DEFINITIVE RODENT HOSTS OF THE RAT LUNGWORM
ANGIOSTRONGYLUS CANTONENSIS

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ABSTRACT. — Under natural conditions, various species of murid rodents are the final or definitive hosts of the rat lungworm *Angiostrongylus cantonensis*. Because of the occurrence of cryptic/sibling species (e.g., *Rattus rattus*, *Maxomys* species), some of the earlier records might be incorrect. Taxonomic revisions have also resulted in name changes of the rodent hosts. This review provides an updated account of the taxonomic names and the geographic occurrence of the definitive rodent hosts. The differentiation within the *Rattus rattus* complex (*R. rattus* and *R. tanezumi*) and the sibling species of *Maxomys* (*M. rajah* and *M. surifer*) are discussed.

KEY WORDS. — Muridae, rats, molecular phylogeny, cytogenetics, cryptic species, taxonomy

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INTRODUCTION

The rat lungworm *Angiostrongylus cantonensis* (Chen, 1935) is a bursate nematode of the family Angiostrongylidae, superfamily Metastrongyloidea. It is a food-borne zoonotic parasite of public health importance in many countries of the tropics and subtropics (Eamsobhana & Tungtrongchitr, 2005; Eamsobhana, 2006). Its occurrence has now been reported in many countries worldwide (Eamsobhana, 2006; Cross & Chen, 2007; Foronda et al., 2010; Maldonado Jr. et al., 2012; Moreira et al., 2013). Humans are a non-permissive, accidental host.

Under natural conditions, various species of murid rodents are the final or definitive hosts of *A. cantonensis* (Eamsobhana, 2006). Because of the occurrence of cryptic/sibling species, identification of the taxa involved has proven problematic based on morphological characters. As such some of the earlier records might be incorrect. Taxonomic revisions have also resulted in name changes of the rodent hosts. This review provides an updated account of the taxonomic names and the geographic occurrence of the definitive rodent hosts (Table 1). The differentiation of the cryptic or sibling species (referred to as species complex), i.e., closely related species that appear as a single species based on morphological characters, are highlighted.

CURRENT TAXONOMIC STATUS

The taxonomy of the murid rodents has undergone considerable revision since the 1960s (for review, see Musser & Carleton, 2005). Of the definitive hosts of *A. cantonensis*, seven species have been assigned new generic status (Table 2). Despite great progress, the systematics of the Black Rat (scientific names reviewed below) is still not fully resolved.

THE BLACK RAT *RATTUS RATTUS* SPECIES COMPLEX

The rat lungworm was first discovered in the lungs of rats *Rattus norvegicus* (Berkenhout, 1769) and *Rattus rattus* (Linnaeus, 1758) in 1933 in Canton (now Guangzhou), China and described as *Pulmonema cantonensis* (Chen, 1933, 1935). The Black Rat is now recognised as a species complex (including, e.g., *R. rattus* and *R. tanezumi* Temminck, 1844); as such, earlier records need clarification concerning the correct taxonomic status of the host.

Cytogenetic data indicate distinct karyotypes between the taxa of Asia and Europe. The karyotype of the typical Asiatic black rat possesses 2n = 42 (e.g., Yong, 1969) while that for the typical European taxon has 2n = 38 (Capanna et al., 1970). Introduced (alien) black rats can therefore be broadly determined as to their origin. For example, chromosomal

Table 1. Geographic distribution of the definitive rodent hosts of *Angiostrongylus cantonensis* (after various sources).

Host	Distribution of Host Records
Bandicota indica	China, India, Indonesia, Sri Lanka, Thailand
Bandicota savilei	Thailand
Berylmys berdmorei	Thailand
Berylmys bowersii	China
Maxomys bartelsii	Indonesia
Maxomys surifer	Thailand
Niviventer fulvescens	China
Niviventer coninga (= *N. coxinga*)	Taiwan
Niviventer lepturus	Indonesia
Rattus argentiventer	Indonesia
Rattus everetti	Philippines
Rattus exulans	Fiji, Hawaii, Indonesia, Peninsular Malaysia, Philippines, Thailand
Rattus losea	China, Taiwan
Rattus nitidus	China
Rattus norvegicus	Australia, Brazil, Cambodia, China, Cuba, Dominican Republic, Egypt, Häiti, India, Indonesia, Jamaica, Japan, Mauritius, Melanesia, Micronesia, Philippines, Polynesia, Puerto Rico, Sri Lanka, Taiwan, Thailand, U.S.A.,Vietnam
Rattus rattus complex	Australia, Brazil, Canary Islands, China, Häiti, Hawaii, Indonesia, Jamaica, Japan, Madagascar, Mauritius, Melanesia, Micronesia, Nigeria, Philippines, Polynesia, Puerto Rico, Taiwan, Thailand
Rattus tiomanicus	Indonesia

Table 2. Changes in taxonomic names of the definitive rodent hosts of *Angiostrongylus cantonensis* (after various sources).

Current name	Previous name
Bandicota indica	*Bandicota malabarica*
Berylmys berdmorei	*Rattus berdmorei*
Berylmys bowersii	*Rattus bowersi*
Maxomys bartelsii	*Rattus bartelsii*
Maxomys surifer	*Rattus surifer*
Niviventer coninga (N. coxinga)	*Rattus coxinga*
Niviventer fulvescens	*Rattus niviventer*
Niviventer lepturus	*Rattus lepturus*
Rattus losea	*Rattus ratoides exiguus*
Rattus rattus complex	*Rattus flavipectus*

studies have shown that the black rats in Oceania have 2n = 38 and the rats in Sri Lanka have 2n = 40 (Yosida et al., 1971). Karyotyping and cytochrome b (cytb) gene sequencing indicated the occurrence of both *R. rattus* (2n = 38) and *R. tanezumi* (2n = 42) in South Africa (Bastos et al., 2010).

Based on cytb nucleotide sequences, four genetic linkages of *R. rattus* complex (RrC) have been recorded: Linkage I (RrC LI) – eastern and southern India; Linkage II (RrC LII) – western part of Indochina and uplands of eastern Indochina; Linkage III (RrC LIII) – Himalayan foothills; and Linkage IV (RrC LIV) – lower Mekong River catchment (Aplin et al., 2011). However, the current taxonomic arrangement of the black rats into two species (*R. rattus* and *R. tanezumi*) does not rest easily atop this model of multi-regional differentiation (Aplin et al., 2011).

Phylogenetic analyses based on mitochondrial genes – cytochrome *c* oxidase subunit I (COI), cytochrome b (cytb) and 16S rRNA – of *R. rattus* and *R. tanezumi* discriminate these cryptic species, as well as differentiating them from other congeneric species such as *R. exulans* (Peale, 1848), *R. norvegicus* and *R. fuscipes* (Waterhouse, 1839); the topologies of the phylogenetic trees are similar as represented by cytb sequences (Fig. 1). Although more than one species may be present for the taxa attributed to *R. tanezumi* (e.g., by Musser & Carleton, 2005), the genetic distance between *R. rattus* and *R. tanezumi* is relatively small compared to that between other congeneric species (Table 3). Population and phylogeography studies, employing morphological, chromosomal, and molecular characters, are needed to determine the species status and species composition for a particular area, such as the presence of a mix of the species complex in port areas, such as in Singapore.

Table 3. Genetic distance (uncorrected 'p' distance) between *Rattus rattus* (NC_012374) and *Rattus tanezumi* (NC_011638) compared to *Rattus exulans* (NC_012389), *Rattus norvegicus* (NC_001665) and *Rattus fuscipes* (NC_014867) based on mitochondrial cytochrome *c* oxidase subunit I (COI, 1618 bp), cytochrome b (cytb, 1187 bp) and 16S rRNA (1603 bp) sequences (from the GenBank) conducted in MEGA5 (Tamura et al., 2011).

| Taxa	COI	CYTB	16S
R. rattus vs R. tanezumi	**0.0337**	**0.0362**	**0.0184**
R. rattus vs *R. exulans*	0.1054	0.0927	0.0630
R. rattus vs *R. norvegicus*	0.1261	0.1104	0.0540
R. rattus vs *R. fuscipes*	0.1234	0.1196	0.0582
R. tanezumi vs *R. exulans*	0.0958	0.0960	0.0658
R. tanezumi vs *R. norvegicus*	0.1238	0.1188	0.0533
R. tanezumi vs *R. fuscipes*	0.1165	0.1188	0.0520
R. exulans vs *R. norvegicus*	0.1164	0.1179	0.0657
R. exulans vs *R. fuscipes*	0.1050	0.1255	0.0685
R. norvegicus vs *R. fuscipes*	0.1020	0.1222	0.0547
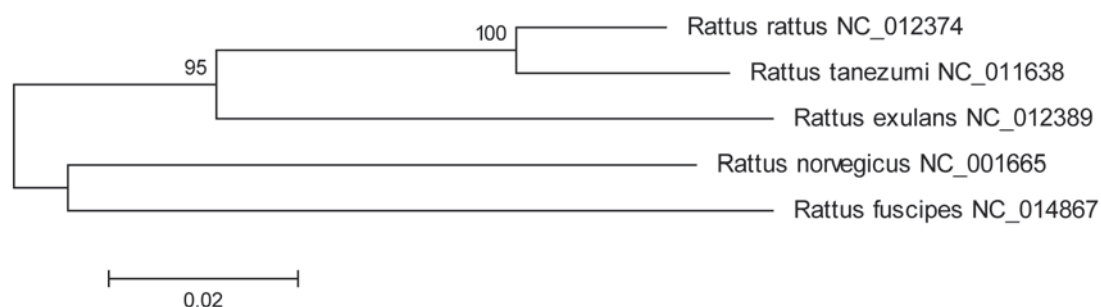
Fig. 1. Phylogenetic tree of five taxa of the genus *Rattus* generated by the Maximum Likelihood method based on partial cytochrome b (cytb) mtDNA nucleotide sequences (from the GenBank) conducted in MEGA5 (Tamura et al., 2011) using the Tamura-Nei model (1993). The tree with the highest log likelihood (–2997.5664) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd. All positions containing gaps and missing data were eliminated. There were a total of 1187 positions in the final dataset.
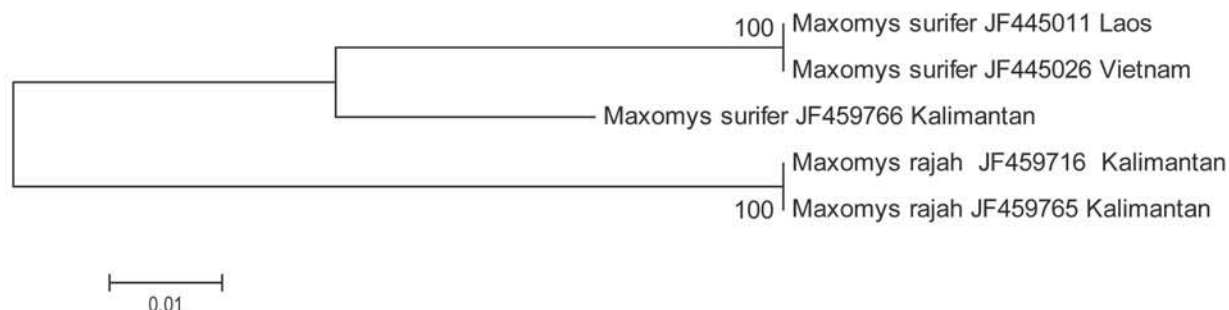

Fig. 2. Phylogenetic relationships between *Maxomys rajah* and *Maxomys surifer* generated by the Maximum Likelihood method based on partial cytochrome *c* oxidase subunit I (COI) mtDNA nucleotide sequences (from the GenBank) conducted in MEGA5 (Tamura et al., 2011) using the Tamura-Nei model (1993). The tree with the highest log likelihood (–1175.2453) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved five nucleotide sequences. Codon positions included were 1st+2nd+3rd. All positions containing gaps and missing data were eliminated. There were a total of 593 positions in the final dataset.

SPINY RATS GENUS *MAXOMYS* SODY, 1936

Spiny rats of the genus *Maxomys* Sody, 1936 are represented by some 18 species (Corbet & Hill, 1992; Musser & Carleton, 2005; Achmadi et al., 2012). Among them are two sibling species, *Maxomys rajah* (Thomas, 1894) and *Maxomys surifer* (Miller, 1900; previously referred to the genus *Rattus*). Cytogenetic, serological (albumin and haemoglobin) and ecological characters show that they are genetically distinct and are valid species (Yong, 1969, 1972). *M. rajah* possesses 36 chromosomes and *M. surifer* 52 chromosomes (Yong, 1969, 1972). Prior to that, these two species had been also regarded as colour phases of one species. These sibling species have perhaps been incorrectly identified in some studies (Yong, 1972).

Among the definitive hosts of *A. cantonensis* in Thailand is a spiny rat of the genus *Maxomys*, reported as *Rattus raja* (see Punyagupta et al., 1970). This taxon is probably *M. surifer* as, based on cytogenetic evidence, *M. rajah* does not occur in Thailand (Marshall, 1977). In addition to karyotypes (Yong, 1969), *M. rajah* and *M. surifer* could be differentiated by molecular markers (Yong, 1972; Chan et al., 1978; Tamrin & Abdullah, 2011). Phylogenetic analyses of COI nucleotide sequences (and other genes) unequivocally separate *M. rajah* and *M. surifer* (Fig. 2). However, the taxon of *M. surifer* from Kalimantan is quite distant from the taxa from Laos and Vietnam, with a genetic distance of 0.1281 (based on COI sequences). Further studies using multiple genes and extensive geographic sampling are needed to determine if the present taxonomic treatment of *M. surifer* consists of a species complex.

CONCLUSIONS

The incrimination of a rodent species as definitive host of the rat lungworm *A. cantonensis* depends on accurate identification. Accurate identification is particularly vital in the case of sibling species which are morphologically very similar but may possess very different genetic constitution and other biological attributes. Cytogenetic and molecular genetic approaches help to resolve component taxa in such species complexes, e.g., the black rat *R. rattus* complex and sibling species of the genus *Maxomys* (e.g., *M. rajah* and *M. surifer*). "The systematist may and should employ any means that are available in order to arrive at a knowledge of biological facts, whether these means be found in morphological, anatomical, physiological, experimental, genetical, or even chemical studies" (Ferris, 1928).

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