REVIEW 10.1111/j.1469-0691.2012.03782.x

Taxonomy of lice and their endosymbiotic bacteria in the post-genomic era

B. M. Boyd[1,2] **and D. L. Reed**[1]

1) Florida Museum of Natural History and 2) Genetics Institute, University of Florida, Gainesville, FL, USA

Abstract

Recent studies of molecular and genomic data from the parasitic lice of birds and mammals, as well as their mutualistic endosymbiotic bacteria, are changing the phylogenetic relationships and taxonomy of these organisms. Phylogenetic studies of lice suggest that vertebrate parasitism arose multiple times from free-living book and bark lice. Molecular clocks show that the major families of lice arose in the late Mesozoic and radiated in the early Cenozoic, following the radiation of mammals and birds. The recent release of the human louse genome has provided new opportunities for research. The genome is being used to find new genetic markers for phylogenetics and population genetics, to understand the complex evolutionary relationships of mitochondrial genes, and to study genome evolution. Genomes are informing us not only about lice, but also about their obligate endosymbiotic bacteria. In contrast to lice and their hosts, lice and their endosymbionts do not share common evolutionary histories, suggesting that endosymbionts are either replaced over time or that there are multiple independent origins of symbiosis in lice. Molecular phylogenetics and whole genome sequencing have recently provided the first insights into the phylogenetic placement and metabolic characteristics of these distantly related bacteria. Comparative genomics between distantly related louse symbionts can provide insights into conserved metabolic functions and can help to explain how distantly related species are fulfilling their role as mutualistic symbionts. In lice and their endosymbionts, molecular data and genome sequencing are driving our understanding of evolutionary relationships and classification, and will for the foreseeable future.

Keywords: γ-Proteobacteria, Insecta, Liposcelidae, *Pediculus*, Phthiraptera, *Riesia*
Article published online: 30 January 2012

Clin Microbiol Infect 2012; **18:** 324–331

Corresponding author: D. L. Reed, Florida Museum of Natural History, University of Florida, PO Box 117800, Gainesville, FL 32611, USA
E-mail: dlreed@ufl.edu

Overview

The parasitic lice are a diverse group of specialized insect parasites of birds and mammals. These parasites belong to a larger group of insects known as book and bark lice. Numerous phylogenies and classification schemes have been proposed for the parasitic lice. Traditional phylogenetics based on morphology suggested that parasitic lice were a monophyletic radiation, because of their permanent parasitic lifestyle [1–3]. However, more recently, molecular data have supported an alternative topology whereby the parasitic lifestyle would have arisen twice within the book and bark lice [4,5]. Molecular data are also revealing the age of this parasitism and the nature of louse–host associations over time [6]. Herein, we will review both old and new phylogenetic hypotheses, and we will illustrate how the recently sequenced genome of the human body louse [7] has already provided, and will continue to provide, additional insights into the evolutionary history of parasitic lice.

Ecology and Morphology of Parasitic Lice

There are *c.* 4500 recognized species of chewing lice (Amblycera, Ischnocera, and Rhyncophthirina) found on both mammals and birds [8]. The sucking lice (Anoplura) are a much smaller group, with 540 described species that occupy 12 mammalian orders [6]. Parasitic lice are generally host-specific, occupying

one or a few closely related species [8]. Many birds and mammals are known to harbour more than one species of louse, and in some cases different louse species may be restricted to only one region of their host. The diet of most chewing lice is dominated by keratin-rich dermal components such as feathers, skin, and hair. A few species of chewing lice (Rhyncophthirina) feed from the pooled blood of their hosts. The sucking lice feed strictly on the blood of their hosts by piercing the skin. The morphology of true lice is highly specialized to suit to an ectoparasitic lifestyle. Lice complete their entire life cycle on their host, and every stage is specialized for parasitism. Eggs of lice, known as nits, are large and affixed to the hair shafts or feather barbules of the host [9]. Lice are hemimetabolous, meaning that the immature stages, or nymphs, look similar to the imago and utilize the same resources. Adult parasitic lice are secondarily apterous, and the body is dorsoventrally flattened. In the Ischnocera, the head is broad and flattened, with the thorax being reduced. In other lice, the head is generally small and the thorax is reduced. The sensory organs are vestigial or absent and antennae are greatly reduced and concealed in the Ambylecera [9]. The tarsi of true lice are modified into claw-like structures to grasp the feathers or hairs of the host. The Amblycera and Ischnocera retain chewing mouthparts with which to feed on the skin, hair, and/or feathers of their hosts; blood feeding is minimal in these two groups. In contrast, the mouth of the Rhyncophthirina has been modified into a long rostrum with the mandibles located at the terminus of the rostrum [9]. The mandibles are rotated to rasp at the skin of the host, causing blood to pool, from which the louse sucks [9]. Mouthparts in the Anoplura have been highly modified to pierce mammal skin and suck blood from the host. Some morphological characteristics of parasitic lice can also be seen in the book louse family Liposcelididae. The Liposcelididae probably represent the closest living relatives of parasitic lice, or they may be part of the parasitic lice (see Discussion below) [1–5]. Liposcelids share similar morphological characteristics with their parasitic relatives, including loss or reduction of wings, eyes, and sensory organs, a smooth broad head, and a reduction of thoracic segments [9]. These small lice have been found in animal nests, feeding on shed fur and feathers, and there are a few documented occurrences within the fur or feathers of birds and mammals (see Grimaldi and Engle [9] for a review).

Higher Taxonomic Placement of Parasitic Lice

The Phthiraptera belong to the order Psocodea, which also includes the book lice (Liposcelidae) and bark lice (Psocoptera) [9]. The book and bark lice are small and often overlooked insects that are diverse and free-living. Many book and bark lice occupy moist areas, where they use modified mouthparts to scrape microorganisms from the surface of detritus [9]. However, some species have acquired the ability to survive desiccation, and feed on organic materials in caves, insect and animal nests, and human habitations [9]. Collectively, the Psocodea form the sister group to the Condylognatha, which includes both thrips (Thysanoptera) and true bugs (Hemiptera). The thrips and true bugs generally feed on the phloem of plants or are generalist insect predators. However, some members of the true bug group feed strictly on vertebrate blood such as bed bugs and kissing bugs. The Psocodea plus the Condylognatha represent a large monophyletic group of insects, known as the Paraneoptera [9]. The Paraneoptera have undergone numerous radiations to occupy niches and utilize numerous food resources. Within this group, feeding by piercing and sucking has arisen multiple times, as has parasitism and feeding on vertebrate blood.

Classification within the Phthiraptera

The evolutionary relationships of lice and their classification have changed considerably over multiple revisions [10]. Kim and Ludwig [1,2] supported two orders, the Mallophaga (all chewing lice) and the Anoplura (all sucking lice). Lyal [3] challenged the monophyly of the Mallophaga, instead supporting a topology of two sister clades. For many years, a phylogeny has persisted that contains two sister clades, one clade containing the Amblycera, and the other containing the Ischnocera, Rhynchopthrina, and Anoplura [10]. Johnson and Whiting [11] and Barker *et al.* [12] were the first to examine the Phthirpateran phylogeny by using molecular data (Fig. 1b). They largely supported Lyal's phylogeny, using both nuclear and mitochondrial markers under a maximum-parsimony criterion. Johnson *et al.* [4] were the first to reconstruct the phylogeny of the Phthirpatera under the maximum-likelihood criterion, using mitochondrial sequence data. Their findings suggested that parasitism had arisen multiple times in non-parasitic book lice, and that Phthiraptera was a polyphyletic classification. Johnson *et al.* [4] supported two families, the Liposcelididae (nest parasites described previously) and the Pachytroctidae, a small group of free-living book lice, as the closest relatives of the Amblycera. Yoskizawa and Johnson [5] further tested the polyphyly of the Phthiraptera under maximum-likelihood and Bayesian frameworks, using multiple nuclear and mitochondrial markers. They also supported the Phthiraptera as polyphyletic when the Liposcelididae and the Pachytroctidae are excluded (Fig. 1a). Both of these studies suggest that parasitism of vertebrates

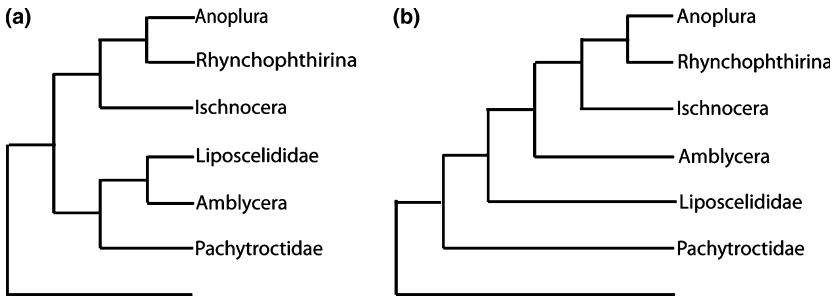

FIG. 1. Comparison of the relationship of phthirpateran families. (a) Traditional classification based on morphological data. (b) Classification based on recent molecular studies. Summarized from Yoskizawa and Johnson [5].

arose twice, once in the Amblycera and again in the common ancestor of the Ischnocera, Rhyncophthirina, and Anoplura. Under this new phylogeny, Smith *et al.* [13] used molecular dating techniques, calibrated to louse and host fossils, to determine the approximate age when major louse clades diverged. They found that all four Phthiraptera families and the Liposcelididae had diverged during the Mesozoic, prior to the K-Pg boundary. Whereas Smith *et al.* [13] found that the major families had Cretaceous origins, major radiations occurred late in the Cretaceous and early in the Cenozoic. Light *et al.* [6] conducted an extensive phylogenetic analysis of the Anoplura, and dated their divergence with a molecular clock calibrated to host divergence. They also found that the Anoplura diversified in the late Cretaceous, but that an additional major radiation occurred after the K-Pg boundary, following the radiation of mammals. Light *et al.* [6] found considerable disagreement with accepted anopluran phylogenies, most importantly demonstrating that host switching had occurred multiple times in anopluran history. This suggests that rapid host switching, and subsequent extinctions in some groups, has played a major role in the post-K-Pg diversification of sucking lice. Whereas host specificity and co-speciation appear to be important in the evolution of the Anoplura, host associations may be less informative for louse phylogeny [6].

Phylogeny and Taxonomy of Human Lice

Humans are parasitized by two species of sucking lice, the pubic louse (*Pthirus pubis* Linnaeus), and head and body lice (*Pediculus humanus* Linnaeus). Recent studies have helped to establish the taxonomic rank of the human body louse and the phylogenetic relationships of *Phtirus* and *Pediculus*. Light *et al.* [14] built a phylogenetic reconstruction of human head and body lice based on mitochondrial sequence data. They found that human body lice did not represent different species, but rather were eco-morphs of a single species. Reed *et al.* [15] investigated the relationships of human, chimp (*Pediculus schaeffi* Farenholz) and gorilla (*Pthirus gorillae* Ewing) lice, using both mitochondrial and nuclear sequence data. Reed *et al.* [15] found that human *Pediculus* species and *P. gorillae* shared a common phylogenetic history with their primate hosts, but that *P. pubis* did not. Light and Reed [16] later supported this topology by using multiple nuclear and mitochondrial markers. Both studies supported a divergence time of the human and gorilla species of *Pthirus* of about 3 million years ago (mya). Reed *et al.* [15] suggested that the human public louse arose from a host switch from gorillas to humans *c.* 3 mya.

Louse Perspective in the Post-genomic Era

The studies surveyed above have utilized molecular data to dramatically change our understanding of louse evolutionary history. The sequencing of the human body louse genome represents new opportunities to understand louse evolution and refine louse classification. The human body louse genome is the second sequenced genome of a hemimetabolous insect, providing opportunities for comparative genomics with more distantly related insect groups [7]. The genome sequence itself revealed numerous interesting characteristics in the nuclear and mitochondrial genomes, and holds potential for phylogenetics and population genetics in lice. The human louse possesses the smallest sequenced insect genome, about 108 Mb, but maintains a complete set of protein-coding genes and RNAs for basic metabolic functions [7]. However, unlike in most other insects, the canalization of lice as obligate ectoparasites has led to the loss of genes associated with detecting and responding to a variable environment [7]. The publication of the genome has also sparked interest and a series of publications on the composition of louse mitochondrial genomes and genome recombination [7,17–19]. Lice are the only insects known to possess mitochondrial genomes that are fragmented into a series of 18

small chromosomes, known as minichromosomes [7,19]. Not all louse species possess mitochondrial minichromosomes, and Cameron *et al.* [19] suspected a link with the functionality of the mitochondrial single-stranded binding protein. Shao and Barker [18] found evidence that louse mitochondrial minichromosomes have undergone multiple instances of non-homologous recombination, resulting in chimeric combinations of minichromosomes. Mitochondrial sequence data have played a major role in recent phylogenetic revisions of lice, and these studies will provide valuable information for the selection of mitochondrial sequence data for phylogenetics and interpretation of the results. The published human louse genome also holds potential for the rapid sequencing of multiple genes to build louse phylogenies and the asking of evolutionary questions. At the University of Illinois, Kevin Johnson and his research group are currently using a new method, the targeted restricted assembly method, to rapidly obtain phylogenetic markers in lice [20]. In this method, conserved gene sequences from the human louse genome are used as a reference for mining high-throughput sequence data from several louse species (K. Johnson, personal communication). The conserved gene library from the human louse allows reads to be readily mapped to genes to generate gene sequences. From the resulting multigene datasets, they plan to build extensive phylogenies of parasitic lice, particularly in the less studied chewing lice. In our laboratory at the University of Florida, we have used the genome data to develop a set of microsatellite markers, non-coding regions and coding regions for population genetics and phylogenetics in human lice and other anoplurans. These data are valuable for looking at population dynamics and migration patterns of human head lice. These lines of research were made possible or greatly accelerated by the release of the human louse genome. This genome has provided a powerful platform for elucidating louse evolutionary history, and will ultimately inform classification.

Endosymbionts of Lice

Insect–bacterium endosymbiosis is a common phenomenon. Numerous insect groups rely on nutritional provisioning by obligate endosymbiotic bacteria to sustain them on nutritionally incomplete diets. Acquisition of an endosymbiont may provide selective advantages to the host, and appears to have facilitated multiple insect groups' invasion of niches with limited diets, and subsequent radiation. Parasitic lice sustain themselves solely on the keratin-rich dermal components, secretions or blood of their hosts, a potentially incomplete diet. Many parasitic louse species have been found to harbour endosymbiotic bacteria that are potentially engaged in nutritional provisioning. Some endosymbionts of lice are found in the gut, whereas others are primary endosymbionts, being intracellular and housed in specialized structures known as mycetomes (Fig. 2). Experimental removal of primary endosymbionts from lice results in increased mortality and reduced fitness. These bacteria are suspected of providing vitamins that are absent in the louse's diet. Recent molecular phylogenetic studies have shown that parasitic lice share endosymbiotic relationships with both α-proteobacteria and γ-proteobacteria. However, all currently known primary endosymbionts (obligate intracellular endosymbionts) of lice belong to the γ-proteobacteria, from the families *Enterobacteriales* and *Legionellales*. Lice and their primary endosymbionts deviate more often from a shared, common evolutionary history than other groups of insects that harbour primary endosymbiotic bacteria. Because primary endosymbiotic bacteria cannot be cultured, genome sequencing and molecular phylogenetics provide the only opportunity to classify these bacteria and develop hypotheses regarding their symbiotic roles.

FIG. 2. Human head louse nymph, showing the white, circular mycetome in the abdomen where primary endosymbionts are housed. Photo credit: J. M. Allen.

The prevalence and complexity of interactions between lice and endosymbionts is varied across parasitic lice. Ries

[21] was the first to make an extensive review of louse mycetome structure and location. Buchner [22] summarized the work of Ries and all subsequent work on mycetome structure and bacterial transmission. From these studies, we learned that amblyceran species appear to have limited or no associations with bacteria [23]. Only α-proteobacteria are known to inhabit the gut of some amblycerans, and defined mycetome structures are absent [23]. The Ischnocera possess mycetomes, but these structures are not well organized [22]. Both the Rhyncophthirina and the Anoplura possess structured mycetomes. The mycetomes of the Rhyncophthirina and the Anoplura vary considerably between species in location, structure, and number [21,22]. Buchner [22] suspected that differences in housing and transmission of endosymbionts suggested that symbiosis between bacteria and lice arose multiple times.

Roles of Endosymbionts

Unfortunately, very little is known about the nutritional provisioning and metabolic roles regarding endosymbionts and lice. Aschner [24] and Puchta [25] conducted experiments with endosymbiont removal in human body lice (Anoplura). They [24,25] found that when endosymbionts were excluded, human body lice showed reductions in survival and fitness. Puchta [25] (as interpreted by Perottii *et al.* [23]) supplemented the diets of lice without endosymbionts, and found that B-vitamins (thiamine, riboflavin, folic acid, pyridoxine nicotinamide, pantothenate, and biotin) increased survival and fitness. Smith *et al.* [26] conducted endosymbiont removal in *Columbicola* species, and found a reduction in fitness when endosymbionts were removed. The endosymbiont of the slender pigeon louse (*Columbicola columbae* [Freire and Duarte]) is closely related to *Sodalis*, a secondary endosymbiont of tsetse flies [27]. *Sodalis* is prototrophic for many cofactors and amino acids [28], and nutritional provisioning is suspected in other *Sodalis*-like endosymbionts. The endosymbiont of *Columbicola* may also be engaged in cofactor or amino acid provisioning. These studies only attempted to address metabolite provisioning from the endosymbiont to the host. No studies have been conducted on provisioning from the host to the endosymbiont. For other insect endosymbionts, provisioning from the host to the endosymbiont can vary considerably between associations. For example, *Carsonella*, an endosymbiont of psyllids, requires provisioning of both metabolites and small proteins [29], whereas *Buchnera*, an endosymbiont of aphids, requires only metabolites from its host [30]. Much remains unknown about the associations between lice and endosymbionts, and in the post-genomic era, there is the potential to understand these relationships.

Taxonomy and Phylogeny of Louse Primary Endosymbionts

The phylogenetic relationships of only a few louse primary endosymbionts have been investigated. Fukatsu *et al.* [31] were the first to characterize the phylogenetic placement of the primary endosymbiont of human body lice. They found this to be a γ-proteobacterium, and named it *Candidatus Riesia pediculicola*. Allen *et al.* [32] further investigated *C. Riesia*, and found that it had co-speciated with its hosts, the lice of great apes (Pediculidae and Pthiridae). Allen *et al.* [32] also described two more species within *C. Riesia*, and noted the close relationship of *C. Riesia* to *Aresnophonus*, an endosymbiont of haematophagous dipterans. Novakova *et al.* [33] conducted an extensive phylogenetic reconstruction of *Arsenophonus* species, sampling from endosymbionts of plants, ticks, and four orders of insect. Novakova *et al.* [33] found that *C. Riesia* belongs within a clade of *Arsenophonus* endosymbionts of dipterans. Allen *et al.* [34] dated the divergence between the *Riesia* and *Arsenophonus* clades at 13–25 mya, making this one of the youngest known insect–primary endosymbiont associations. The next youngest involves the primary endosymbiont of the grain weevil, which is estimated to have diverged from the secondary endosybmiont of tsetse flies, *Sodalis*, 25 mya [30]. Most insect–primary endosymbiont associations range from 50 to 350 mya [30], which is considerably older than the louse–endosymbiont association.

A sister clade to the hominid lice is the Pedicinidae, comprising the lice of cercopithecid primates [6]. Fukatsu *et al.* [35] were the first to investigate the phylogenetic placement of primary endosymbionts in the Pedicinidae. They found that the endosymbiont of *Pedicinus obtusus* represented a primary endosymbiont independent of *C. Riesia*, and proposed the name *Candidatus Puchtella*. Interestingly, phylogenetic reconstruction placed *C. Puchtella* close to *Wigglesworthia*, the primary endosymbiont of tsetse flies [35]. Like *C. Riesia*, *C. Puchtella* is another louse primary endosymbiont that is closely related to an endosymbiont of a non-louse blood-feeding insect.

Hypsa and Krizek [36] sampled primary endosymbionts from the anopluran genera *Haematopinus*, *Solenoptes*, *Pediculus*, and *Polyplax*, and from the rhyncophthirinan genus *Haematomyzus* (lice of ungulates, hominids, rodents, and elephants). They found that these louse primary endosymbionts represented five independent clades of endosymbionts. Whereas the primary endosymbionts of

Haematopinus, *Solenoptes*, *Pediculus* and *Haematomyzus* were from the *Enterobacteriales*, the endosymbionts from *Polyplax* were members of the *Legionellales*. Collectively, with *C. Puchtella*, this suggests six known independent lineages of louse primary endosymbionts within the Anoplura and the Rhyncophthirina. Allen *et al.* [37] presented the first attempt to determine how many times louse primary endosymbiosis has arisen in γ-proteobacteria. They conducted a large-scale phylogenetic analysis of thousands of bacterial strains, supporting additional lineages. They found that there are at least ten distinct lineages of endosymbionts in mutualistic relationships with lice.

Although they are much more diverse, very little is known about primary endosymbiosis in the Ischnocera. The primary endosymbiont of *Columbicola columbae* (slender pigeon louse) was investigated for its phylogenetic placement in γ-proteobacteria by Fukatsu *et al.* [27]. They found it to be closely allied with *Sodalis glossinidius*, a secondary endosymbiont of the tsetse flies (Diptera). Additional *Sodalis*-like endosymbionts have recently been described from multiple distantly related insect groups, including a primary endosymbiont in the weevil genus *Sitophilus* (Coleoptera) [38], a secondary endosymbiont of the parasitic fly *Craterina melbae* (Rondani; Diptera) [39], and a primary endosymbiont of the stinkbug *Cantao ocellatus* (Thunberg; Hempitera) [40]. Additional studies would improve our understanding of ischnoceran endosymbiosis and determine whether polyphyly is present in this group as well.

Perspectives for the Post-genomic Era

The post-genomic era holds great potential for identifying and classifying the γ-proteobacterial primary endosymbionts of true lice. Unlike free-living and pathogenic bacteria, louse primary endosymbionts cannot be cultured and classified with traditional microbiological methods. Sequencing of the 16S rRNA gene by PCR has provided valuable insights into the diversity of primary endosymbionts of all insects. Recent studies have shown that lice house distantly related primary endosymbionts that are closely related to other insect symbionts and pathogens. Although this work has added to our understanding of louse primary endosymbiosis, the A/T-rich and low-complexity regions prevalent in insect endosymbiont genomes often limit PCR techniques. The recent publication of the genome of *C. Riesia pediculicola* revealed a small genome, 574 kB, similar to what is found in other insect primary endosymbionts [7]. Genomes of this size can easily be sequenced at low cost with current high-throughput sequencing technologies. Although primary endosymbiont bacteria cannot easily be separated from louse tissues, supercomputers and metagenomic algorithms allow for parsing of mixed short-read pools. These technologies have brought whole genome sequences of louse primary endosymbionts within reach, with respect to both budget and time. An initiative to sequence multiple genomes of louse primary endosymbionts would provide additional markers for phylogenetic analysis and insights into the symbiotic interaction between louse and bacteria. Although 16S rRNA is a valuable resource, Novakova *et al.* [33] and Comas *et al.* [41] have both highlighted the importance of using multiple phylogenetic markers when reconstructing the evolutionary history of endosymbionts. Additional markers would provide additional resolution in closely related taxa, and the recent explosion of publically available bacterial genomes would make multigene phylogeny building feasible. Unlike the recent queries into the evolutionary history of louse primary endosymbionts, very few attempts have been made to describe the nutritional role that the primary endosymbiont provides for its louse host, and vice versa. Past endosymbiont removal experiments, such as that conducted by Puchta [25], may not be possible for many species of lice. Whole genome sequences would provide new insights on which we can build hypotheses of metabolic provisioning via metabolites (and potentially proteins) to both the louse host and primary endosymbiont. Collectively, these two lines of study would provide insights into how distantly related endosymbionts come to inhabit louse mycetomes and act as primary endosymbionts engaged in metabolite provisioning. Ultimately, we will learn whether these disparate bacteria have used similar means to provide for their host.

Conclusions

Recent molecular data and increasingly sophisticated phylogenetic analyses are challenging our hypotheses of the evolutionary history of parasitic lice. It appears that parasitism has arisen twice within lice, and that host switching has played an important role in louse speciation. Previous proposals of louse phylogenies based on morphology have been contentious at times. Next-generation sequencing and genome assembly technologies offer an opportunity to test current phylogenetic hypotheses. Rapid sequencing and efficient gene mapping to the human louse genome will allow for extensive multigene phylogenies to be developed and improve our understanding of the evolutionary history and classification of lice.

Molecular data have provided the first insights into louse primary endosymbiont evolutionary history. Parasitic lice and their primary endosymbionts do not share a completely

overlapping evolutionary history, which is largely unique in insect–endosymbiont systems. Additionally, parasitic louse primary endosymbionts are among the youngest known insect primary endosymbionts. Whether these endosymbionts are being replaced by new endosymbionts or whether louse endosymbiosis has arisen multiple times independently remains an important evolutionary question. Whether these bacteria are fulfilling precisely the same roles in symbiosis is also an intriguing question. The recent surge in available γ-proteobacteria genomes and advances in next-generation sequencing technologies will bring whole genome sequencing within the time and budget limitations of most laboratories. Whole genome sequences will provide additional markers for phylogenetics and help us to understand the roles of primary endosymbionts in lice by comparative genomics, improved phylogenies, and understanding genome evolution.

Acknowledgements

We would like to thank J. A. Soto-Centeno and M. Ascunce for editing early drafts of this manuscript. We also thank V. S. Smith for reviewing the manuscript. This work was supported in part by grants from the National Science Foundation to D. L. Reed (DEB 0717165 and DEB 0845392).

Transparency Declaration

The authors declare no conflicts of interest.

References

1. Kim KC, Ludwig HW. The family classification of Anoplura. *Syst Entomol* 1978; 3: 249–284.
2. Kim KC, Ludwig HW. Parallel evolution, cladistics, and classification of parasitic Psocodea. *Ann Entomol Soc Am* 1982; 75: 537–548.
3. Lyal CHC. Phylogeny and classification of the Psococea, with particular reference to the lice (Psocodea, Phthiraptera). *Syst Entomol* 1985; 10: 145–165.
4. Johnson KP, Yoshizama K, Smith VS. Multiple origins of parasitism in lice. *Proc R Soc B* 2004; 271: 1771–1776.
5. Yoshizawa K, Johnson KP. How stable is the 'polyphyly of lice' hypothesis (Insecta: Psocodea)?: a comparison of phylogenetic signal in multiple genes. *Mol Phylogenet Evol* 2010; 55: 939–951.
6. Light JE, Smith VS, Allen JM, Durden LA, Reed RL. Evolutionary history of mammalian sucking lice (Phthiratpera: Anoplura). *BMC Evol Biol* 2010; 10: 292–306.
7. Kirkness EF, Haas BJ, Sun W *et al.* Genome sequences of the human body louse and its primary endosymbiont provide insights into the permanent parasitic lifestyle. *Proc Natl Acad Sci USA* 2010; 107: 12168–12173.
8. Price RD, Hellenthal RA, Palma RL, Johnson KP, Clayton DH. The chewing lice: world checklist and biological overview. *Illinois Nat Hist Special Publication* 2003; 24: i-501.
9. Grimaldi D, Engel MS. *Evolution of the insects*. Cambridge: Cambridge, University Press, 2005.
10. Smith VS. Lousy phylogenies: Phthiraptera systematic and the antiquity of lice. *Entomol Abh* 2004; 61: 150–151.
11. Johnson KP, Whiting MF. Multiple genes and the monophyly of Ischnocera (Insecta: Phthiraptera). *Mol Phylogenet Evol* 2002; 22: 101–110.
12. Barker SC, Whiting M, Johnson KP, Murrell A. Phylogeny of the lice (Insecta, Phthirpatera) inferred from small subunit rRNA. *Zool Scr* 2003; 32: 407–414.
13. Smith VS, Ford T, Johnson KP, Johnson PCD, Yoshizawa K, Light JE. Multiple lineages of lice pass through the K-Pg boundary. *Biol Lett* 2011; 7: 782–785. 10.1098.
14. Light JE, Toups MA, Reed DL. What's in a name: the taxonomic status of the human head louse and body louse. *Mol Phylogenet Evol* 2008; 47: 1203–1216.
15. Reed DL, Light JL, Allen JM, Kirchman JJ. Pair of lice lost or parasites regained: the evolutionary history of anthropoid primate lice. *BMC Biol* 2007; 5: 7–17.
16. Light JE, Reed DL. Multigene analysis of phylogenetic relationships and divergence times of primate sucking lice (Phthirpatera: Anoplura). *Mol Phylogenet Evol* 2009; 50: 376–390.
17. Shao R, Kirkness EF, Barker SC. The single mitochondrial chromosome typical of animals has evolved into 18 minichromosomes in the human body louse, *Pediculus humanus*. *Genome Res* 2009; 19: 904–912.
18. Shao R, Barker SC. Chimeric mitochondrial minichromosomes of the human body louse, *Pediculus humanus*: evidence for homologous and non-homologous recombination. *Gene* 2011; 473: 36–43.
19. Cameron SL, Yoskizawa K, Mizukoshi A, Whiting MF, Johnson KP. Mitochondrial genome deletions and minicircles are common in lice (Insecta: Phthiraptera). *BMC Genomics* 2011; 12: 394–408.
20. Johnson KP, Walden KKO, Robertson HM. A targeted restricted assembly method (TRAM) for phylogenetics. *Nat Proc* 2011; npre20104612-1.
21. Ries E. Die symbiose der lause und federlinge. *Aus dem Zoologischen der Universitat Breslau* 1930;mit 74.
22. Buchner P. *Endosymbiosis of animals with plant microorganisms*, revised English version. New York, NY: Interscience Publishers, 1965.
23. Perotti MA, Kirkness EF, Reed DL, Braig HR. Endosymbionts of lice. In: Bourtzis K, Miller TA, eds. *Insect symbiosis*, Vol. 3. Boca Raton, FL: CRC Press, 2009; 205–219.
24. Aschner M. Studies on the symbiosis of the body louse: I Elimination of the symbionts by centrifugalisation of the eggs. *Parasitology* 1934; XXVI: 309–314, plate XII.
25. Puchta O. Experimentelle untersuchungen uber die bedeutung der symbiose der kleiderlaus *Pediculus vestimenti* Burm. *Z. Parasitenkunde* 1955; 17: S1–S40.
26. Smith WA, Dale C, Clayton DH. Determining the role of bacterial symbionts with the genus *Columbicola*. *Turkiye Parazitol Derg* 2010; 34 (suppl 1): 67.
27. Fukatsu T, Koga R, Smith WA *et al.* Bacterial endosymbionts of the slender pigeon louse, *Columbicola columbae*, allied to endosymbionts of the grain weevils and tsetse flies. *Appl Environ Microbiol* 2007; 73: 6660–6668.
28. Toh H, Weiss BL, Perkin SAH *et al.* Massive genome erosion and functional adaptations provide insights into the symbiotic lifestyle of *Sodalis glossinidius* in the tsetse host. *Genome Res* 2006; 16: 149–156.
29. Tamames J, Gil R, Latorre A, Pereto J, Silva FJ, Moya A. The frontier between cell and organelle: genome analysis of *Candidatus* Carsonella ruddii. *BMC Evol Biol* 2007; 7: 181–187.

30. Gosalbes MJ, Latorre A, Lamelas A, Moya A. Genomics of intracellular symbionts in insects. *Int J Med Microbiol* 2010; 300: 271–278.
31. Fukatsu KS, Koga R, Nikoh N *et al.* Symbioitic bacteria associated with stomach discs of human lice. *Appl Enivon Microbiol* 2006; 72: 7349–7352.
32. Allen JM, Reed DL, Perotti MA, Braig HR. Evolutionary relationships of '*Candidatus* Riesia spp.,' endosymbiotic *Enterobacteriaceae* living within hematophagous primate lice. *Appl Environ Microbiol* 2007; 73: 1659–1664.
33. Novakova E, Hypsa V, Moran NA. *Arsenophonus*, an emerging clade of intracellular symbionts with a broad host distribution. *BMC Microbiol* 2009; 9: 143–156.
34. Allen JM, Light JE, Perotti MA, Braig HR, Reed DL. Mutational meltdown in primary endosymbionts: selection limits Muller's ratchet. *PLoS One* 2009; 4: e4969.
35. Fukatsu T, Hosokawa T, Koga R *et al.* Intestinal endocellular symbiotic bacterium of the macaque louse *Pedicinus obtusus*: distinct endosymbiont origins in anthropoid primate lice and the old world monkey louse. *Appl Environ Microbiol* 2009; 75: 3796–3799.
36. Hypsa V, Krizek J. Molecular evidence for polyphyletic origin of the primary symbionts of sucking lcie (Phthiratpera, Anoplura). *Microb Ecol* 2007; 54: 242–251.
37. Allen JM, Light JE, Reed DL. Coevolutionary history of sucking lice and their primary endosymbionts. *Turkiye Parazitol Derg* 2010; 34((suppl 1): 68.
38. Charles H, Condemine G, Nardon C, Nardon P. Genome size characterization of the principal endocullular symbiotic bacteria of the weevil *Sitophilus oryza*e, using pulsed field gel electrophoresis. *Insect Biochem Mol Biol* 1997; 27: 345–350.
39. Novakova E, Hypsa V. A new *Sodalis* lineage from bloodsucking fly *Caterina melbae* (Diptera, Hippoboscoidea) originated independently of the tsetse fly symbiont *Sodalis glossinidius*. *FEMS Microbiol Lett* 2007; 269: 131–135.
40. Kiawa N, Hosokawa T, Kikuchi Y *et al.* Primary gut symbiont and secondary, *Sodalis*-allied symbiont of the Scutellerid stinkbug *Cantao ocellatus*. *Appl Environ Microbiol* 2007; 76: 3486–3494.
41. Comas I, Moya A, Gonzales-Candelas F. From phylogenetics to phylogenomics: the evolutionary relationships of insect endosybiotic γ-proteobacteria as a test case. *Syst Biol* 2007; 56: 1–16.