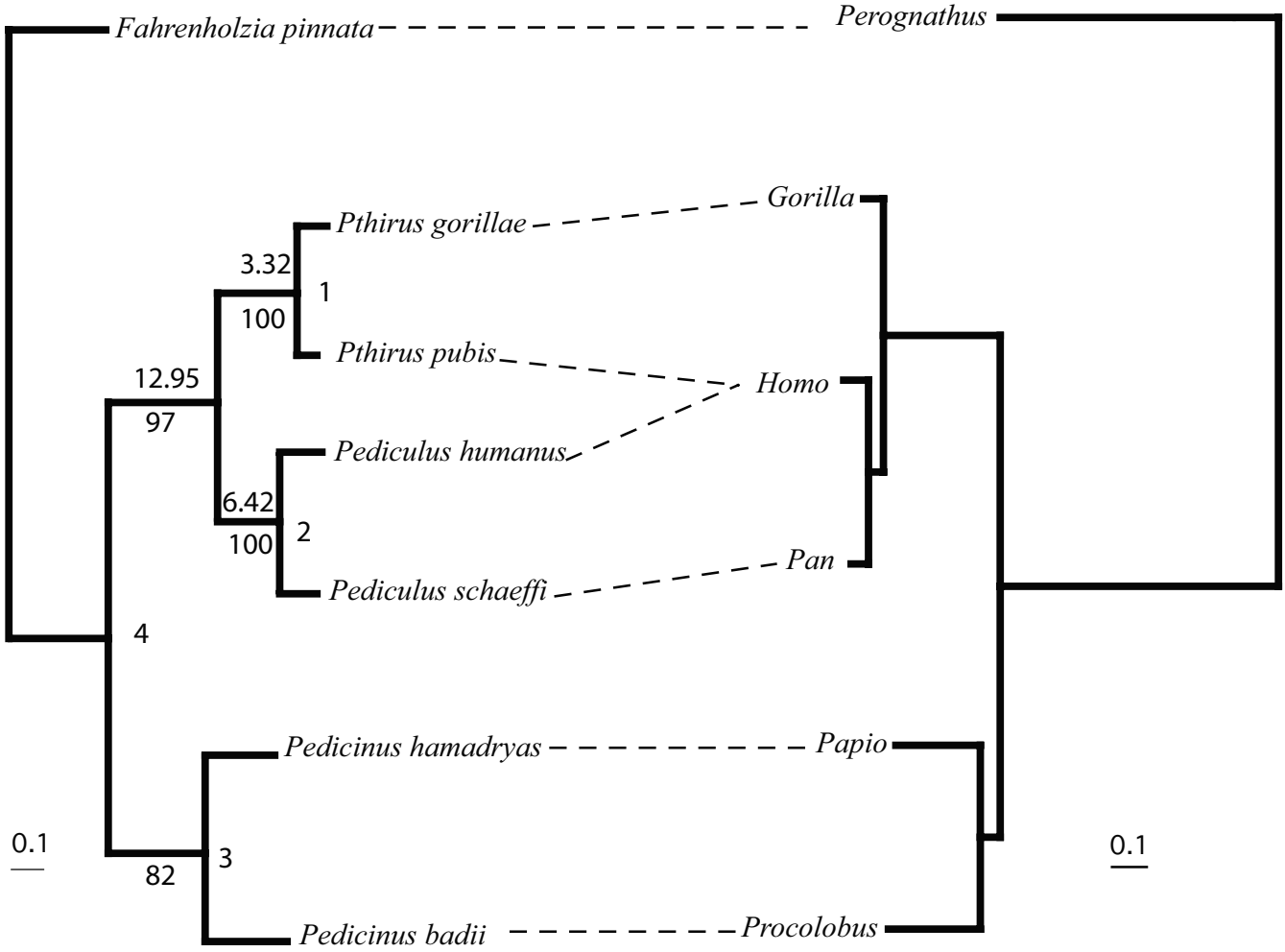

Figure 3

Maximum likelihood (ML) phylogeny of primate lice using the combined Cox1 and EF-1α dataset with a best-fit ML model of nucleotide substitution (left). Bootstrap values are indicated below the nodes and divergence estimates are given above. Clade numbers used in Table 1 are provided to the right of each node. The ML phylogeny of the Cox1 gene from host taxa is indicated on the right. Branch lengths are drawn to the same scale (substitutions/site), and are based on the best-fit ML model of nucleotide substitution. Dashed lines connect hosts and their associated parasites.

To further examine the validity of the 'pair of lice lost' hypothesis, we assessed whether the divergence of the two *Pthirus* species was contemporaneous with the host divergence of roughly 7 MYA. The branch length between the two *Pthirus* species in the best-fit ML louse tree was artificially lengthened to approximate a branch that is the same age as the branch between *Pediculus schaeffi* and *Pediculus humanus*, thereby representing the split between chimp and human lice. It is widely known that the gorilla, chimpanzees, and human divergence times are very close in age, so if our estimate is contemporaneous with the divergence of chimpanzees and humans (the most conservative expected age), then we cannot rule out true contemporaneous times of divergence between the two *Pthirus* species and their hosts. The likelihood score of this constrained tree (with a branch length approximating a 6 million year divergence between *Pthirus gorillae* and *Pthirus pubis*) was significantly worse than the true louse tree (where the estimated divergence was closer to 3–4 MYA; d.f. = 6, χ^2 = 12.91, p = 0.044). We can therefore reject contemporaneous divergence events and we can conclude that the split between the human and gorilla species of *Pthirus* diverged much more recently than the split between humans and gorillas or even humans and chimpanzees. The divergence within *Pthirus* is therefore the result of a host switch from gorillas to humans (loosely defined) roughly 3–4 MYA, as predicted in the 'recent host switch' hypothesis.

We can similarly examine the divergence date estimation for the branch between *Pthirus* and *Pediculus*, which the

Table 1: Divergence date estimates.

MRCA (clade number)	Cox1 and EF1α	Cox1	EF1α	Cox1 and EF1α (r8s, 20 MYA)	Cox1 and EF1α (r8s, 25 MYA)	Cox1 (r8s, 20 MYA)	Cox1 (r8s, 25 MYA)
Pedicinus (3)	10.63 (7.08–14.94)	10.23 (6.56–15.17)	10.91 (3.02–18.87)	11.52	13.21	11.53	14.40
Pthirus (1)	3.32 (1.84–5.61)	3.86 (2.05–7.49)	1.76 (0.05–6.75)	3.56	4.45	3.48	4.35
P. schaeffi and *P. humanus* (2)	6.39 (3.94–9.96)	6.87 (4.07–11.65)	6.65 (1.72–14.70)	5.03	6.28	4.92	6.15
Pediculus and *Pthirus* (4)	12.95 (9.42–17.38)	13.03 (9.25–18.21)	14.66 (7.26–22.23)	10.56	14.41	10.45	13.06
OWM/Ape Calibration	22.50 (20.13–24.87)	22.39 (20.12–24.84)	22.48 (20.12–24.87)	20.00	25.00	20.00	25.00

Mean divergence date estimates (in millions of years) for the clades shown in Figure 3 (see Figure 3 for clade numbers). Divergence estimates using a 20–25 calibration point for the split between Old World primate lice (*Pedicinus*) and Anthropoid primate lice (*Pediculus* and *Pthirus*) in *multidivtime* are given in the first three columns (95% credibility intervals in parentheses). Mean divergence date estimates using 20 MYA and 25 MYA calibration points for the split between Old World and Anthropoid primate lice using the Langley Fitch model in r8s are indicated in the final four columns for the Cox1+EF1α combined analysis and the Cox1 gene alone.

'recent host switch' model would predict to be 7 MYA. Our estimates were much older (Table 1), and shortening the branch length artificially between *Pthirus* and *Pediculus* to resemble a divergence near 7 MYA can be easily rejected ($p < 0.01$). These analyses support an ancient duplication at that node, consistent with the 'pair of lice lost' model. Therefore, contrary to the results of the reconciliation analysis, the divergence date estimates predict a much less parsimonious explanation of current primate louse distributions: a combination of the 'recent host switch' and 'pair of lice lost' hypotheses.

Given our estimates of divergence dates, the most likely evolutionary history is that *Pthirus* and *Pediculus* diverged on an ancestor of chimpanzee, human, and gorilla roughly 13 MYA (a duplication event), with each genus then having the potential to cospeciate with descendent hosts (Figure 4). However, only the gorillas retained *Pthirus* with an extinction of *Pthirus* on the branch leading to both humans and chimpanzees (Figure 4). *Pediculus* was maintained on the lineage leading to humans and chimpanzees but lost from the gorilla lineage, and the two resulting species (*Pediculus schaeffi* and *Pediculus humanus*) diverged in tandem with their primate hosts roughly six million years ago (Figure 4). Approximately 3–4 MYA, a *Pthirus* species switched from the gorilla lineage to the lineage leading to modern humans. It is important to note that this happened after the divergence of chimpanzees and humans and that these data suggest humans acquired their pubic louse from gorillas not recently, but rather 3–4 million years ago. In total, this coevolutionary scenario requires four evolutionary steps (one duplication, two losses, and one host switch), and is a combination of both the 'recent host switch' and the 'pair of lice lost' hypotheses.

Conclusion

Evidence suggests that *Pthirus pubis* has been associated with humans for several million years, and likely arrived on humans via a host switch from gorillas. Despite the fact that human pubic lice are primarily transmitted via sexual contact, such contact is not required to explain the host switch. Parasites often switch from a given species to a predator of that species [17], and are sometimes found to switch to unrelated hosts in communally used areas, such as roosting or nesting sites [18]. The host switch in question could have resulted from any form of contact between archaic humans and gorillas including, but not limited to, feeding on or living among gorillas. Regardless of how the transfer occurred, suitable habitat had to be available on the new human host for the host switch to be successful. For example, it is possible that the switch of *Pthirus* from gorillas to humans coincides with a change in available niche space in humans, such as the loss of body hair. Further study, however, is required to test such a hypothesis.

Because *Pthirus* has been associated with humans for several million years, this taxon can be examined in the same way that *Pediculus humanus* has to study the evolutionary history of its human host [9,19,20]. *Pthirus pubis* represents an independent, ecological replicate that went through the same evolutionary history on humans as their head/body lice, and can be used to test predictions made from *Pediculus humanus*. *Pediculus humanus* shows genetic evidence of population expansion out of Africa roughly 100,000 years ago, which is concordant with host evolutionary history [9]. However, in contrast to the shallow mitochondrial DNA (mtDNA) gene history of humans (human mtDNA coalesce to a common ancestor within 200,000 years, [21-23]), *Pediculus humanus* has three deeply divergent mtDNA lineages that share a MRCA ca. 2 million years ago, which is far older than the age of their modern human hosts [9]. Perhaps a worldwide sample of *Pthirus pubis* will mirror that of *Pediculus humanus*, and show both the population expansion 100,000 years ago and the three deeply divergent mtDNA lineages. Understanding human evolutionary history from the perspective of its parasites may provide useful insight into a brief period of history that is not fully recorded in the host fos-

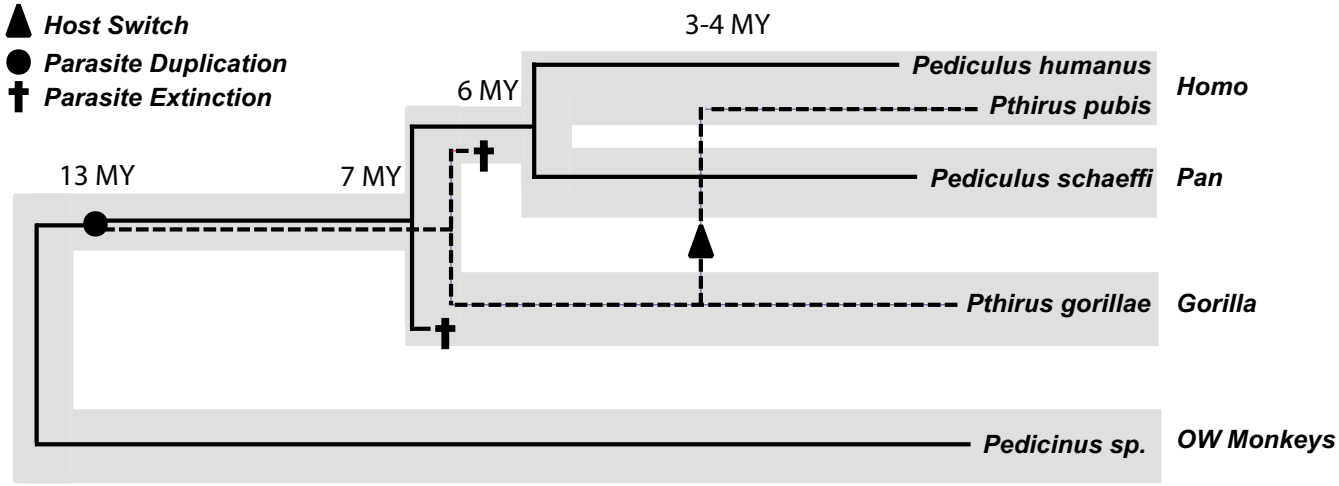

Figure 4
Coevolutionary reconstruction of primate lice and their hosts based on reconciliation analysis and divergence date estimation. Thick grey lines represent the host phylogeny for humans, chimpanzees, gorillas, and Old World monkeys. Thin black lines (solid and dashed) represent the louse lineages. This evolutionary scenario depicts a parasite duplication ca. 13 MYA leading to the extant genera *Pediculus* (solid lines) and *Pthirus* (dashed lines). One species from each lineage is depicted as having gone extinct (dagger), and a single host switch ca. 3–4 MYA is shown by an arrow within the *Pthirus* lineage. The divergence of the chimpanzee and human lice (*Pediculus spp*.) are shown as having diverged in tandem with their hosts.

sil record or in host DNA [24]. However, if parasites are to provide much clarity, it will likely be only after many human parasites have been examined.

The advent of parsimony-based reconciliation analysis has permitted many researchers to assess phylogenetic congruence in a wide array of host-parasite assemblages. However, this method is more limited than Bayesian approaches [25] to studying cophylogenetics, which evaluate not only topological congruence but also the comparative timing of host and parasite divergences. It is imperative that we continue to put into practice the theoretical work that has propelled systematists forward in recent years. Only then can we hope to uncover the more complex interactions between hosts and parasites.

Methods

Specimen collection and preparation

Samples of *Pthirus gorillae* (from gorillas), *Pthirus pubis* (from humans), *Pediculus humanus* (from humans), *Pediculus schaeffi* (from chimpanzees), *Pedicinus hamadryas* (from baboons), *Pedicinus badii* (from red colobus monkeys), and one outgroup species (*Fahrenholzia reducta*) were collected for this study (Table 2). Lice in the genus *Pedicinus* parasitize only Cercopithecoid monkeys (Old World Monkeys; OWM) whereas the genera *Pediculus* and *Pthirus* parasitize only the Anthropoid primates (apes). All lice were preserved in 95% EtOH and stored at -80°C. DNA was extracted from louse specimens using the technique of Johnson and Clayton [26] and Reed *et al*. [9], which enabled extraction of whole genomic DNA from each louse while retaining the entire louse body as a voucher specimen. The Qiagen DNeasy Tissue Kit (QIAGEN Inc., Valencia, California) was used to isolate genomic DNA from the body of each louse according to louse-specific protocols [9,26,27]. After DNA extraction, lice were mounted on slides and retained as vouchers. Voucher specimens will be deposited in the Price Institute for Phthirapteran Research collection (University of Utah).

PCR and sequencing

PCR amplification and sequencing of a portion of the mitochondrial cytochrome *c* oxidase subunit I gene (Cox1; 858 bp) was performed using the primers LCO1718 [9] and H7005 [16]. PCR amplification and sequencing of 345 bp of the nuclear elongation factor 1 alpha (EF-1α) gene were performed using the primers For3 and Cho10 [28]. Double-stranded PCR amplifications for both Cox1 and EF-1α were performed in 25 μl reaction volumes using 10 μl of Eppendorf HotMaster PCR Mix (Fisher Scientific), 1 μl of each primer (at 10 mM), and 2 μl of DNA template. The amplification protocol required an initial denaturation step of 94°C for 10 min, followed by 5 cycles of 94°C (1 min), 48°C (1 min), and 65°C (2 min), then 30 cycles of 94°C (1 min), 52°C (1 min), and 65°C (2 min) and a final extension of 65°C for 10 minutes. Amplified fragments were purified using ExoSAP-IT (USB Corporation) and sequenced in both directions. Sequences were edited using Sequencher v.

Table 2: Specimens examined. Louse taxa included in phylogenetic and cophylogenetic analyses.

				Genbank Accession Numbers	
Louse Species	Host Species Voucher ID	Collection Locality	Host Identification	Cox1	EF1
Pediculus humanus	Pdcap9.20.05.25	USA, Florida, West Palm Beach	*Homo sapiens* (WP007)	EF152552	EF152558
Pediculus schaeffi	Pdsch5.23.05	Uganda	*Pan troglodytes*	EF152553	EF152559
Pthirus pubis	Ptpub1.19.06.3	UK, Scotland, Glasgow	*Homo sapiens* (GLA 140)	EF152554	EF152560
Pthirus gorillae	Ptgor8.1.06.6	Uganda	*Gorilla gorilla* (051122CAWBB001)	EF152555	EF152561
Pedicinus hamadryas	Qnham2.4.01.2	Captive (SW Found for Biomed. Res.)	*Papio hamadryas*	AY696007	EF152562
Pedicinus badii	Qnbad7.24.06.9	Uganda	*Procolobus badii*	EF152556	EF152563
Fahrenholzia pinnata	Fzpin163	USA, Nevada, Tonopah	*Perognathus longimembris* (MLZ 2039)	EF152557	EF152564

Abbreviations are as follows: Moore Laboratory of Zoology (MLZ), Page Lab, University of Glasgow (GLA), Lice Solutions, West Palm (WP), and Maryland Gorilla Veterinary Project (MGVP).

4.2.2 (Gene Codes Corporation, Ann Arbor, Michigan) and aligned by eye using Se-Al v2.0a11 http://evolve.zps.ox.ac.uk/Se-Al/Se-Al.html. Primer sequences were removed and sequences were trimmed in reference to the translated protein sequence using Se-AL v2.01a11 and MacClade 4.0 [29]. All sequences were submitted to [Genbank: EF152552-EF152564] and alignments to [TreeBase: #SN3269]. Sequences of the Cox1 gene from the primate host taxa were downloaded [Genbank: NC001807, NC001643, NC001645, NC001992, NC008219]. EF-1α sequences were not available for several primate taxa, and were therefore not examined.

Phylogenetic and cophylogenetic analyses

The partition homogeneity test [30] in PAUP*4.0b10 [31] was used to evaluate phylogenetic congruence of the louse Cox1 and EF-1α data sets. One thousand partition replicates were analyzed by maximum parsimony (heuristic search option with random addition replicates and tree bisection-reconnection branch swapping). Modeltest [32] was used to determine the best-fit ML model for the molecular data. Phylogenetic analyses were conducted on host and parasite data sets using maximum likelihood (ML) with branch and bound searches using the best-fit model in PAUP* 4.0b10 [31]. Nonparametric bootstraps (100 replicates) were performed to assess nodal support for the louse phylogeny. ML searches were performed with and without the 'enforce clock' constraint in order to test the hypothesis of a molecular clock in the Cox1 and EF-1α louse datasets. The resulting ML host and parasite trees with branch lengths estimated from the best-fit ML model were then used in cophylogenetic analyses.

TreeMap (v. 2.0.2; [7]) was used to determine whether host and parasite trees were more similar to one another than would be expected by chance. Default costs for evolutionary events (codivergence = 0, host switching = 1, duplication = 1, and loss = 1) were used. Significance values were calculated from a sample of 1,000 randomly generated trees.

Divergence date estimation

Because the lice and their primate hosts showed significant codivergence and because molecular data did not differ significantly from clocklike behavior, divergence dates were estimated using methods that both adhere to and relax the molecular clock. Divergence dates were estimated in the program r8s [33], using the Langley and Fitch (LF) model which assumes a molecular clock. Dates were also estimated using a parametric Bayesian approach [34] in the program *multidivtime*. This method relaxes the molecular clock and allows rate variation among genes and lineages, and it is therefore appropriate for datasets that utilize more than one molecular marker. The topology resulting from ML analysis of the combined 2-gene data set was used in *multidivtime*. The Cox1 best-fit ML tree and the combined 2-gene topologies were used in r8s. Divergence dates were estimated using each individual gene (with branch lengths optimized on the best ML tree) as well as a combined 2-gene dataset.

For the parametric Bayesian analysis, model parameters for the F84+Γ model were estimated for each gene separately using the *baseml* program in PAML v3.14 [35]. These parameters were then used in the program *estbranches* [34,36] to estimate the ML and the variance-covariance matrix of the branch length estimates for each gene. Lastly, the program *multidivtime* [34,36], utilizing the output files from *estbranches* and implementing Markov chain Monte Carlo (MCMC) sampling, was used to estimate prior and posterior distribution of rates and divergence time estimates among lineages. The prior assumption for the mean and standard deviation of the time of the ingroup root node (rttm) was set to 3.0 time units, where 1 time unit represents 10 million years. This value corresponds to the upper limit of the split between hominoid and cercopithecoid primates. The mean and standard deviation for the prior distribution of the rate of evolution at the ingroup node (rtrate and rtratesd) was determined following the protocol of Jansa *et al.* (rttm; [37]). To avoid violation of the definition of the prior, rtratesd was set to

its maximum value (equal to rtrate). The Markov chain was initialized by randomly selecting the initial parameter value and each Markov chain was sampled every 100 cycles for 1,000,000 generations with a burn in of 100,000 cycles.

A calibration point of 22.5 ± 2.5 MYA was used for the split between *Pedicinus* and *Pediculus+Pthirus*. This divergence of 20–25 MYA corresponds to the split between OWM and apes [38-41]. Since lice and their primate hosts show significant cospeciation, we can use this well-established host calibration based on fossil data to calibrate the louse phylogenetic trees. It is preferable to use more than one calibration point when estimating divergence dates [42,43], however the small number of nodes in our trees make that impossible. Furthermore, Reed *et al*. [9] showed that the calibration point of 20–25 MYA yielded estimated clade ages that were very similar to those estimated from a calibration of 5–7 million years between the human and chimpanzee lice (*P. humanus* and *P. schaeffi*, respectively).

Authors' contributions

JEL and JMA collected specimens. JEL, JMA and JJK performed molecular lab work. DLR and JEL analyzed data and wrote the manuscript. All authors provided comments on initial and final drafts of the manuscript.

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