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Secondary structure construction and molecular identification of rRNA 18S V4 region E23-5–E23-6 of parasitic lice of Hominidae

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Abstract

The parasitic lice of Hominidae are a class of blood-sucking insects, having a large fragment expansion region in ribosome 18S V4 region. In this study, the value of the E23-5–E23-6 stem-loop structure in the insertion region for molecular identification of lice were explored through motif analysis and secondary structure construction. Five pubic lice samples from China were morphologically identified, and primers for the rRNA 18S V4 region were designed for molecular identification. The V4 sequence of the parasitic lice of Hominidae was retrieved from GenBank for sequence analysis. The five samples were identified as pubic lice based on V4 region, which were of the same specie but geographically different from Australian strains in Genbank, with the identity of 99.06-99.46%. Compared with the human lice, both the chimpanzee lice and pubic lice had large indel fragments in the V4 region. Comparison results showed that Muscle and MAFFT had better alignment and phylogeny results than Clustal. The large expansion region, comprising E23-5 and E23-6, was located between E23-4 and E23-7. The V4 secondary structure showed that the stem-loop structures of the lice parasitizing on pubic area, human, and chimpanzee were different in the E23-5 and E23-6, which could effectively distinguish the three parasitic lice and divide the human lice into five genotypes. This is suitable not only for the identification of three lice species in higher taxonomic ranks but also for genotype identification of human lice in lower taxonomic ranks. The difference between the stem-loop structure is more intuitive than that between the primary sequences.

Introduction

The lice are the oldest permanent ectoparasite of humans and have a high degree of host specificity throughout its life history. Lice parasitizing the human body can be divided into three categories based on the parasitic site: head lice (*Pediculus humanus capitis* Linnaeus, 1758) (Anoplura: Pediculidae), body lice (*Ped. h. corporis* or *Ped. humanus* Linnaeus, 1758) (Anoplura: Pediculidae), and pubic lice (*Pthirus pubis* Linnaeus, 1758) (Anoplura: Phthiridae). Head lice are mainly

parasitic on the head, behind the ears, and on hair roots. Body lice are mainly found in the folds of intimate underwear. The adults of head and body lice are so similar that it is difficult to distinguish their morphology. They are collectively referred to as human lice. As a vector, human lice can transmit infectious diseases, such as epidemic typhus, trench fever, and louse-borne relapsing fever, posing a notable risk to human health (Bragg and Wills, 2022). Compared to human lice, pubic lice are easily distinguished by their short size and crab-like shape and are usually found in the pubic hair of the perineum and perianal or axillary hair areas, with the main symptoms being itching and rash of the vulvar skin. In 1975, pubic lice infection was recognized as a secondary sexually transmitted disease by the World Health Organization (Anderson and Chaney, 2009). Besides infecting adults, it also occurs in adolescents, especially infants, which has become a potential public health problem.

Ribosomal 18S is a common gene for molecular identification, with a slow rate of nuclear gene evolution and relatively conservative sequences and is mainly used for higher taxonomic categories studies (Wu et al., 2015), while the 18S V4 region can be used for lower taxonomic rank species identification, owing to its large sequence variation (Chatanga et al., 2021). Stephen et al. (2003) analyzed the phylogeny of 33 species of the four suborders of lice (Insecta: Phthiraptera) using small subunit rRNA sequences (SSU rRNA). Unexpectedly, compared to insects such as *Drosophila melanogaster* Linnaeus, 1758 (Diptera: Drosophilidae), large expansion regions were observed in the 18S V4 region between E23-4 and E23-7. Accordingly, here, the 18S V4 region was selected as the target gene fragment, the nucleotide sequences were obtained, and the construction of the secondary structure of the large expansion regions of parasitic lice of Hominidae was attempted for molecular identification. However, the sequence of 18S V4 region in GenBank is limited, and most of them belong to human lice(Leo and Barker, 2005, Light and Reed, 2009). In contrast, pubic lice and chimpanzee lice have one sequence each, and more sequences of related species need to be obtained, to acquire sufficient samples for taxonomic identification. At the same time, it is difficult to use conventional software to compare the large fragment expansion regions of E23-4 and E23-7. It is necessary to explore appropriate software for sequence alignment to determine the location of the expansion region and implement secondary structure construction and molecular identification.

In this study, *Pth. pubis* samples were first obtained from Shaanxi, China for morphological and molecular identification, the full-length sequences of rDNA 18S V4 region were downloaded from GenBank, suitable software was selected for sequence alignment, and the location of the large expansion region was determined by MEME motif analysis. Finally, the

secondary structure of the V4 regions of the lice parasitizing on pubic area, human and chimpanzee were reconstructed and the E23-5–E23-6 was compared to identify parasitic lice of Hominidae.

Section snippets

Sample collection and morphological identification

Pubic lice samples were collected from outpatients of a county hospital in Shaanxi, China. Samples were obtained from patients' shaved pubic hair and intimate clothing and examined under a Motic light microscope (Motic, Xiamen, China), and morphologically intact insects were collected, identified, and photographed for retention.…

DNA extraction, PCR amplification, cloning, and sequencing

Genomic DNA was extracted from the front feet of individual lice using the Chelex-100 method. PCR amplification, cloning, and sequencing were performed on five samples…

Morphological identification

To the naked eye, the samples were grayish-white and resembled a small flat piece of scurf. Microscopic observation, as shown in Fig. 1, showed that the insect body is broad and crab-like; the idiosoma is about 2 mm long and 1.5 mm wide; the head has a pair of antennae; the eyes are located on both sides of the head; the thorax and abdomen are wide at the front and narrow at the back; the lateral edge of the body has a conical lateral protrusion with setae on it; and three pairs of feet, the…

Discussion

In this study, the secondary structure construction and molecular identification of parasitic lice of Hominidae based on the rRNA 18S V4 region had the following highlights. (1) Based on morphological identification, the only sequence from the Australian pubic lice in GenBank was used for the first time to design primers for the 18S V4 region, and the molecular identification of five Chinese pubic lice strains was completed for the first time, found that there were 8 base differences between…

Supplementary data

Figure S1. Sequence alignment of ribosome 18S V4 variable region of parasitic lice of Hominidae based on Clustal algorithm.

Figure S2. Sequence alignment of ribosome 18S V4 variable region of parasitic lice of Hominidae based on Muscle algorithm.

Figure S3. Sequence alignment of ribosome 18S V4 variable region of parasitic lice of Hominidae based on MAFFT algorithm.…

CRediT authorship contribution statement

Wanyu Zhang: Methodology, Data curation, Software, Visualization, Writing – original draft. **Haoruo Li:** Resources, Investigation. **Yae Zhao:** Conceptualization, Funding acquisition, Project administration, Writing – review & editing. **Chenglin Guan:** Visualization, Investigation. **Rong Chai:** Visualization, Investigation. **Chenxi Yang:** Visualization, Investigation. **Li Hu:** Writing – review & editing.…

Declaration of Competing Interest

None of the authors have conflicts of interest to report.…

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