学位論文内容の要旨
Abstract of the dissertation

博士の専攻分野の名称：博士（獣医学）　　氏名： Chukwunonso Onyemaechi Nzelu
Name

学位論文題名
The title of the doctoral dissertation

Molecular epidemiological study of *Leishmania* and sand fly vectors

（リーシュマニアおよび媒介昆虫サシチョウバエに関する分子疫学的研究）

Leishmaniasis is a vector-borne parasitic disease caused by intracellular protozoa of the genus *Leishmania,* transmitted by female phlebotomine sand flies. Early and accurate diagnosis of infected patients and the identification of *Leishmania* species are crucial for treatment and prognosis. Moreover, effective control and monitoring of leishmaniasis transmission require the identification of circulating sand fly species and vectors in endemic areas.

In Chapter 1, the study provided the cytochrome c oxidase subunit 1 (COI) barcodes for several Peruvian sand flies and showed their effectiveness in discriminating species recognized through prior conventional taxonomic work. Neighbor-joining analysis based on Kimura 2-Parameter genetic distances formed non-overlapping clusters for all species. The levels of intraspecific genetic divergence ranged from 0 to 5.96%, whereas interspecific genetic divergence among different species ranged from 8.39 to 19.08%. The generated COI barcodes could discriminate between all the sand fly taxa. In addition, the COI gene was

found to be useful in revealing population differentiation and cryptic diversity, and thus promises to be a valuable tool for vector surveillance.

In Chapter II, the study detected for the first time the DNA of *L*. (*L*.) *tropica*, *L*. (*L*.) *major* and *Trypanosoma* species within *Sergentomyia* sand flies in Ghana, and suggests that *S. ingrami* and *S. hamoni* are potential vectors of cutaneous leishmaniasis (CL) in the outbreak area of Ghana. The detection of *L*. (*L*.) *tropica* DNA in the CL outbreak focus is a novel finding in Ghana as well as West Africa. Additionally, the detection of *Trypanosoma* species in this study also provides the first report on infection of sand fly by trypanosomes other than *Leishmania* in West Africa. Significantly, the study supports the possibility of *Sergentomyia* sand flies as the vectors of CL in Ghana other than *Phlebotomus*, which contains all currently known vectors for *Leishmania* in the Old World.

In Chapter III, a novel loop-mediated isothermal amplification (LAMP) method for the mass screening of sand flies for *Leishmania* infection based on 18S rRNA gene was developed. The LAMP method could amplify *Leishmania* DNA from crude sand fly template within 60 min using a normal water bath or heating block. The assay is highly sensitive enough to detect 0.01 *Leishmania* parasites. Amplicon detection could be accomplished by the newly developed colorimetric malachite green dye-mediated naked eye visualization. The study represents the first report on the use of malachite green dye in the visualization of LAMP products. The field applicability of the assay was demonstrated using field captured sand fly specimens from Ecuador, and showed to be a reliable rapid surveillance tool for leishmaniasis.

In Chapter IV, a one-step, single-tube, field applicable LAMP assay in combination with FTA card as a direct sampling tool for diagnosis of cutaneous leishmaniasis was established. The LAMP assay could reliably amplify 0.01 *Leishmania* parasites from FTA card DNA template and showed no cross reactivity with *Leishmania* related human pathogenic

trypanosomes. The colorimetric malachite green based FTA-LAMP assay is rapid and simple to perform and may provide an important tool for diagnosis of cutaneous leishmaniasis.