

MORPHOSPESIES AND
PHYLOGENETIC TREE
ANALYSES OF
LEUCOCYTOZOOM
CAULLERYI FROM CHICKENS
LEUCOCYTOZOOMONOSIS
CASES IN PASURUAN, EAST
JAVA

Submission date: 21-Aug-2020 02:50PM (UTC+0800)
by Endang Suprihati

Submission ID: 1372142005

File name: Morphospesies_and_phylogenetic....pdf (2.25M)

Word count: 2010

Character count: 12577

MORPHOSPECIES AND PHYLOGENETIC TREE ANALYSES OF LEUCOCYTOZOOM CAULLERYI FROM CHICKENS LEUCOCYTOZOOONOSIS CASES IN PASURUAN, EAST JAVA

Endang Suprihati

Department of Parasitology, Faculty of Veterinary Medicine, Universitas Airlangga

ABSTRACT

The current taxonomy of leucocytozoids is based on the morphology of blood stages of the parasites and on limited information about their specificity. Recent molecular studies have revealed a remarkable genetic diversity of leucocytozoids, indicating that their taxonomic diversity may be greater than in the current classifications. We addressed this issue using morphological data and phylogenetic analysis of the cytochrome b gene of 4 positively identified species of Chickens Leucocytozoonosis in Pasuruan, East Java. Based on the current taxonomy, *Leucocytozoon caulleryi* is the sole species of leucocytozoids parasitizing chicken. We investigated the morphology of blood stages of leucocytozoids of 4 haplotypes and concluded that these parasites can be readily distinguished due to length of the cytoplasmic processes of their host cells; therefore, they do represent distinct morphospecies. Morphology of the cytoplasmic processes of host cells warrants more attention in the taxonomy of *Leucocytozoon* species. *Leucocytozoon caulleryi* indeed is a species group that currently can be separated from other *Leucocytozoon* by morphospecies analysis. It is can be concluded by phylogenetic tree analysis that 4 *Leucocytozoon caulleryi* from Pasuruan closely related strain.

Keywords: *Leucocytozoon caulleryi*, morphospecies, phylogenetic tree, cytochrome b gene

INTRODUCTION

Leucocytozoon in Pasuruan, East Java is one of the parasites that can attack the chickens throughout the year although the frequency is not fixed, and in certain areas are endemic. Services for Pasuruan Animal Husbandry has never reported a poultry disease caused by *Leucocytozoon* although the disease is highly detrimental to farmers so that there is no government intervention associated with the control and eradication of this disease. Hence the publication of this research is expected to provide input to the government to *Leucocytozoonosis* be one of the diseases included in control programs.

Leucocytozoon spp morphological identification is often not determined until the determination of species due to the occurrence of morphological variation so that the difficulties in characterizing the morphology of the parasite. Phenotypic characters can overlap among species and the real changes that can occur among isolates of the same species. With advances in biomolecular engineering, the morphological diversity can be searched directly cause the gene level, the material responsible for the occurrence of morphological variation. The use of DNA sequences in population genetic studies has grown rapidly providing more in-depth information about the evolution and phylogenetic relationships. Series of genetic information contained in mitochondrial DNA has been reported to describe the characteristics of a population, phylogenetic and reconstruct the evolutionary history (Kvist, 2000). The use of phylogenetic tree can be useful to know the causative agent relationship in *Leucocytozoonosis* cases that happened in Pasuruan.

2 MATERIALS AND METHODS

Study Sites and Blood Samples

Blood samples were collected in Pasuruan, East Java from September to December 2011, as described by Sehgal *et al.* (2006). Blood was taken by venipuncture of the medial metatarsal vein. Extraction of DNA and PCR and sequencing was done during our recent study (Sehgal *et al.*, 2006). Blood smears were fixed in methanol and stained with Giemsa, as described by Valkiunas (2005). Positive blood samples, as determined by PCR-based analysis (Sehgal *et al.*, 2006), from 10 chickens that have sign of Leucocytozoonosis were used for investigations of the morphology of leucocytozoids and their host cells. In morphological analysis, we used only a good-quality blood film, which is essential for taxonomic studies (Valkiunas *et al.*, 2008). Sehgal *et al.* (2006) described details of the study sites and collection of the material.

Morphological Analysis

An Olympus BX61 light microscope (Olympus, Tokyo, Japan) equipped with Olympus DP70 digital camera and imaging software ANALYSIS FIVE (Olympus Soft Imaging Solution, GmbH, Munster, Germany) was used to examine blood films, prepare illustrations, and to take measurements. Intensity of infection was estimated as a percentage by actual counting of the number of parasites per 1,000 red blood cells, or per 10,000 red blood cells if infections were light, i.e., 0.1%, as recommended by Valkiunas *et al.* (2010). Blood films with intensity of Leucocytozoon spp. Intensity of parasitemia in the investigated chickens was light (0.01%), so this sample was not used for morphometric analysis. The morphometric features studied (Fig.1) were those defined by Valkiunas (2005).

Phylogenetic Analysis

The phylogenetic analysis was based on sequences (500 bp) of the cyt b gene PCR product compare to sequences of GenBank. Phylogenetic analyses using maximum parsimony techniques were conducted using PAUP*4.0b10 (Swofford, 2002). Searches used the bootstrap search option, with 1,000 stepwise addition replicates, using the TBR branch-swapping algorithm. In addition, we performed distance analyses using the Kimura 2-parameter distance model and taxa were joined using neighbor-joining analysis (Perkins and Schall, 2002; Saito *et al.*, 2007). Simple consensus trees were constructed to summarize the results: both maximum-parsimony and neighbor-joining analyses resulted in trees with identical topologies. The sequence divergence between the different lineages (Fig. 2) was calculated with the use of a Jukes-Cantor model of substitution, with all substitution weighted equally, implemented in the program CLUSTALW (Kumar *et al.*, 2004).

RESULTS AND DISCUSSION

Morphological Analysis

There were no significant differences in all morphometric features of Leucocytozoon sp. gametocytes and their host cells in different individual hosts belonging to the same species of chicken, so we pooled morphometric data for parasite measurements in different individual hosts belonging to the same chicken species. These data are given in Figure 1.



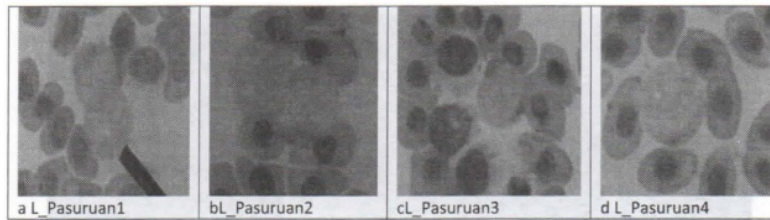


Figure 1. a-d. Gametocytes of *Leucocytozoon caulleryi* in fusiform host cells from the blood of Chickens

Phylogenetic Analysis

Parasites from 4 Pasuruan chickens cluster together, and form distinct on clades in the phylogenetic tree (Fig. 2). Sequence divergence between 4 positively identified as *Leucocytozoon caulleryi*.
Scores

Table 1. The sequence divergence (in percentage) between mitochondrial cytochrome b lineages of positively identified species of *Leucocytozoon caulleryi* GenBank, *L. caulleryi* Pasuruan and *Plasmodium.juxtranucleare*.

SeqA	Name	Length	SeqB	Name	Length	Score
1	<i>Leucocytozoon_ caulleryi_AB302215</i>	493	2	<i>Leucocytozoon_ Pasuruan1</i>	493	99.0
1	<i>Leucocytozoon_ caulleryi_AB302215</i>	493	3	<i>Leucocytozoon_ Pasuruan2</i>	493	100.0
1	<i>Leucocytozoon_ caulleryi_AB302215</i>	493	4	<i>Leucocytozoon_ Pasuruan3</i>	493	97.0
1	<i>Leucocytozoon_ caulleryi_AB302215</i>	493	5	<i>Leucocytozoon_ Pasuruan4</i>	493	99.0
1	<i>Leucocytozoon_ caulleryi_AB302215</i>	493	6	<i>Plasmodium_ juxtanucleare_AB302893</i>	478	88.0

We encourage using morphological analysis of the cytoplasmic processes during description of *Leucocytozoon* spp. Such processes develop only in some species of *Leucocytozoon*; the function of the processes remains unknown. It is important to note that the character presence or absence of the cytoplasmic processes can hardly be applied in the taxonomy of leucocytozoids, on either the level of genera or subgenera, because lineages of parasites possessing the cytoplasmic processes of *Leucocytozoon caulleryi* are paraphyletic in the phylogenetic analysis (Fig. 2). This conclusion is in accord with former taxonomic conclusions which are based on morphology and life histories of leucocytozoids (Fallis *et al.*, 1974). The present study shows that morphology of the cytoplasmic processes of host cells warrants more attention in the taxonomy of *Leucocytozoon* species. Detailed analysis of morphology of the processes has not been applied in taxonomy of these parasites, so far. It is worth noting that development of the fusiform processes of host cells is induced by developing gametocytes. Because the development of the fusiform processes is parasite species-specific (Valkunas, 2005), it should be genetically determined and might reflect evolutionary adaptations and, thus, be applied in *Leucocytozoon* taxonomy at the species level.



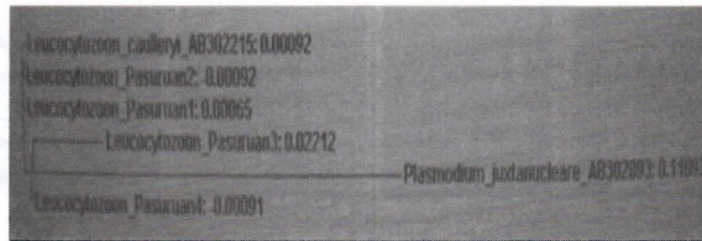


Figure 2. Maximum-parsimony phylogeny of 4 mitochondrial cytochrome b lineages of *Leucocytozoon* species. Lineages of 1 species of avian *Plasmodium juxtranucleare* from GenBank were used as an outgroup. GenBank accession number of the *L. caulleryi* sequences for controlling analysis. The names of *Leucocytozoon* species, which develop in fusiform host cells, are given in bold. The branch lengths are drawn proportionally to the amount of change.

We suggest the validation of these names by BLAST software and use them to identify morphologically similar parasites (Figs. 1) of closely related lineages (Fig. 2). This conclusion is in accord with the hypothesis of Omori *et al.*, (2008) that *L. caulleryi* might represent morphologically similar taxa comprising a species complex. We agree, and suggest identifying parasites of this complex as a group of *L. caulleryi* species by using Basic Local Alignment Search Tool (BLAST).

It is probable that the criterion of a genetic difference in *cyt b* gene effects interspecific divergence in many groups of avian haemosporidians, and so can be used for better understanding of phylogenetic trees based on this gene. It should be noted, however, that genetic divergence in the *cyt b* gene between some readily distinguishable morphospecies of avian haemosporidian parasites is some readily distinguishable morphospecies (Hellgren *et al.*, 2007; Valkiunas *et al.*, 2009). Thus, the molecular criterion of sequence divergence in the *cyt b* gene for identification of haemosporidian species should be developed and applied carefully, preferably by linking molecular and microscopical data. To fully accept this criterion, additional information about genetic distances between lineages of positively identified species of haemosporidians is needed (Table 1). The conclusion on phylogenetic tree analysis that 4 *Leucocytozoon caulleryi* in Chicken Leucocytozoonosis from Pasuruan, East Java is one strain of species *Leucocytozoon caulleryi*.

It is important to note that PCR detects very small numbers of unknown sporozoites in the peripheral circulation (Valkiunas *et al.*, 2009), so detection of lineages of hemosporidians in vertebrate hosts should be carefully considered in ecological and evolutionary biology studies. To be accepted as the lineages of successfully developing species of hemosporidians, such PCR-based information could be supported with the detection of blood stages of the parasites. We thus emphasize an urgent need for the synthesis of information provided by tools of traditional parasitology and molecular biology in studies of haemosporidians, particularly *Leucocytozoon caulleryi* in Indonesia.

REFERENCES

- Fallis, A. M., S. S. Desser, and R. A. Khan. 1974. On species of *Leucocytozoon*. *Advances in Parasitology* 12: 1–67.
- Hellgren, O., A. Krizanauskiene, G. Valkiunas, and S. Bensch. 2007. Diversity and phylogeny of mitochondrial cytochrome b lineages from six morphospecies of avian Haemoproteus (Haemosporida, Haemoproteidae). *Journal of Parasitology* 93: 889–896.
- Kumar, S., K. Tamura, and M. Nei. 2004. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics* 5: 150–163.



- Kvist L, 2000. Phylogeny and Phylogeography of European Parids. Departement of Biology, Oulu University.
- Omori,S., Y. Sato, S. Hirakawa,T. Isobe , M. Yukawa and K. Murata. 2008. Two extra chromosomal genomes of *Leucocytozoon caulleryi*; complete nucleotide sequences of the mitochondrial genome and existence of the apicoplast genome. *Parasitol Res.* 103:953–957.
- Perkins,S.L. and J.J.Schall.2002. A molecular phylogeny of malarial parasites recovered from cytochrome b gene sequences. *J.Parasitol.* 88: 972-978.
- Perkins, S. L., I. N. Sarkar, and R. Carter. 2007. The phylogeny of rodent malaria parasites: Simultaneous analysis across three genomes. *Infection, Genetics and Evolution* 7: 74–83.
- Sato, Y,Hagahira M, Yamaguchi,T, Yukawa,M, Murata,K, 2007, Phylogenetic Comparison of *Leucocytozoon Spp* from Wild Birds of Japan, *J.Vet.Med.Sci* 69: 55-59, 2007.
- Sehgal, R N M, G Valkiunas, T A. Iezhova, and T B. Smith, 2006, Blood Parasites Of Chickens In Uganda And Cameroon With Molecular Descriptions Of *Leucocytozoon Schoutedeni* And *Trypanosoma Gallinaru*. *J. Parasitol.*, 92: 1336–1343.
- Sehgal, R. N. M. Angus C. Hull, Nancy L. Anderson, Gediminas Valkiunas, Michail J. Markovets ,Shiho Kawamura, and Lisa A. Tell. 2006, Evidence for cryptic speciation of *Leucocytozoon spp.*(haemosporida, leucocytozoidae) in diurnal raptors, *J. Parasitol.*, 92(2), 2006, pp. 375–379.
- Swofford, D. L. 2002. PAUP*. Phylogenetic analysis using parsimony (and other methods). Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.
- Valkiunas, G. 2005. Avian malaria parasites and other haemosporidia. CRC Press, Boca Raton, Florida, 946 p.
- Valkiunas, G., T. A. Iezhova, C. Loiseau, and R. N. M. Sehgal. 2009. Nested cytochrome b polymerase chain reaction diagnostics detect sporozoites of hemosporidian parasites in peripheral blood of naturally infected birds. *J. of Parasitol* 94: 1512–1515.
- Valkiunas, G., A. Krizanauskiene, T. A. Iezhova, O.Hellgren, And S. Bensch. 2007. Molecular phylogenetic analysis of circumnuclear hemoproteids (Haemosporida: Haemoproteidae) of sylviid birds, with a description of *Haemoproteus parabelopolskyi* sp. nov. *J. Parasitol* 93: 680–687.
- Valkiunas, G.,Ravinder N. M. Sehgal, Tatjana A. Iezhova, and Angus C. Hull. 2010. Identification of *Leucocytozoon toddi* Group (Haemosporida: Leucocytozoidae), With Remarks On The Species Taxonomy Of Leucocytozoids. *J. Parasitol.*, 96: 170–177.



MORPHOSPESIES AND PHYLOGENETIC TREE ANALYSES OF LEUCOCYTOZOOM CAULLERYI FROM CHICKENS LEUCOCYTOZOONOSIS CASES IN PASURUAN, EAST JAVA

ORIGINALITY REPORT

19%

SIMILARITY INDEX

4%

INTERNET SOURCES

17%

PUBLICATIONS

2%

STUDENT PAPERS

PRIMARY SOURCES

- 1** RAVINDER N. M. SEHGAL. "Molecular evidence for host specificity of parasitic nematode microfilariae in some African rainforest birds", *Molecular Ecology*, 11/2005

Publication

2%
- 2** Erika Walther, Gediminas Valkiūnas, Elizabeth A. Wommack, Rauri C. K. Bowie, Tatjana A. Iezhova, Ravinder N. M. Sehgal. "Description and molecular characterization of a new *Leucocytozoon* parasite (Haemosporida: Leucocytozoidae), *Leucocytozoon californicus* sp. nov., found in American kestrels (*Falco sparverius sparverius*)", *Parasitology Research*, 2016

Publication

2%
- 3** Submitted to Universitas Airlangga

Student Paper

2%
- 4** Gediminas Valkiūnas, Tatjana A. Iezhova, Daniel R. Brooks, Ben Hanelt, Sara V. Brant,

2%

Marie E. Sutherlin, Douglas Causey. "Additional Observations on Blood Parasites of Birds in Costa Rica", Journal of Wildlife Diseases, 2004

Publication

5

Gediminas Valkiūnas, Tatjana A. Iezhova. "Keys to the avian malaria parasites", Malaria Journal, 2018

Publication

2%

6

Palinauskas, Vaidas, Tatjana A. Iezhova, Asta Križanauskienė, Mikhail Yu. Markovets, Staffan Bensch, and Gediminas Valkiūnas. "Molecular characterization and distribution of Haemoproteus minutus (Haemosporida, Haemoproteidae): A pathogenic avian parasite", Parasitology International, 2013.

Publication

2%

7

Jan Hanel, Jana Doležalová, Šárka Stehlíková, David Modrý, Josef Chudoba, Petr Synek, Jan Votýpka. "Blood parasites in northern goshawk (Accipiter gentilis) with an emphasis to Leucocytozoon toddi", Parasitology Research, 2015

Publication

1%

8

www.labome.org

Internet Source

1%

9

Gediminas Valkiūnas, Mikas Ilgūnas, Dovilė Bukauskaitė, Carolina Romeiro Fernandes

1%

Chagas et al. "Molecular characterization of six widespread avian haemoproteids, with description of three new Haemoproteus species", Acta Tropica, 2019

Publication

10

Dimitar Dimitrov, Pavel Zehtindjiev, Staffan Bensch, Mihaela Ilieva, Tatjana Iezhova, Gediminas Valkiūnas. "Two new species of Haemoproteus Kruse, 1890 (Haemosporida, Haemoproteidae) from European birds, with emphasis on DNA barcoding for detection of haemosporidians in wildlife", Systematic Parasitology, 2014

Publication

1%

11

Lane, C.E.. "A molecular assessment of northeast Pacific Alaria species (Laminariales, Phaeophyceae) with reference to the utility of DNA barcoding", Molecular Phylogenetics and Evolution, 200708

Publication

1%

12

Donald J. Forrester. "Leucocytozoonosis", Parasitic Diseases of Wild Birds, 12/19/2008

Publication

1%

13

www.cambridge.org

Internet Source

<1%

14

M. A. Jasper, J. M. Hull, A. C. Hull, R. N. M. Sehgal. "Widespread lineage diversity of

<1%

leucocytozoon blood parasites in distinct populations of western Red-tailed Hawks", Journal of Ornithology, 2014

Publication

15

Hanan Abo El-Kassem Bosly. "Molecular Identification of Musca domestica L. from Jazan (KSA) Based on Partial Mitochondrial Cytochrome Oxidase Gene Sequencing", Journal of Entomology, 2020

Publication

<1%

16

Margaret K. Butler, Huub J. M. Op den Camp, Harry R. Harhangi, Feras F. Lafi, Marc Strous, John A. Fuerst. "Close relationship of RNase P RNA in Gemmata and anammox planctomycete bacteria", FEMS Microbiology Letters, 2007

Publication

<1%

Exclude quotes Off

Exclude matches Off

Exclude bibliography On

MORPHOSPESIES AND PHYLOGENETIC TREE ANALYSES OF LEUCOCYTOZOOM CAULLERYI FROM CHICKENS LEUCOCYTOZOOMOSIS CASES IN PASURUAN, EAST JAVA

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5
