Molecular Characterization and Genetic Diversity of *Entamoeba* spp. from Free-ranging Macaque in Thailand

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Introduction

- *Entamoeba* is protozoan
  - Phylum Sarcomastigophora
  - Subphylum Sarcodina

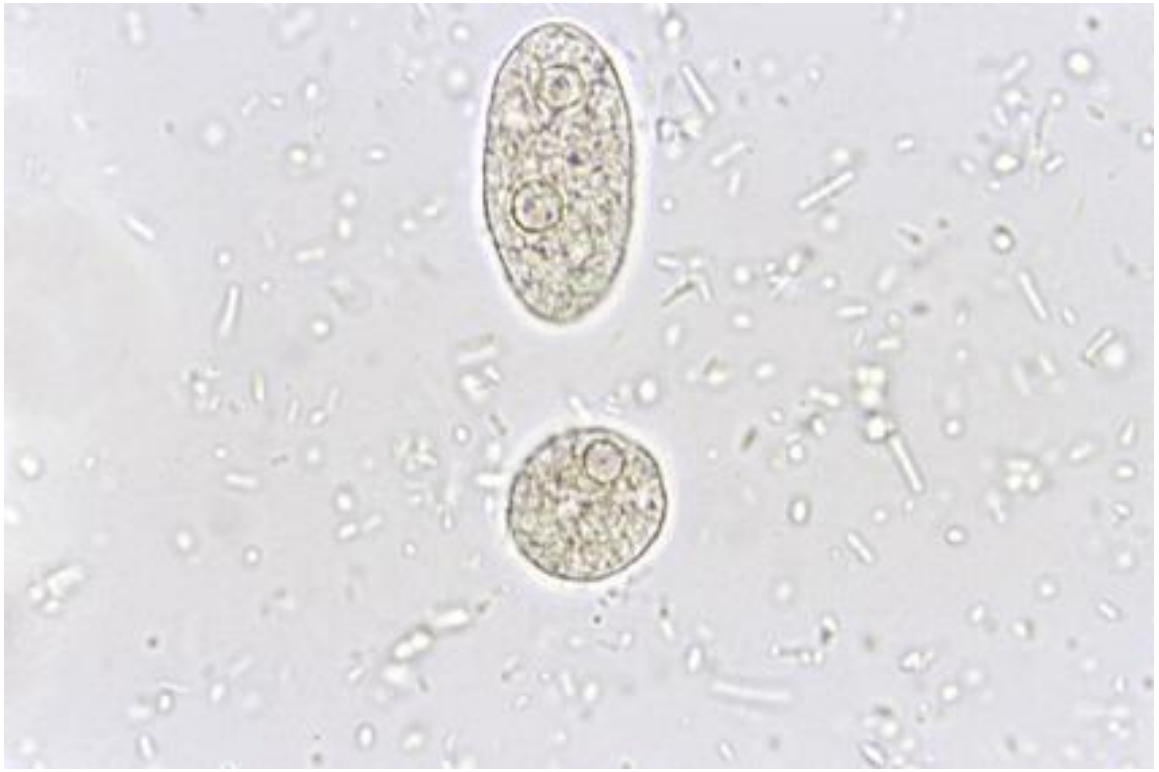


Figure 1 *Entamoeba histolytica/dispar*. SAF-fixed specimen, wet mount examination. Above, an elongated trophozoite with two nuclei (telophase). Below, a rounded one (pre-cystic form)

Figure 1 resource: D. (2014). Entamoeba histolytica. Retrieved January 22, 2017, from http://www.medical-labs.net/entamoeba-histolytica-2-2281/

- Cause of dysentery and invasive disease in animals and human (Hoare, 1959).

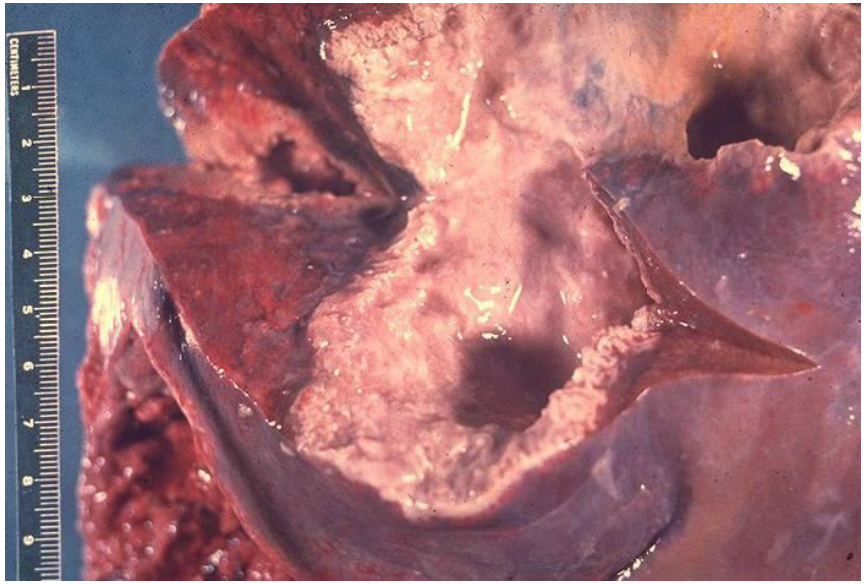


Figure 3 Gross pathology of liver containing amebic abscess

Figure 3 resource:: Melvin, D., & West, D. (2006, May 31). Liver containing amebic abscess, gross pathology 3MG0042 lores [Digital image]. Retrieved May 19, 2017, from https://en.wikipedia.org/wiki/File:Liver_containing_amebic_abscess,_gross_pathology_3MG0042_lores.jpg

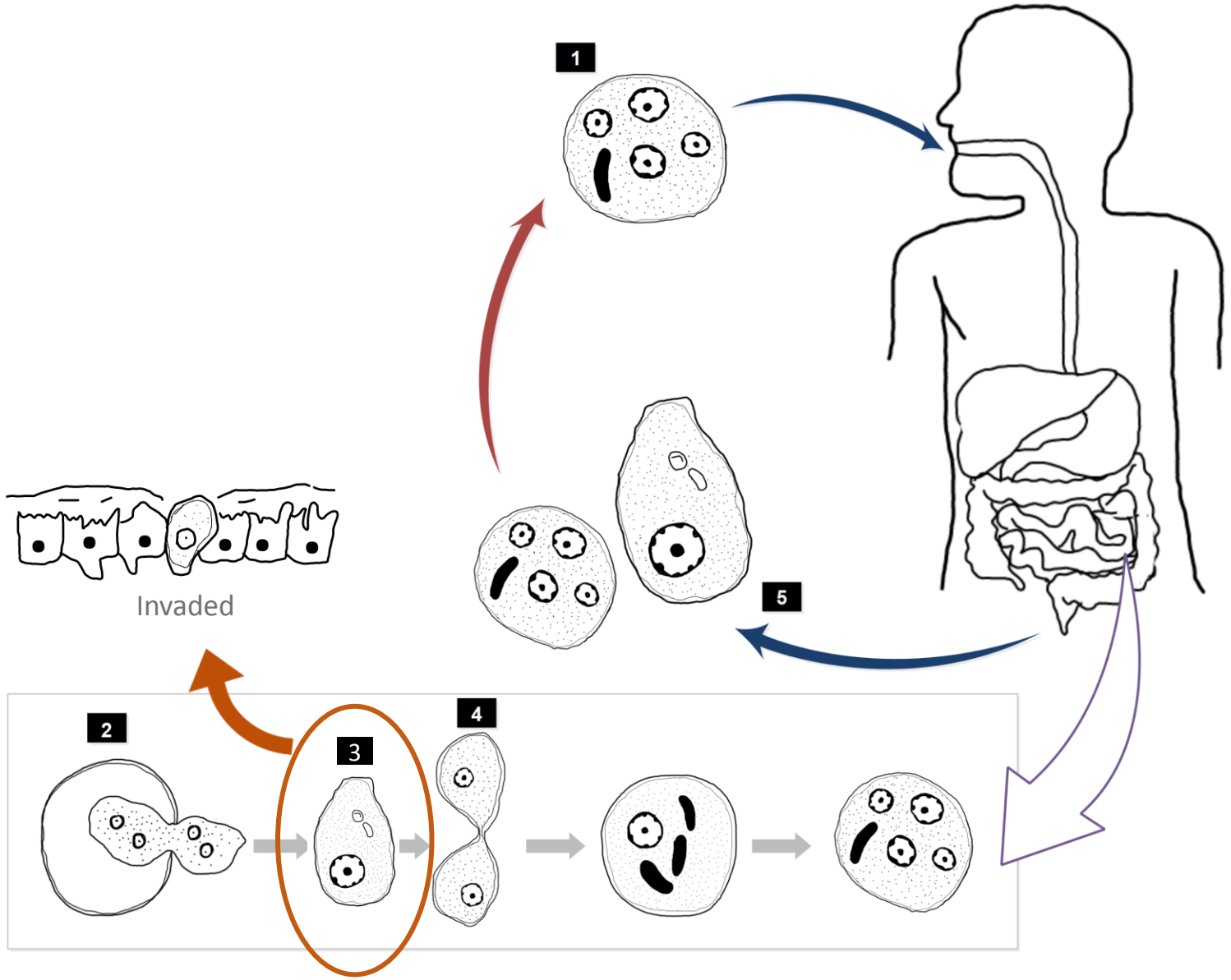


Figure 2 Life cycle of Entamoeba (1) *Entamoeba histolytic*a infections occur by ingestion of mature cysts in contaminated food, water, or dirty hands. (2) Cyst go through small intestine and excyst to be trophozoite. (3) Trophozoites migrate to large intestine. (4) Trophozoites multiply by asexual replication (binary fission) and encyst. (5) Trophozoites and cysts were excreted with feces.

- The six species of *Entamoeba* can be found in human feces

- *Entamoeba histolytica*
- *Entamoeba dispar*
- *Entamoeba moshkovskii*
- *Entamoeba hartmanni*
- *Entamoeba coli*
- *Entamoeba polecki*

← Pathogenic →

- The seven species of *Entamoeba* can be found in macaque feces

- *Entamoeba histolytica*
- *Entamoeba nuttali*
- *Entamoeba dispar*
- *Entamoeba hartmanni*
- *Entamoeba coli*
- *Entamoeba polecki*
- *Entamoeba chattoni*

- ***E.* nuttali**; In Belgium and Netherlands had the report of *E. nuttali* infection in zookeeper who work with the infectious animals (Levecke et al., 2015).

Epidemiology

- This disease spread worldwide, commonly found in the developing countries (Acha & Szyfres, 2001; Samad, 2013).

- From 2007 to 2011, the samples of returned traveler were collected and showed positive results with *E. histolytica* from the travelers, most of them came back from Mexico, India, Indonesia, or Thailand ("Amebiasis - Chapter 3 - 2016 Yellow Book | Travelers' Health | CDC," n.d.).

- In 2015 from the annual epidemiology surveillance report showed 10.78 of dysentery cases per 100,000 inhabitants, may has cause from amoebic 42.58%.
- Most of cases were found in the border area and north part of Thailand, especially in remote rural area.
- The highest incidence occurred in May and June period, and lowest incidence occur in September. (Bureau of Epidemiology, Department of Disease Control, n.d.).

Transmission

- The ingestion of cyst contaminated in food or water, especially the raw vegetables and undercooked food (Acha & Szyfres, 2001; Samad, 2013).

- Oral-anal sexual (Lim & Vythilingam, 2013; Tanyuksel & Petri, 2003; Ximénez et al., 2009).

- Globalization (Kruse & others, 2004; Schuster & Visvesvara, 2004).

Zoonosis

- Especially in non-human primates
  - They have similar genetic with human
  - they are known as naturally harbor of *Entamoeba* with the same strains in human infection.

It demonstrated by the experiment in 1934 about the cross-infection of *E. histolytica* in simian strains and human strains (*Advances in Parasitology*, 1967; Hoare, 1959).

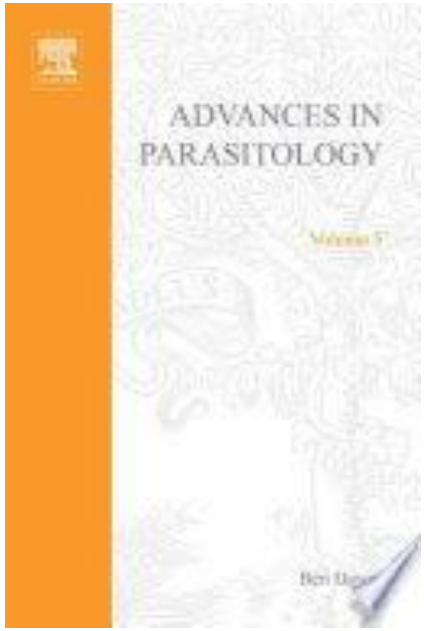


Figure 4 Advances in Parasitology, 1967

Figure 4 resource: [Digital image]. (n.d.). Retrieved May 19, 2017, from https://books.google.co.th/books/content?id=EmvRNxOgpTQC&printsec=frontcover&img=1&zoom=5&edge=curl&imgtk=AFLRE721qMG-UfoWCQZwYVYYErAosLO2IkQ516Rr4IBYOYw7ZzNm0Wk0XI6czOB1HudspdC4P2mgncnOPKRaQQ1GTAo__SdNSnViiAVLZa92JE6POdWMNGaMN_EhjuTiL6DkjdenaGXv

“The transmission of zoonotic diseases often occurs when human and animals coexist and share the resources together”
(Lane-deGraaf et al., 2014)

Figure 5 Increasing of macaque population in Lopburi

Figure 6 Monkey buffet in Thailand

Figure 5 resource: http://www.mediastudio.co.th/2016/07/11/29851/
Figure 6 source: https://articlebio.com/know-about-the-monkey-buffet-festival-in-thailand-where-monkeys-are-fed-2000-kilograms-of-food

Diagnosis

- Molecular method
  - Gold standard (Fletcher et al., 2012; Tanyuksel & Petri, 2003).
  - High sensitivity and specificity more than other method.
  - It can detect pathogens from a variety of clinical specimens.
  - Need the specialized equipment, technically staff, and high costs

- The 18s rRNA is widely used as gene target for Entamoeba identification because of the ubiquitous and conserved within species (Fotedar et al., 2007).

- Project

Objectives of study

- To examine pathogenic *Entamoeba* spp. exist in monkey population in Lopburi province, Thailand.
- To establish the study on molecular characterization and genetic diversity of *Entamoeba* spp. infection in NHPs.

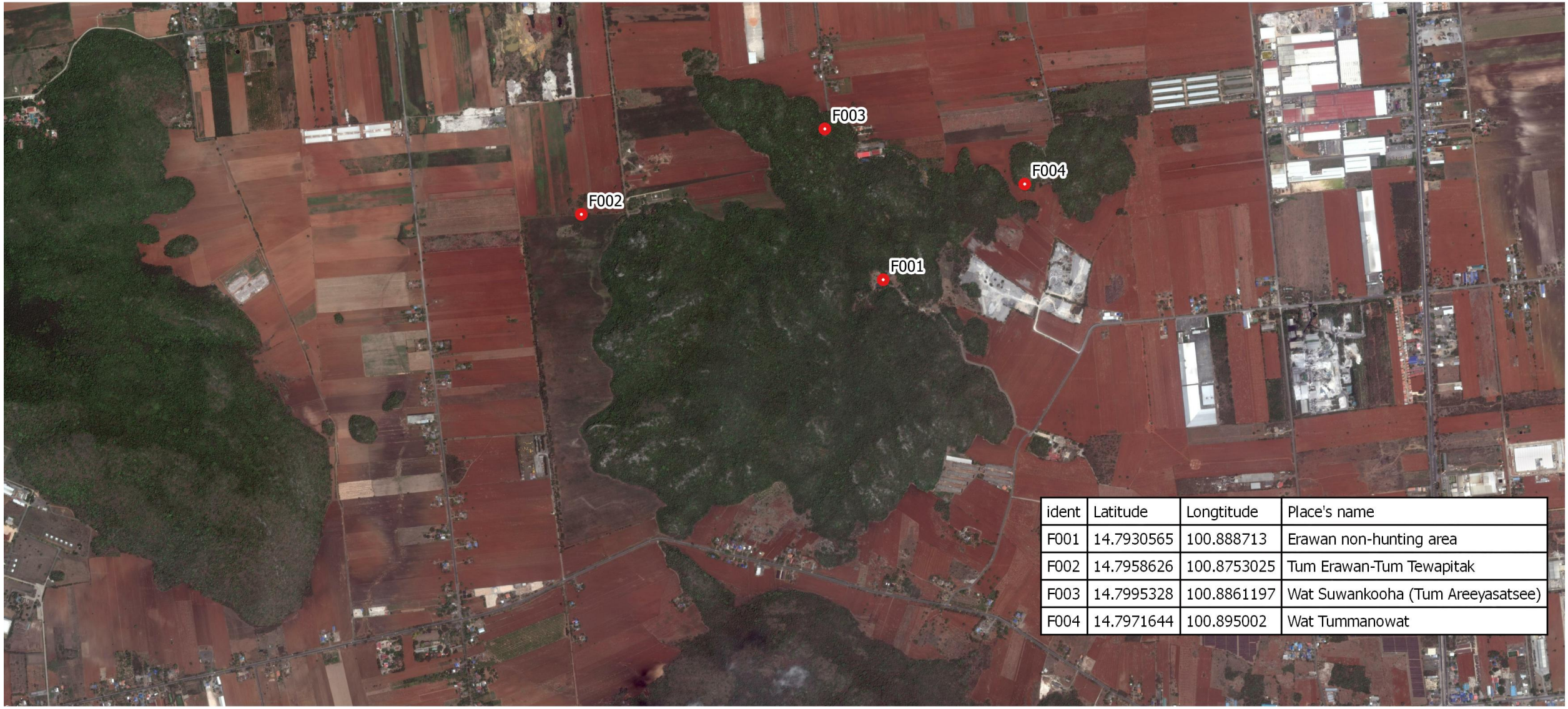
Material and Methods

- The 456 free-ranging macaque stools were collected from the city center and Erawan non-hunting area, Lopburi province, Thailand.
- The samples were preserved in D-solution and kept at 4°C
- Extraction by the phenol-chloroform method and storing at -20°C until use.

Figure 7 *Macaca leonina* in the community around Erawan non-hunting area, Lopburi province

ID	Latitude	Longtitude	Place's name
C001	14.8010418	100.6097499	Sengheng-Pinya Shop
C002	14.8028599	100.6113922	Meung Tong Hotel
C003	14.803035	100.6118071	Phra Prang Sam Yod
C004	14.8035952	100.6120225	Manora market
C005	14.803833	100.6116413	Malai Rama Theater
C006	14.8002773	100.6368557	Chayowanich Shop
C007	14.7997437	100.6371754	Arunee van's station

Figure 8 Sample collection points at City center area, Lopburi province, Thailand



ident	Latitude	Longtitude	Place's name
F001	14.7930565	100.888713	Erawan non-hunting area
F002	14.7958626	100.8753025	Tum Erawan-Tum Tewapitak
F003	14.7995328	100.8861197	Wat Suwankooha (Tum Areeyasatsee)
F004	14.7971644	100.895002	Wat Tummanowat

Figure 9 Sample collection points at Erawan non-hunting area, Lopburi province, Thailand

• PCR by using forward primer Entam1 and reverse primer Entam2 (Table 1) with pre-denature 3 minutes at 95°C, follow by 40 cycles of 40 seconds at 94°C, 30 seconds at 50°C, 30 seconds at 72°C for, and the last extension 5 minutes at 72°C.

Table 1 Primers use for the conventional PCR for *Entamoeba* spp. (Suzuki, Kobayashi, Murata, Yanagawa, & Takeuchim, 2007)

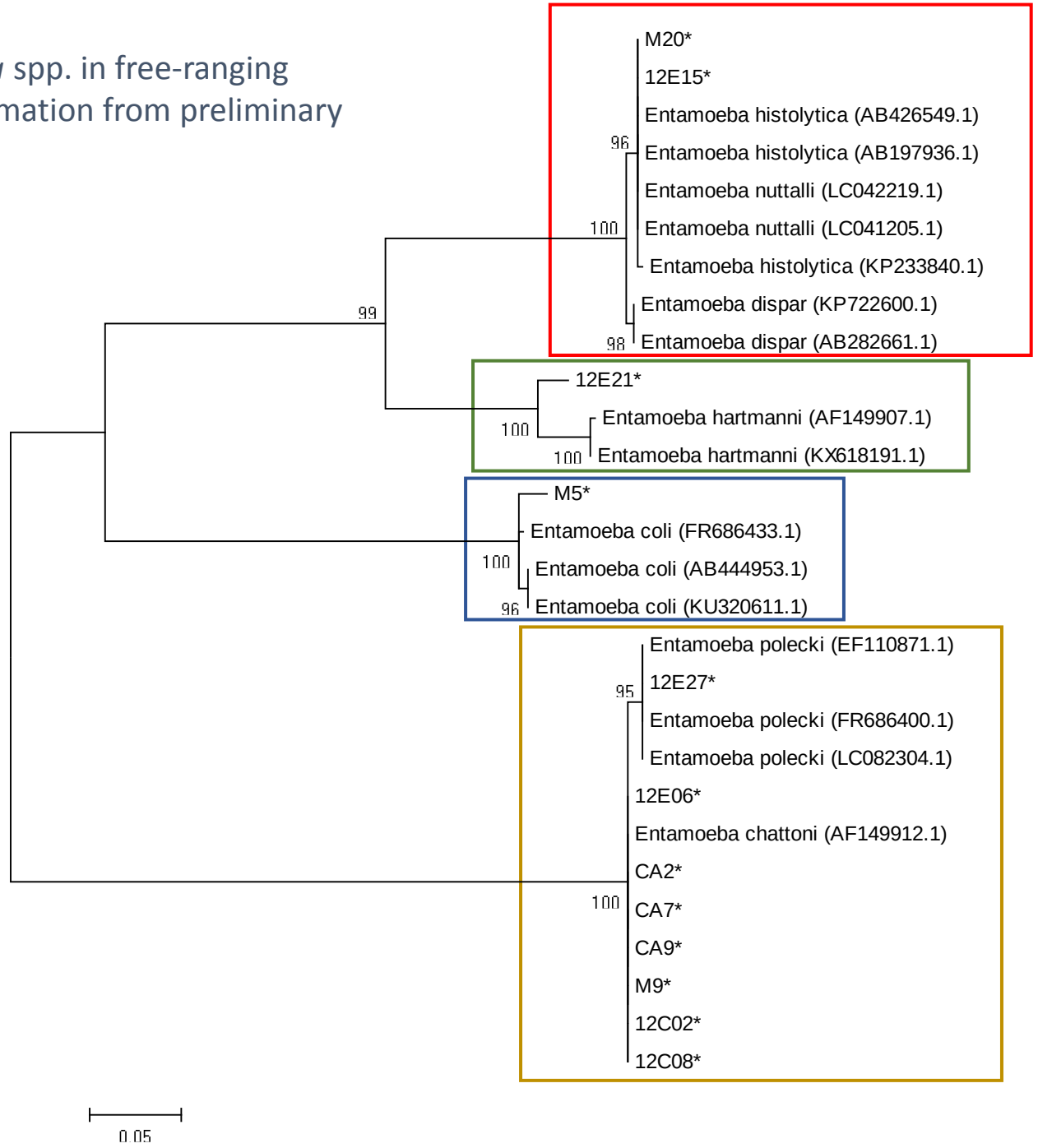
Primers	Sequences	Amplification product (bp)
Entam1(Fw)	5'- GTT GAT CCT GCC AGT ATT ATA TG-3'	506
Entam2(Rw)	5'-CAC TAT TGG AGC TGG AAT TAC-3'	

• The positive samples were done again with the new forward primer (18s-entam-ku-1w) and three new reverse primers (18s-entam-poc/chat-ku-2w, Entam-coli-2w, and 18s-entam-path-ku-2w) (Table 2).
• PCR parameters were started by 3 minutes at 98°C, follow by 40 cycles of 40 seconds at 98°C, 30 seconds at 58°C, 30 seconds at 72°C for, and the last extension 5 minutes at 72°C.

Table 2 The new forward and reverse primers for specific group of *Entamoeba* spp.

Primers	Sequences	Amplification product (bp)
18s-entam-ku-1w	5’-ATC TGG TTG ATC CTG CCA GTA TT-3’	
18s-entam-poc/chat-ku-2w	5’-CCT TCA AGT TGA TAG GTC AGA TAT TTA AAA CAG-3’	315
Entam-coli-2w	5’-CAC TAC CTC CTT CAG TTG TTA AGA T-3’	516
18s-entam-path-ku-2w	5’-CAT TTT GTA CTA ATA CAA ACT GGA TCG TC-3’	199

Figure 10 Phylogenetic tree of *Entamoeba* spp. in free-ranging macaques based on 18S rRNA gene (information from preliminary study)



Result and Discussion

- The 190 from 456 fecal samples showed positive results with primer Entam1 and Entam2.
- All of those were detected again with new specific primers. The result showed 170 positive samples that separated to 8 group results (Table 3).

Table 3 The result of new specific primers

	Number of samples
E. polecki or E. chattoni **(group I)**	40
E. coli **(group II)**	4
E. histolytica**,** ***E. nuttali*****, or** ***E. dispar*** **(group III)**	1
Coinfection with group I and II	30
Coinfection with group I and III	37
Coinfection with group II and III	4
Coinfection with group I, II, and III	54
Negative with new primers	18

New primers

- The negative results of new primers showed the sequencing of plant, fungi, and *E. hartmanni*
    - The new primers are specific more than the old primer

Future plan

- The specific primer for *E. hartmanni* group should be created and add in the set of new specific primers.
- Need more sequencing results and phylogenetic tree
- Analyze the information
- Publish

THANK YOU

FOR YOUR ATTENTION