TAXONOMY AND PHYLOGENETIC CHARACTERIZATION OF PARASITIC NEMATODES IN LIZARDS FROM VIETNAM

(ベトナム産トカゲ寄生線虫の分子系統学的分類に関する研究)

DISSERTATION

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ABSTRACT

Parasites and parasitic fauna of lizards have been explored for a long time in the world, particularly in Europe, Russia and North America, whereas little is known about them in Vietnamese lizards. Considering that the territory of Vietnam is of special interest from the zoogeographical viewpoint and has a great richness of the fauna of lizards in the territory, exploration and understanding of the substantial diversity of lizard parasites in Vietnam are critically important, which may bring more information on the ecological relationships among lizards and biogeographical history of them in Vietnamese nature. In this thesis, I focused on nematodes of three categories (Pharyngodonidae, Cosmocercoidae, and Heterakidae) in Vietnamese lizards to clarify their taxonomical statuses with an aid of phylogenetic characterizations based on the ribosomal RNA gene (rDNA). Although morphological characters of taxonomic importance have been refined step by step to identify the species or make a border between different species during a long history of parasite descriptions, often the range of useful criteria varied by researchers or by observed parasites. In this sence, nucleotide sequencing provides additional data as well as phylogenetic positions of observed specimens even if a part of them show exceptional morphological features.

In Chapter 1, I charaterized two nematode species of Pharyngodonidae (Oxyuroidea) collected from golden geckos, *Gekko badenii* (syn. *Gekko ulikovskii*; Sauria: Gekkonidae), which are currently known only from the highland in the central part of Vietnam. Geckos had abundant oxyurid nematodes in the large intestine with a high prevalence (90%), and based on morphological criteria I identified two new species, i.e., *Pharyngodon duci* and *Spauligodon vietnamensis,* as the 35th and 44th species assigned for each genus, respectively. A large number of immature oxyurids dwelled in the large instestine as well, indicating active and continuous infection with these two oxyurid species occurred among golden geckos. Records

of two new species from an isolated lizard host population stimulate the understanding of their phylogenetic relationships with congeners, and this point will be pursued in future works.

In Chapter 2, I characterized morphologically and phylogenetically a new *Cosmocercoides* species, i.e., *C. tonkinensis*, in the scale-bellied tree lizard, *Acanthosaura lepidogaster* (Squamata: Agamidae), from the northern and central parts of Vietnam. Currently 19 nomial *Cosmocercoides* spp. have been recorded, mainly from amphibian host, and only two species, *C. variabilis* in North America and *C. sauria* in South America, have been recorded from lizards. Therefore, this new species is the third species from reptilian hosts. The 18S rDNA of the new species is almost identical to that of a unique congener *C. dukae* from land snails and slugs in North America. Similarly, between the present new species and *C. pulcher* from a toad (*Bufo japonicus*) in Japan, few nucleotide substitutions were noticed in the 18S to 28S rDNA sequence including the internal transcribed spacer (ITS) regions. It is needed to collect more specimens of diverse *Cosmocercoides* spp. to elucidate the significance of appreciated morphological criteria with reference to phylogenetic datasets.

In Chapter 3, I observed and compared the morphology of *Strongyluris calotis*, a heterakid nematode with cuticular flanges extend from the inner surface of lips, in the large intestine of agamid lizards (*Japalura* spp. and *Calotes emma*) from the Oriental region (Japan, Taiwan and Singapore) by SEM. It was for the first time to clarify the accurate arrangement of cephalic and caudal papillae in *Strongyluris* nematodes. Furthermore, although the species was described as having 10 pairs of caudal papillae in the previous works, we noticed a pair of united papilla structures and a pair of phasmids in this count of caudal papillae. When I collected and examined *S. calotis* specimens from *C. emma* living in the plain forest at low altitude, and from *Pseudocalotes brevipes* living in the mountainous forest at high altitude in the northern part of Vietnam, the arrangement of caudal papillae in male worms from *C. emma* was found to be comparable to classical *S. calotis* specimens, while male worms from

P. brevipes did not have a pair of united papillae but had 10 pairs of independent caudal papillae with a pair of phasmids. Molecular genetic analyses of the rDNA from worms of the classical *S. calotis* morphotype from Japan and Singapore and two *S. calotis* morphotypes from Vietnam demonstrated absolutely identical nucleotide sequences of 18S rDNA and 5.8S rDNA with moderate nucleotide diversities in the ITS regions (96.6– 98.5 %) and 28S rDNA (99.6–99.7 %). These results indicate the usefulness of molecular genetic analyses to know the relationships among multiple isolates of different origins, particularly when the isolate shows extraordinary morphological characters. In Chapeter 4, I tried to clarify the relationships of multiple morphotypes of *Meteterakis* spp. (Heterakidae) found in the large intestine of scale-bellied lizards, and identified at least two species.

In Chapter 5, I list up recorded paraites from Vietnamese lizards, which include a total of 45 parasite species (11 cestode species, 12 trematode species, 18 nematode species, 1 acanthocephalan species, and 3 pentastomida species) of 34 genera in 27 families. These parasites were recorded only from 10 out of more than 120 lizard species in Vietnam. Therefore, we can easily suppose that we know an absolutely limited number of parasitic helminths in lizards distributed in Vietnam. More efforts should be paid to understand the real diversity of parasites in Vietnamese lizards.

GENERAL INTRODUCTION

Topography

Vietnam is located on the eastern coast of the Indochinese peninsula, in the area of Southeast Asia, where is known as one of the biodiversity hotspots of the world. The country S-shaped bend, expand in two flat deltas in the north and the south, connected by a narrow strip of land in the central region, stretching from north to south over 1,648 km, with a wide range of latitude and elevation. From west to east, and extends southward, especially with lowlands, range of mountains and many plateaus with thick forests. With a mainland area of 330,591 square kilometer (127,240 square miles), Vietnam is less than two-thirds of the size of Thailand and slightly smaller than Japan. Description of Vietnam often emphasizes the hilly and mountainous nature of three quarters of the country, though much of the land lies at moderate elevations. Vietnam's mountainous areas lie in the country's northern and central region. West of the Red River, the Hoang Lien Son Range represents the southeastern-most extension of the Himalayas. It runs northwest to southeast, paralleling the river's course. Vietnam's highest peak, Fan Xi Pan, is found here, rising to 3,143 meter above sea level. Several smaller ranges lie in northeastern Vietnam, including the Viet Bac and Bac Son uplands and large areas of limestone are exposed here. Central Vietnam's highland areas are part of the Truong Son (also called the Annamite). Range, which stretches for 750 miles (1,200 km) from 20° N, along Vietnam's western border with Laos, ending south of the Da Lat Plateau in south-central Vietnam (Sterling et al. 2006).

Climate

Because of Vietnam's shape, topography, and location along mainland Asia's southeastern edge, the country experiences many climatic regimes. Southeast Asia's climate is closely associated with monsoons, major wind systems that reverse direction seasonally. The dynamic monsoon circulation patterns produce two main seasons, a dry, cool winter and a warm, wet summer, that are interrupted by short transitional periods.

The winter monsoon season begins in mid-November and last until the end of March. During this time, monsoon winds arise from cold high-pressure zones over the eastern Asian continent and flow southward toward hot, low-pressure zones over Australia. These polar air currents arcing across Siberia and China bring chilly conditions to northern Vietnam. April and May are transitional months and are followed by the summer monsoon season, which

lasts through September. In summer, monsoon winds from high-pressure areas in the southwest bring moist air from the Indian Ocean and the Gulf of Thailand toward China's interior. This cause heavy rains to fall in Vietnam, particularly in mountainous areas. October and November mark the end of the rainy season and the transition to another winter. Vietnam's broad variation in the timing and amount of rain falling in different parts of Vietnam is due largely to these circulation patterns.

Climate plays a substantial role in determining the plant and animal communities in an area. Both extended dry seasons and particularly cold winters stress plants and animals and set climatic boundaries for species unable to survive under these conditions (Sterling et al. 2006). In northern Vietnam, from the Chinese border south to 18° N (around the Ngang Pass), both temperature and rainfall are noticeably seasonal. Cold, humid winters with occational light rains last from November to April, and frost is not unusual in high mountain regions. Depending on the location, dry periods can vary from zero to six months. Summer is hot, muggy, and rainy and last from May to October. The hottest months in the north are June, July, and August, when humidity reaches 80 to 100 percent. Further south (to 16° N), temperatures are less seasonal, and the timing of the wet season varies, particularly between coastal and inland areas. Winters are cool, with rains extending from summer though autumn and into winter and a dry season of zero to three months. On the high plateaus of the central region, temperatures are lower and conditions wetter, with dry seasons lasting for only three months. Coastal areas experience a rainy season in the autumn and winter followed by a dry period of up to seven months. Farther south in the Mekong Delta, temperatures are quite warm and stable year-round. Rains fall in the summer from May to October, with the heaviest rains occurring in July and August. The dry season varies from two to six months. The hottest period is from March to May, with high humidity in the latter month.

Biodiversity and Biogeography

Vietnam lies north of a well known area of biotic transition, sometimes known as Wallacea, that bridges the different plant and animal communities found in Asia and Australia. Within Vietnam, transition occurs across both elevation and latitude. For instance, east of the Red River, the tropical plants and animals of northeastern Vietnam's limestone range are similar to the biota of southern China, as well as their close evolutionary relatives. To the river's west, the Hoang Lien Son Range resembles the subtropical southeastern foothills of the Himalayas. By contrast, southern Vietnamese vegetation shares its dry deciduous forests

and peat swamp communities with the lowland tropics of Southeast Asia's mainland and island archipelago. Central Vietnam's Truong Son Range is a transitional region between these subtropical and tropical communities, and harbors many endemic species. In each, the northern and central representatives are related more closely to each other than they are to their southernmost counterpart. However, not all of Vietnam's species occupy ranges that overlap with or abut close evolutionary relatives. Large distances separate some species from their relation, a distribution pattern referred to as disjunct. It is often complex histories that underli the evolution and distribution of each species. Current topographic and climatic conditions are major components determining recent species distribution patterns as are past events, including the movement of continents over geological time, climate change and the fluctuation of sea levels (Sterling et al. 2006).

Herpetofauna of Vietnam

Vietnam represents one of the global hot spots of reptilian diversity; although its herpetofauna is still incompletely known, as revealed by numerous new species and even genera which have been discovered during the past decades.

Herpetofaunas are often the least-known group of terrestrial vertebrates and their status in Vietnam is not exceptional. Over the past few decades, however, knowledge on the diversity of Vietnamese herpetofauna has remarkably increasing, from 340 species by Nguyen VS and Ho (1996) to 458 species by Nguyen VS et al. (2005) and 545 species by Nguyen VS et al. (2009). Even so, the herpetofauna of Vietnam remains incompletely studied, particularly regarding to the lizard groups such as the agamids, geckos, and skinks. Recently, a total of 120 species of lizards have been recorded in Vietnam, including 47 newly described species during the period from 1996 to 2009 (Nguyen VS and Ho 1996; Nguyen VS et al. 2005; Nguyen QT 2006, 2011; Nguyen VS et al. 2009; Sterling et al. 2006).

Patterns of species richness and endemism vary between reptile groups. The number of endemic snakes appears to peak in montane areas, whereas for lizards there seem to be more endemics in the south, including eight on the Con Dao Archipelogo off southeastern Vietnam. Snake and lizard species richness is evenly distributed across Vietnam's lowlands and uplands, whereas turtles are generally lowland species, and peak species richness is found in this habitat in both the north and the south. Among the most species-rich reptile lineages are those of two families of lizards; the geckos (Family Gekkonidae: 42 species) and skinks (Family Scincidae: 46 species).

Vietnamese False Bloodsucker, *Pseudocalotes brevipes* (Werner 1904): *P. brevipes* is known from Cao Bang, Lang Son, Vinh Phuc, Hai Duong, Bac Kan, Bac Giang, Quang Ninh, Hai Phong, Ha Tinh, and Ha Noi (Ba Vi) Provinces. Elsewhere, this species is recorded from Guangxi, southern China (Nguyen VS et al. 2009).

Emma Gray's forest lizard, *Calotes emma* Gray 1845: *C. emma* is distributed in the following provinces of Vietnam: Cao Bang, Bac Kan, Thai Nguyen, Vinh Phuc, Ninh Binh, Thanh Hoa, Nghe An, Ha Tinh, Quang Binh, Quang Tri, Thua Thien-Hue, Quang Nam, Kon Tum, Dak Lak, Dong Nai, Ba Ria-Vung Tau. Elsewhere, this species is recorded from Eastern India, China, Myanmar, Laos, Thailand, Cambodia, Malaysia (Ananjeva et al. 2007; Nguyen VS et al. 2009).

Garden fence lizard, *Calotes versicolor* (Daudin 1802): *C. versicolor* is distributed in the following provinces of Vietnam: Lao Cai, Son La, Yen Bai, Lang Son, Vinh Phuc, Hai Duong, Ninh Binh, Thanh Hoa, Nghe An, Ha Tinh, Quang Tri, Thua Thien-Hue, Da Nang, Quang Nam, Kon Tum, Gia Lai, Dak Lak, Dak Nong, Lam Dong, Ninh Thuan, Dong Nai, Tay Ninh, Ba Ria-Vung Tau, Can Tho, Kien Giang, and Ca Mau. Elsewhere, this species is recorded from India, Srilanka, Afghanistan, Nepal, Bhutan, Southern China, Myanmar, Laos, Thailand, Cambodia, Malaysia, and Indonesia (Nguyen VS et al. 2009).

Scale-bellied Tree Lizard, *Acanthosaura lepidogaster* Cuvier 1829: *A. lepidogaster* is distributed in southern China, southern Myanmar, Thailand, Laos, Cambodia, and Vietnam. The most widely-distributed species of the genus (Nguyen VS et al. 2009).

Eastern butterfly lizard, *Leiolepis reevesii* Gray 1831: *L. reevesii* is distributed in Thanh Hoa, Nghe An, Ha Tinh, Quang Tri, Thua Thien-Hue, Gia Lai, Kien Giang. Elsewhere, this species is recorded from Southern China (including Hainan) Thailand, Laos, Cambodia (Nguyen VS et al. 2009).

Ulikovski's gecko, *Gekko ulikovskii* Darevsky et Orlov 1994: *G. ulikovskii* is currently known only from Vietnam. The type locality is northwest Gia Lai province in central Vietnam near border with Laos and Cambodia (Darevsky and Orlov 1994). Nguyen QT et al. (2010) regarded the species as a junior synonym of *Gekko badenii* Szezerbak et Nekrasova 1994.

Spiny-tailed house gecko, *Hemidactylus frenatus* Schlegel 1836: *H. frenatus* is distributed throughout India, Nepal, Sri Lanka, Maldives, China, Taiwan, Myanmar, Thailand, Malaysia, Indonesia, Philippines, New Guinea, Japan, Polynesia, Micronesia, Melanesia, Solomon Island, Somalia, Madagascar, Mauritius, Reunion, Rodrique, Comoro Island, Samoa,

New Caledonia and Autralia. The species is very common in entire Vietnam (Nguyen VS et al. 2009).

Long-tailed mabuya, *Eutropis longycaudata* Hallowell 1856: *E. longycaudata* is distributed in southern China (including Hainan), Taiwan, Laos, Thailand, and Malaysia. The species is common thoroughly in Vietnam (Nguyen VS et al. 2009).

Clouded monitor, *Varanus nebulosus* Gray 1831: *V. nebulosus* is distributed in Quang Tri, Thua Thien-Hue, Da Nang, Quang Nam, Kon Tum, Gia Lai, Dak Lak, Lam Dong, Binh Phuoc, Dong Nai, Ba Ria-Vung Tau, Kien Giang. Elsewhere, this species is recorded from Myanmar, Thailand, Laos, Cambodia, Malaysia, Indonesia (Nguyen VS et al. 2009).

Water monitor, *V. salvator* Laurenti 1786: *V. salvator* is distributed in India, Sri Lanka, Bangladesh, southern China, Myanmar, Laos, Thailand, Singapore, Malaysia, Indonesia, Philippines. This species is common in entire of Vietnam (Nguyen VS et al. 2009).

Exploitation and trade in reptiles

Reptiles make up a significant portion of Vietnam's vertebrates whose survival in the wild is threatened by exploitation and wildlife trade. The IUCN (2010) listed over three-quarters of turtle species as globally threatened, two monitor lizards, the Bengal monitor (*Varanus bengalensis*) and Water monitor (*V. salvator*), which are of great concern and under pressure for both their meat and hides.

The demand that drives wildlife exploitation has deep cultural roots formed over the past two millennia. Traditional Asian values regarding wildlife consumption are based on an intricate combination of nutrition and medicine. Eating wildlife is thought to have a tonic effect, providing restorative and stimulative benefits as the consumer draws into himself the particular energy that formerly flowed through the reptile. Strong or unusual looking species such as monitor lizards apparently offer greater energy flow. Individuals also believe that specific characteristics and strengths associated with animals can be transferred to them through consumption, correcting internal imbalances thought to be responsible for affliction or disease. For example, the natural attributes of snakes – their flexibility and periodic shedding of skin – are tapped for the treatment of arthritis and skin disease.

For reptiles, the demands that drive hunting and live animal collection range from subsistence-level requirement for food and medicine through an ever-widening circle of local and domestic markets to the booming international trade. This demand is met by an almost

equally wide variety of methods and trading ingenuity. Most major cities and towns in Vietnam have wildlife markets that tender an enormous of live animals representing every major reptile group, from geckoes, snakes, turtles and skinks. Reptile products are also well represented in marketplaces. Consumed primarily for pharmacological and restorative benefits, these secondary items include: wines made by steeping wild-caught venomous snakes or geckoes.

As of June 2001, the export of all wild animals and rare and precious plants is prohibited, but not all wildlife trade is illegal. Vietnam permits the export of live wildlife and wildlife products that are farmed. Legal exports include the burmese (*Python molurus*) and reticulated pythons (*P. reticulatus*), crocodiles (siamese crocodiles, *Crocodylus siamemsis*; saltwater crocodiles, *C. porosus*; Cuban crocodiles, *C. rhombifer*; and their hybrids) (CITES Scientific Authority of Vietnam 2008). However, farmed reptiles are a nonviable replacement source of wildlife for many reasons. Often poorly managed, unsanitary, and overcrowded, these farms frequently rely on wild populations to provide breeding and replacement animals, and in some case they merely launder wild-caught specimens. It is also an incomplete substitute, since consumers often prefer wild-caught reptiles, a desire expressed in continuing price differentials between the wild and farmed options. Wild-caught venomous snakes are known to fetch twice the price of farm-raised counterparts. These preferences reflect a belief in the greater potency, and hence presumed health benefits, of food and medicine derived from wild-caught reptiles.

The transportation, trade, consumption and breeding wildlife reptiles spread disease from one species to another, one region to another. The reptiles are usually concentrated and mixed farming in small cages, and food is often live fishes, frogs and insects, and in fact it has happened many serious diseases in small farms throughout Vietnam.

State of researches on lizard helminths in South-Eastern Asia

Systematic studies of parasites in lizards were carried out since the end of 19th century (some works were published even earlier) and their results were published in a very large number of parasitological books and journal papers published by many experts around the world, especially in Europe, Russia and North America (Skrjabin et al. 1961; Anderson et al. 2009). Recently, Bursey C. R. and Goldberg S. R published hundreds of article on helminth parasites of lizards around the world with description of numerous new heminth species as well as redescription of known species.

In the South-Eastern Asia, however, this is still a rather new object for basic research, and studies of the parasites of reptiles have been so far highly sporadic and inadequate, despite the fact that these animals are being farmed for sale and this business is rapidly developing. There are about 10 reptile farms that breed crocodiles, snakes, turtles, varanids in Thailand, Malaysia, Indonesia, and Philippines. Up to now, however, there is only some information available on their parasites, e.g. only some publications in Laos (Scholz and Ditrich 1991), Malaysia (Palmieri and Sullivan 1977), and Thailand (Saehoong and Wongsawad 1997). In the last few years, increasing numbers of researches on reptile parasites in some South-Eastern Asian countries have been published (Bursey et al. 2005a, b, 2013, 2014, 2015a, b, c; Tkach et al. 2013), but still in an insufficient state.

Researches on lizard helminths in Vietnam

To date, researches on veterbrate parasites in Vietnam have been limited; most studies focused on the parasite fauna of birds and mammals, while researches on other groups such as reptiles and amphibians were scarce. Vietnam is well-known as harboring a rich lizard fauna, with more than 120 species. Given this rich assemblage of lizard species, it is also likely that they harbor a diverse array of endoparasites. However, current research efforts on helminth fauna of Vietnamese lizards so far is very limited, with only 10 species out of more than 120 species of Vietnamese lizards that have been examined for parasite infections. Recent studies indicated that the parasite species composition in Vietnamese lizards is very diverse with many new species have been found along with new hosts records.

During the recent decades, the knowleage of the parasite fauna of Vietnamese lizards is remarkably increasing from the fisrt species was reported by Le and Nguyen (1966) with the description of a new species of nematode, *Abbreviata deschiensi,* in *Calotes versicolor* from South Vietnam. Thirty-six years later the second study on lizard helminths in Vietnam was reported with 9 species of parasitic nematodes in giant lizards from Vietnam (Nguyen TM 2002) or 7 species of cestodes in giant lizards (Tran 2009). Currently, a total of 45 species of parasites in lizards have been reported, including 6 new species (*Abbreviata deschiensi, Pharyngodon duci, Spauligodon vietnamensis, Thelandros vietnamensis, Cosmocercoides tonkinensis, Pseudoacanthocephalus nguyenthileae*) (Le and Nguyen 1966; Nguyen TL et al. 2003, 2005, 2008; Amin et al. 2008; Bui et al. 2009; Nguyen TM 2002; Tran 2009; Tran et al. 2005, 2007, 2015a,b, 2016). Thus, the parasitic fauna of lizards remains far limited, particularly on parasitic nematodes. So, researches undertaken in this thesis have focused on

nematodes of three families such as Pharyngodonidae (*Pharyngodon* and *Spauligodon*), Cosmocercidae (*Cosmocercoides*) and Heterakidae (*Strongyluris* and *Meteterakis*). Not only morphological but also phylogenetic analyses based on the rDNA were performed on the specimens since the latter approach enables the understanding of the parasites from the biogeographical viewpoints and phylogenetic relationships with closely related taxa in morphology. In addition, the use of the latter methodology provides sometimes intraspecific variation related to possible cryptic species which cannot be detected solely by morphology.

Classification and distribution of three families, Pharyngodonidae, Cosmocercidae and Heterakidae

The family Pharyngodonidae Travassos 1919, parasitic to cold-blood verterbrates, rarely archaic mammals, is characterized by the number of cephalic papillae, four pairs or eight joined in pairs, which distinguished originally the family from Thelastomatidae parasitic to invertebrates and bearing eight separate cephalic papillae, although the recent taxonomical notions mention that the separation of these two families is rather arbitrary due to the fact that some Thelastomatidae have only four papillae (Petter and Quentin 2009). Structure of the family in male caudal extremity of the family seems to show best of the phylogenetic relationship of the group (Petter and Quentin 2009). Currently, it contains 25 genera, in which three genera (*Pharyngodon* Diesing 1861, *Spauligodon* Skrjabin, Schikhobalova et Lagodovskaja 1960 and *Thelandros* Wedl 1861) are known in lizards from Vietnam. The genus *Pharyngodon* Diesing, 1861 occurs primarily in lizards of the families Gekkonidae, Phrynosomatidae, Scincidae and Teiidae (Bursey and Goldberg 1996, 1999a, b). The genus *Spauligodon* Skrjabin, Schikhobalova et Lagodovskaja 1960 was established during the revision of *Pharyngodon* by Skrjabin et al. (1961); they separated some species assigned to *Pharyngodon* at that time, in which male worms had distinct caudal alae that envelop only the anterior 2 pairs of the 3 pairs of caudal papillae, and placed them in the new genus. The genus contains currently 51 species, including two species collected from Oriental region, *S. vietnamensis* and *S. bintangensis.* The genus occurs mainly in lizards of the families Gekkonidae, Phrynosomatidae, Scincidae, Teiidae, Agamidae, Chamaleonidae, Obluridae, Polychrotidae and Tropiduridae (Ramallo et al. 2002).

The family Cosmocercidae Travassos 1925 contains three subfamilies, members of which are parasites of amphibians and/or reptiles (Chabaud 2009a); Cosmocercinae Raillet 1916, Maxvachniidae Chabaud et Brygoo 1960, and Gyrinicolinae Yamaguti 1938. The subfamily

Cosmocercinae contains 7 genera, and characterized by the presence of caudal rosette papillae, either on plectanes or not on them. Currently, 19 *Cosmocercoides* spp. have been recorded mainly from amphibian hosts with exceptional three species in South American lizards (*C. sauria*), North American snails and slugs (*C. dukae*), and a wide spectrum of North American amphibians and lizards (*C. variabilis*).

The family Heterakidae Railliet et Henry 1912 is characterized by the esophagus with tri-balved posterior bulb, and rounded lips not connected by lateral lobes (Chabaud 2009a), containing three subfamilies (Spinicaudinae Travassos 1920; Meteterakinae Inglis 1967; and Heterakinae Railliet et Henry 1912). Heterakinae has a head with interlabia or their homologues, and caudal alae supported by long, narrow papillae, whereas Spinicaudinae and Meteterakinae has a head without interlabia, and caudal alae supported by stout papillae (Chabaud 2009a). Parasites with lips off-set from the body are classified in Spinicaudinae, which contains the genus *Strongyluris* Mueller 1894, and those with lips not off-set from the body are classified in Meteterakinae, which contains *Meteterakis* Karve 1930.

CHAPTER 1

Two new species of Pharyngodonidae (Nematoda: Oxyuroidea) in *Gekko ulikovskii* from Vietnam

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1.1. Abstract

Pharyngodon duci sp. n. and *Spauligodon vietnamensis* sp. n. (Nematoda: Pharyngodonidae) from the large intestine of a gecko, *Gekko ulikovskii* (Sauria: Gekkonidae), from Vietnam are described and illustrated. *Pharyngodon duci* is the 35th species assigned to the genus and is separated from its congeners based upon the lack of spicule, egg morphology, mouth morphology and cloacal lip morphology. *Spauligodon vietnamensis* is the 44th species assigned to the genus and is separated from its congeners by lack of a spicule, egg morphology, and tail filament morphology.

1.2. Introduction

During a helminthological examination of golden gekkos, *Gekko ulikovskii* Darevsky et Orlov 1994, 9 of 10 (90%) individuals were found to harbor nematodes representing an undescribed species of *Pharyngodon* Diesing 1861 and an undescribed species of *Spauligodon* Skrjabin, Schikhobalova et Lagodovskaja, 1960. *Gekko ulikovskii* is currently known only from Vietnam. The type locality is northwest Gia Lai Province in central Vietnam near the border with Laos and Cambodia (Darevsky and Orlov 1994).

The genus *Pharyngodon* was established by Diesing (1861) with *P. spinicauda* (Dujardin 1845) (originally *Oxyuris spinicauda* Dujardin 1845 but reclassified by Diesing 1861) from a lizard *Podarcis muralis* (= *Lacerta muralis)* collected at St. Malo, France, as the type species. Skrjabin et al. (1961) revised the genus to retain only those species in which males have well-developed caudal alae forming a genital bursa enveloping the 3 pairs of caudal pedunculate papillae. Currently, 34 species are assigned to the genus; however, 4 are known from female specimens only, i.e., *P. boulengerula* Ubelaker 1965; *P. elongata* Markov et Bogdanov 1961; *P. sphaerodactyli* Barus et Coy Otero 1974; and, *P. polpedatis* Yamaguti 1941 (see Bursey and Goldberg 1996, 1999a). The genus *Spauligodon* was established during the revision of *Pharyngodon* by Skrjabin et al. (1961) in that they removed those species assigned to *Pharyngodon* in which males have distinct caudal alae that envelop only the anterior 2 pairs of the 3 pairs of caudal papillae and placed them in the new genus. *Spauligodon extenuatus* (Rudolphi 1819) [originally, *Ascaris extenuatus* Rudolphi 1819, then *Pharyngodon extenuatus* (Rudolphi 1819) Seurat 1917] was made the type species. Currently 43 species are assigned to the genus (Bursey et al. 2005a). The purpose of this paper is to describe the 35th species assigned to *Pharyngodon* and the 44th species assigned to *Spauligodon*.

1.3. Materials and Methods

Ten *G. ulikovskii* (mean snout-vent length 180 ± 20 mm) were collected by hand on Baden Mountain, Tay Ninh Province (30°06'N, 123º01'W) October 2004 by TTB, and fixed in 4% formalin. The body cavity was opened by a longitudinal incision and the gastrointestinal tract was removed by cutting across the esophagus and rectum. The esophagus, stomach, small intestine, and large intestine of each gecko were examined separately for endoparasites. Nematodes were placed in lactophenol, allowed to clear and examined under a light microscope. Drawings were made with the aid of a microprojector. Measurements are in μm with mean ± 1 standard deviation (SD) and range in parenthesis unless otherwise stated. Lizards were deposited in the Institute of Ecology and Biological Resources, Hanoi, Vietnam. Nematodes were deposited in the United States National Parasite Collection (USNPC), Beltsville, Maryland, USA.

1.4. Results and Discussion

One thousand three nematodes were found. Of these, 75 male and 52 gravid females fit the description of *Pharyngodon* and 52 male and 43 gravid females fit the description of *Spauligodon*. The remaining 781 were immature oxyurid females, which we did not attempt to identify to genus.

Description

***Pharyngodon duci* sp. n.** (Figs. 1)

General: Oxyuroidea Railliet 1916; Pharyngodonidae Travassos 1919; *Pharyngodon* Diesing 1861. Males with caudal alae that envelop the 3 pairs of caudal papillae; females with vulva in anterior half of body. Sexual dimorphism evident, males shorter and thinner than females. Mouth bounded by 3 lips; dorsal lip with 2 papillae, each sublateral lip with 1 papilla and 1 amphid. Esophagus composed of anterior cylindrical corpus, short isthmus and posterior valved bulb. Tail in both sexes forming flexible terminal process.

Male (holotype and 9 paratypes): Small, cylindrical nematodes, distinctly truncated posterior end. Cuticle with transverse annulations approximately 20 apart. Length including tail filament 2.34 ± 0.10 mm (2.18–2.50 mm), width at excretory pore 154 ± 7 (140–166). Lateral alae approximately 10 in width extending from level of nerve ring to approximately

60 anterior to cloaca. Buccal cavity 17 ± 1 (15–18). Oesophageal corpus 290 ± 19 (244–305) in length, isthmus 26 ± 3 (21–31) in length, bulb 75 ± 5 (67–82) long, 70 ± 7 (61–82) wide. Nerve ring 145 ± 8 (128–153) and excretory pore 747 ± 40 (663–791) from anterior end. Three pairs of caudal papillae; precloacal pair situated on slightly inflated portion of caudal end, adcloacal pair posteriorly directed, and postcloacal pair enclosed by caudal alae. Prominent V-shaped cloacal lips. Spicule absent. Body terminates in flexible, smooth, filiform process, 146 ± 14 (122–171) in length.

Female (allotype and 9 paratypes): Slender, white, cylindrical nematodes, cuticle with transverse annulations approximately 20 apart. Length including tail filament 4.29 ± 0.29 mm (3.97–4.99 mm), width a level of vulva 325 ± 12 (306–344), body cylindrical, tapering sharply posterior to anus to form flexible, smooth, process 472 ± 34 (434–534) in length. Lateral alae absent. Buccal cavity 14± 3 (12–18). Oesophagus consisting of corpus 316 ± 7 (300–323) in length, isthmus 23 ± 3 (18–27) in length, bulb 113 ± 7 (104–128) long, 121 ± 9 (104–128) wide. Nerve ring 119 ± 6 (113–134), excretory pore 835 ± 49 (765–931), and vulva 924 ± 47 (842–999) from anterior end. Vulva slitlike, salient. Thick-walled vagina extending 505 ± 41 (459–587) posteriorly, joining 2 uteri, which extend posteriorly and when filled with eggs reach posterior end of body cavity. In non-gravid individuals, posterior ovary at midbody, anterior ovary parallel to vagina. Egg barrel-shaped, ends truncated, slightly flattened on one side, 138 ± 4 (134–143) by 54 ± 3 (49–58); egg shell thin, punctated. Eggs not larvated when released.

Taxonomic summary

Type host: Gekko ulikovskii Darevsky et Orlov 1994, collected October 2004.

Type locality: Baden Mountain, Tay Ninh Province, Vietnam (30°06'N, 123°01'W, 100-400 m elevation).

Site of infection: Large intestine.

Type specimens: Holotype male USNPC 99774; allotype female USNPC 99775; paratypes USNPC 99776; voucher specimens USNPC 99777.

Etymology: The new species is named for Nguyen Van Duc, Head of Parasitology Department, Institute of Ecology and Biological Resources, Hanoi, Vietnam.

Remarks

Species of *Pharyngodon* are separated on the presence or absence of a spicule, the morphology of the caudal alae, the shape of the egg, the presence or absence of spines on the tail filament of adults, and their distribution (Bursey and Goldberg 1996). One additional species, *P. oceanicus* Bursey et Goldberg 1999, should be added to Table I of Bursey and Goldberg (1996): male, spicule absent, tail filament longer than bursa; female, tail subulate with 5–7 spines, bottle-shaped egg.

Eleven species, i.e., *P. asterostoma* Adamson 1984; *P. australis* Johnston et Mawson 1942; *P. brevibursata* Caballero Rodriguez 1968; *P. cesarpintoi* Pereira 1935; *P. cnemidopori* Read et Armein 1953*, P. hierrensis* Solera-Puertas et al. 1988; *P. hispanicus* Astasio-Arbiza, Zapatero-Ramos et Solera Puertas 1987; *P. kirbii* Specian et Ubelaker 1974*, P. tiliquae* Baylis 1930; *P. warneri* Harwood 1932; and *P. yucatanensis* Chitwood 1938, have been described as lacking a spicule but having eggs with truncated ends. Of these, *P. hispanicus* and *P. yucatanensis* differ from the new species in that tails of females have cuticular spines; *P. cesarpintoi, P. cnemidopori, P. kirbii* and *P. warneri* differ in that tails of females have inflexible tail spikes; *P. australis* differs in that the tail of the male is shorter than the bursa; and *P. brevibursata* and *P. hierrensis* differ in that males have tails equal to bursa. The remaining 2 species, *P. asterostoma* and *P. tiliquae* are most similar to the new species. However, the mouth of *P. asterostoma* has a six-pointed star-shaped opening in contrast to the triangular opening of *P. duci* n. sp. and the anterior cloacal lip in *P. tiliquae* is echinate in contrast to the smooth anterior cloacal lip of *P. duci* sp. n.

***Spauligodon vietnamensis* sp. n.** (Fig. 2)

General: Oxyuroidea Railliet 1916; Pharyngodonidae Travassos 1919; *Spauligodon* Skrjabin, Schikhobalova et Lagodovskaja 1960. Males with caudal alae that do not envelop posterior postcloacal pair of pedunculate papillae; females with vulva in anterior half of body. Sexual dimorphism evident, males shorter and thinner than females. Mouth bounded by 3 lips; dorsal lip with 2 papillae, each sublateral lip with 2 papillae and 1 amphid. Oesophagus composed of cylindrical corpus, short isthmus and valved bulb. Males and females with narrow lateral alae extending from level of isthmus to approximately 60 anterior of base of tail filament.

Male (holotype and 9 paratypes): Small, cylindrical nematodes, distinctly truncated posterior end. Cuticle with transverse annulations approximately 9 apart. Length including tail filament 1.61 ± 0.14 mm (1.39–1.76 mm), width at excretory pore 155 ± 18 (128–179). Buccal cavity 11 ± 3 (6–15). Oesophageal corpus 247 ± 12 (220–262) in length, isthmus 36 ± 2 (34–40) in length, bulb 59 ± 3 (52–61) long, 60 ± 5 (52–67) wide. Nerve ring 120 ± 6 (110-128) and excretory pore 510 ± 42 (446–587) from anterior end. Truncate posterior end terminating dorsally in elongated smooth filiform tail filament 111 ± 8 (100–122) in length and laterally in narrow caudal alae. Three pairs of caudal papillae present; precloacal pair situated at anterior ventral inlet of caudal bursa; adcloacal pair bifurcated laterally and directed posteriorly; postcloacal pair not enclosed by caudal alae and situated on base of tail filament. Postcloacal papillae separated from caudal alae by narrow cleft. Spicule absent.

Female (allotype and 9 paratypes): Slender, white, cylindrical nematodes, cuticle with transverse annulations approximately 20 apart. Length including tail filament 3.25 ± 0.23 mm (2.88–3.58 mm), width a level of vulva 288 ± 32 (230–357), body cylindrical, tapering sharply posterior to anus to form smooth spike 248 ± 33 (204–306) in length. Anus 234 ± 17 (204–255) from base of terminal spike. Esophagus consisting of corpus 271 ± 19 (244–305) in length, isthmus 24 ± 2 (21–27) in length, bulb 106 ± 5 (101–116) long, 109 ± 7 (92–119) wide. Nerve ring 110 ± 6 (98–116), excretory pore 576 ± 53 (510–650), and vulva 665 ± 65 (561–740) from anterior end. Vulva slitlike, slightly salient. Thick-walled ovijector extending 266 ± 21 (244–293) posteriorly, opening onto thin walled vagina 135 ± 16 (122–161), which in turn joins 2 uteri. Uteri extend posteriorly and when filled with eggs reach posterior end of body cavity. In non-gravid individuals, ovaries lie at midbody. Egg fusiform, knobed at each end, slightly flattened on one side, 138 ± 5 (131–146) by 57 ± 3 (52–61), thin shell. Eggs larvated when released.

Taxonomic summary

Type host: Gekko ulikovskii Darevsky et Orlov, 1994, collected October 2004.

Type locality: Baden Mountain, Tayninh Province, Vietnam, (30°06'N, 123°01'W, 100-400 m elevation).

Site of infection: Large intestine.

Type specimens: Holotype male, USNPC 99778; allotype female, USNPC 99779; paratypes USNPC 99780; voucher specimens USNPC 99781.

Etymology: The new species is named for the country of collection.

Remarks

Species of *Spauligodon* are distinguished on the basis of the presensce or absence of a spicule, the presence or absence of spines on the tail filament of adults, egg morphology, and geographical distribution (see Table I of Bursey et al. 2005a). Of the 43 species listed, *S. blydeensis* Hering-Hagenbeck 2001; *S. cubensis* (Read et Amrein 1953); *S. maytacapaci* (Vicente et Ibanez 1968); *S. morgani* (Fitzsimmons 1961); *S. oxkustzcabiensis* (Chitwood 1938) and *S. petersi* Bursey, McAllister et Freed 1997 are described as lacking a spicule and having spindleform eggs with knobs at each end. *S. blydeensis*, *S. morgani* and *S. oxkutzcabiensis* differ from the new species in that the tail of females has cuticular spines; *S. cubensis* and *S. petersi* differ in that females have a filiform tail; and females of *S. maytacapaci* have a bifid tip to the tail spike.

CHAPTER 2

A new *Cosmocercoides* species (Nematoda: Cosmocercidae), *C. tonkinensis* n. sp., in the scale-bellied tree lizard (*Acanthosaura lepidogaster*) from Vietnam

This work described in the chapter has been published as follows:

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2.1. Abstract

A new cosmocercid nematode species, *Cosmocercoides tonkinensis* n. sp., is described from the scale-bellied tree lizard (*Acanthosaura lepidogaster*) in the northern and central parts of Vietnam. The new species is characterized by medium-sized male worms (4.2–5.1 mm in length and 0.34–0.37 mm in width) relative to known members of the genus, with lateral alae, two sharply pointed spicules of equal length (0.22–0.26 mm in length), a gubernaculum (0.113–0.122 mm in length), 16 or 17 pairs of caudal rosettes, and the presence of somatic papillae. Female worms are slightly larger than male worms (5.3–5.5 mm in length and 0.32–0.42 mm in width), with the vulva situated at 3/5 from the anterior end, and elliptical embryonated eggs, 0.064–0.084 mm long by 0.040–0.048 mm wide. From 19 recorded species of the genus, the morphology of *C. tonkinensis* n. sp. is closest to *C. multipapillata*, *C. bufonis*, and *C. pulcher* reported from toads and frogs in East Asia. The present new species is differentiated from them by the number of caudal rosettes, tail length relative to body length, presence of somatic papillae and lateral alae, and embryonated eggs. Furthermore, after *C. variabilis* in North America and *C. sauria* in Brazil, this new species is only the third species to be recorded from a reptilian host. The 18S ribosomal RNA gene (rDNA) of the new species is almost identical to that of *C. dukae* infecting land snails and slugs in North America. Between the present new species and *C. pulcher* from a toad (*Bufo japonicus*) in Japan, remarkably fewer nucleotide changes were noticed in the 18S to 28S rDNA including the internal transcribed spacer regions. The molecular phylogenetic position of the genus *Cosmocercoides* is briefly discussed.

2.2. Introduction

The scale-bellied tree lizard, *Acanthosaura lepidogaster* (Cuvier 1829), is common in Southeast Asia, being distributed widely in Vietnam, southern provinces of China, Myanmar, Laos, Thailand, and Cambodia (Ananjeva et al. 2008; Nguyen VS et al. 2009). To the best of our knowledge, the parasite fauna of this lizard species has not yet been clarified. Further, only a few researchers have conducted parasitological surveys for any lizards in Vietnam (The study of Chapter 2; Bui et al. 2009). Recently, we examined 32 scale-bellied tree lizards in Vietnam and detected several nematode species in the intestine of 29 (90.6%) lizards. Collected parasites included multiple *Meteterakis* spp. and a single *Cosmocercoides* sp. The latter genus is classified in the family Cosmocercidae of the order Ascaridida, and

characterized by the presence of caudal rosette papillae, distinct from the caudal papillae on plectanes of the genus *Cosmocerca* (Chabaud 2009a). Currently, 19 *Cosmocercoides* spp. have been recorded, mainly from amphibian hosts. Two species, *C. dukae* and *C. sauria,* have been recorded from North American land snails and slugs and from Neotropical lizards, respectively (Vanderburgh and Anderson 1986, 1987; Ávila et al. 2010). *Cosmocercoides variabilis* has been reported to infect not only a wide spectrum of amphibians but also lizards in North America (Bursey et al. 2007). Due to an exceptional host specificity of our nematode specimens and occurrence in the Oriental region, we conducted morphological and molecular genetic characterizations of the *Cosmocercoides* sp. from scale-bellied tree lizards. Following comparison with its congeners, it is described as a new species, *C. tonkinensis* n. sp.

2.3 Materials and Methods

Animals and parasitological examination

Thirty-two scale-bellied tree lizards were collected by hand during the period February 2012 to February 2013 from 5 localities in the northern region (17 lizards), 1 locality in the central region (11 lizards), and 2 localities in the southern region (4 lizards) of Vietnam (Fig. 3). Details of the collection sites are shown in Table 1. Body cavities of the lizards were opened by a longitudinal incision, and the gastrointestinal tracts were removed by cutting across the esophagus and rectum. The esophagus, stomach, small intestine, and large intestine of each lizard were examined separately for endoparasites. Collected nematodes were killed in hot water at 70 ℃, fixed, and preserved in 70% ethanol for later use. For morphological observation, the nematode was placed in a clearing solution with glycerin and lactic acid, and examined under a light microscope. Drawings were made with the aid of a camera lucida. Measurements are in millimeters (mm) unless otherwise stated, with the range followed by the mean in parentheses. Nematodes were deposited in the Vietnam National Museum of Nature, VAST, Hanoi, Vietnam, under specimen numbers VNMN-2013-01001–1003. *Cosmocercoides pulcher* from a toad (*Bufo japonicus)* caught in Oita Prefecture, Japan, was provided by Dr. Y. Ikeda, Oita University, and examined similarly for morphological and genetic comparison with the cosmocercid species from the scale-bellied tree lizards. Examined specimens of *C. pulcher* were deposited in the Meguro Parasitological Museum, Tokyo, Japan, under specimen number MPM Coll. No. 20968.

Scanning electron microscopy (SEM)

Samples preserved in 70% ethanol were washed 3 times in 0.2 M Na_2HPO_4 – NaH_2PO_4-buffered solution (PB), pH 7.8, and immersed in 2.5% glutaraldehyde in PB overnight. Following 3 washes in PB, the samples were post-fixed in 1% (w/v) osmium tetroxide in PB for 1 hr. After washing 3 times in PB, the samples were dehydrated through a graded alcohol series, immersed in warmed *t-butyl-alcohol,* and cooled at 4 ℃ for 2 hr. The nematode samples were then freeze-dried (model JFD-300; JEOL, Akishima, Tokyo, Japan), mounted on stubs, and sputter-coated with gold-palladium at 200Å (model JFC- 1500; JEOL). Samples were examined using a scanning electron microscope (model JSM-6100; JEOL) at an accelerating voltage of 15 kV.

DNA extraction, polymerase chain reaction (PCR), and sequencing

Individual nematodes stored in 70% ethanol were cut into three longitudinally equal parts. The middle 1/3 portion was washed with distilled water and used for DNA extraction using an IllustraTM tissue and cells genomicPrep Mini Spin Kit (GE Healthcare UK, Buckinghamshire, UK) according to the manufacturer's instructions. PCR amplification of overlapping fragments of the 18S to 28S ribosomal RNA gene (rDNA) was performed in a 20µl volume using a DNA polymerase, Blend Taq-Plus- (TOYOBO, Dojima Hama, Osaka, Japan), and primer combinations as previously described (Makouloutou et al. 2013). The PCR cycling protocol was 3 min at 94°C, then 40 cycles of 45 sec at 94°C, 60 sec at 64°C or 62°C, and 60 sec at 72°C, followed by a final extension at 72°C for 7 min. PCR products for sequencing were purified using a FastGene Gel/PCR Extraction Kit (Nippon Genetics, Bunkyo-ku, Tokyo, Japan). Following direct sequencing of PCR amplicons, sequences were assembled manually with the aid of the CLUSTAL W multiple alignment program (Thompson et al. 1994). For rDNA segments containing internal transcribed spacers (ITS) 1 and 2, the amplicon was cloned into a plasmid vec- tor, pTA2 (Target CloneTM; TOYOBO), and transformed into *Escherichia coli* JM109 (TOYOBO) according to the manufacturer's instructions. Following propagation, plasmid DNA was extracted using a FastGene Plasmid Mini Kit (Nippon Genetics) and inserts from multiple independent clones were sequenced using universal M13 forward and reverse primers. Specimens excluding the middle 1/3 portion used for DNA extraction were deposited in the Meguro Parasitological Museum, Tokyo, Japan, under specimen number MPM Coll. Nos. 20966 and 20967 (C. *tonkinensis* n. sp.) and 20969 (C. *pulcher).*

Phylogenetic analysis

For phylogenetic analysis, the newly obtained 18S and 28S rDNA sequences of *Cosmocercoides* spp. examined in the present study (DDBJ/EMBL/GenBank accession nos. AB908160, AB908161 and LC018444) and related sequences of ascarid and spirurid nematodes retrieved from the DDBJ/EMBL/GenBank databases were aligned using the CLUSTAL W multiple alignment program, with subsequent manual adjustment. The accession numbers of the sequences analyzed in the present study are given in the figure showing the phylogenetic trees. Regions judged to be poorly aligned and characters with a gap in any sequences were excluded from subsequent analyses (Smythe et al. 2006); 1,544 characters, of which 264 were variable, remained for subsequent analysis for 18S rDNA, and 652 characters, of which 323 were variable, remained for subsequent analysis for 28S rDNA. Maximum likelihood (ML) analysis was performed with the program PhyML (Guindon and Gascuel 2003; Dereeper et al. 2008) provided on the 'phylogeny.fr' website (http://www.phylogeny.fr/). The probability of inferred branch was assessed by the approximate likelihood- ratio test, an alternative to the non-parametric bootstrap estimation of branch support (Anisimova and Gascuel 2006).

2.4 Results

Parasite recovery

A single *Cosmocercoides* sp. was found in the large intestine of 10 lizards collected from four localities in the northern (localities A, C, and E in Fig. 3) and central (locality F in Fig. 3) regions of Vietnam (Table 1). Worm recovery ranged from 1 to 29 (4.1 geomean).

Description

Cosmocercoides tonkinensis n. sp. (Ascaridida: Cosmocercidae) (Figs. 4A-H and 5A-D)

General: Stout, medium-sized among recorded *Cosmocercoides* spp. Sexual dimorphism not hugely evident, male worms a little smaller than female worms. Anterior end blunt and posterior end pointed. Head not separated from body, and mouth with 3 lips of equal size; dorsal lip with 2 papillae, each subventral lip with 1 papilla and 1 amphid. On buccal wall, 1 thin but long lingual projection corresponding to each lip. Lateral alae throughout the length of the body except for both ends. Esophagus following a short pharynx, consisting of

cylindrical corpus and posterior bulb containing valves. Nerve ring at the middle of the esophageal corpus, and excretory pore at the midpoint between the nerve ring and anterior margin of the esophageal bulb.

Male (3 specimens): 4.17–5.06 (4.65) in body length, with 0.34–0.37 (0.35) in maximum width. Pharynx 0.060–0.064 (0.061) long and esophagus 0.79–0.87 (0.82) long; corpus 0.58–0.67 (0.61) long, connected to bulb 0.136–0.152 (0.141) long by 0.136–0.144 (0.139) wide. Nerve ring 0.31–0.36 (0.34) from anterior end, and similarly excretory pore 0.44–0.55 (0.49). Two spicules equal in length, lightly sclerotized, curved ventrally, and sharply pointed at the distant end, 0.222–0.256 (0.243) long. Gubernaculum well sclerotized, 0.113–0.122 (0.117) long. Tail conical, sharply pointed at the tip, 0.211–0.233 (0.226) long. Ventral surface of posterior 1/3 of body with 16 or 17 pairs of caudal rosettes in double rows, but with the first one of either row frequently missing then just one at either of the rows, 12 or 13 subventral precloacal pairs, 1 adcloacal pair, and 3 postcloacal pairs, in which 2 pairs subventral in position and 1 pair lateral in position. Each rosette is composed of about 13–16 punctations around the central papilla. Tail with 1 pair of somatic papillae and 4 pairs of small and simple papillae, including 3 pairs of subventral papillae and 1 pair of lateral papillae.

Female (3 specimens): Body length 5.28–5.50 (5.40) with 0.32–0.42 (0.37) in maximum width. Pharynx 0.064–0.068 (0.067) long and esophagus 0.82–0.91 (0.88) long; corpus 0.62–0.69 (0.66) long, connected to bulb 0.136–0.152 (0.144) long by 0.152–0.168 (0.157) wide. Nerve ring 0.34–0.35 (0.35) from anterior end, and similarly excretory pore 0.51–0.54 (0.53). Vulva at the posterior part of the body, 2.96–3.12 (3.05), corresponding to 1.27–1.36:1 point from the anterior end. Vagina running anteriorly from vulva for a short distance, then turning posteriorly and dividing into two didelphic uteri. Ovary and uterus not extending anteriorly beyond esophageal bulb and posteriorly beyond anus. The uterus filled with embryonated eggs at different stages of development. Intrauterine eggs elliptical with thin egg shell, 0.064–0.084 (0.072) long by 0.040–0.048 (0.043) wide. Tail conical but sharply pointed, 0.256–0.304 (0.276) long.

Taxonomic summary

Type specimens: Holotype, male (VNMN-2013-001); allotype, female (VNMN-2013-002); and paratypes (VNMN- 2013-003) in Vietnam National Museum of Nature, VAST, Hanoi, Vietnam.

Other materials: Voucher IEBR (51 contracted specimens as the hosts were fixed in 70% ethanol). MPM Coll. Nos. 20966 and 20967 (female and male specimens excluding the middle 1/3 part of body, used for DNA analysis).

Type host: Acanthosaura lepidogaster (Cuvier, 1829).

Type locality: Trung Khanh, Cao Bang Province (22°40′N, 106°38′E).

Distribution: Phu Yen, Son La Province (21°13′N, 104°40′ E); Tay Yen Tu, Bac Giang Province (21°11′N, 106°42′E); and Pu Hu, Thanh Hoa Province (20°28′N, 104°55′E), Vietnam.

Site of infection: Large intestine.

Prevalence: 10 (31.3%) of 32 lizards examined.

Intensity of infection: 1–29 (4.1 geomean).

Etymology: This species is named referring to the area where infected lizards were collected, i.e. the northern part of Vietnam. The area is called 'Tonkin' in Vietnamese.

Remarks

To date, 17 valid species of the genus *Cosmocercoides* have been described from amphibians worldwide. Additionally, *C. dukae* and *C. sauria* have been reported from land snails and slugs in North America and from gymnophthalmid lizards in Brazil, respectively (Rizvi 2009; Ávila et al. 2010). Among these 19 recorded species, *C. tonkinensis* n. sp. shows the closest morphology with *C. multipapillata* Khera 1958, *C. bufonis* Karve 1944, and *C. pulcher* Wilkie 1930. The morphometric values and some morphological characters of these species are compared in Table 2. The new species is differentiated from *C. multipapillata* by smaller body size, smaller size of gubernaculum (0.11–0.12 vs. 0.13–0.14), different numbers of postcloacal rosettes (3 vs. 4 pairs; the latter species has an additional pair of rosettes near the posterior end), longer tail of females, and embryonated eggs in the uterus; from *C. bufonis* in smaller body width, longer esophagus in males, smaller size of gubernaculum (0.11–0.12 vs. 0.12–0.15), presence of somatic papillae, and number and position of small and simple papillae; and from *C. pulcher* in smaller sizes of the body and gubernaculum, and smaller eggs. Critical variation of morphometric values of *C. pulcher* is seen in different reports (Wilkie 1930; Hsü 1933; Yamaguti 1941; Hasegawa 1984), and sometimes significant overlapping of morphometric values of *C. tonkinensis* n. sp. and *C. pulcher*, particularly that

reported by Hasegawa (1984), might make species differentiation difficult (see Table 2). At that time, the arrangement of caudal rosette papillae, particularly adcloacal and postcloacal ones, could be used for morphological species differentiation. As mentioned later, molecular genetic characterization of these two species supported our species differentiation based on minor but critical morphological differences. With regard to the 16 other described species, differentiation is more straightforward due to different body sizes, different lengths of spicules and gubernacula, different numbers of caudal rosette papillae, or presence/absence of lateral alae and somatic papillae (refer to Table I provided by Ramallo et al. 2007, and Table II and 'Species key for *Cosmocercoides*' provided by Rizvi 2009).

Molecular genetic analysis

Near complete lengths of the 18S rDNA (1,780 bp), ITS-1 (415 bp), 5.8S rDNA (158 bp), and ITS-2 (248 bp), and partial 28S rDNA (1,147 bp) of *C. tonkinensis* n. sp. were successfully sequenced using a female worm collected at Phu Yen, Son La Province (DDBJ/EMBL/GenBank accession no. AB908160). Another 2,057-bp long sequence from the 18S rDNA to 28S rDNA of a male worm collected at Tay Yen Tu, Bac Giang Province (accession no. AB908161) was completely identical with the aforementioned sequence. Similarly, near complete lengths of the 18S rDNA (1,780 bp), ITS-1 (415 bp), 5.8S rDNA (158 bp), and ITS-2 (246 bp), and partial 28S rDNA (1,433 bp) of *C. pulcher* from a toad in Japan were successfully sequenced (DDBJ/EMBL/GenBank accession no. LC018444).

Prior to the present study, only a single nucleotide sequence, i.e. 1,644-bp long 18S rDNA sequence of *C. dukae*, has been deposited in the DDBJ/EMBL/GenBank database (accession no. FJ516753). Intriguingly, alignment of the 18S rDNA of *C. tonkinensis* n. sp. and *C. dukae* showed that the 18S rDNA of these two species does not differ to a great extent (only 3 gaps and 2 substitutions over a comparable 1,647bp length), despite different host specificities (lizards vs. land snails and slugs) and different geographical distributions (East Asia vs. North America). Between *C. tonkinensis* n. sp. and *C. pulcher*, there were only a few nucleotide differences: a single nucleotide substitution each in the 18S rDNA, ITS-1, and 5.8S rDNA, 4 nucleotide substitutions and 2 gaps in the ITS2, and 2 nucleotide substitutions in the partial 28S rDNA of 1,147-bp length.

In a phyML tree based on the 18S rDNA, members of the superfamily Cosmocercoidea (*Cosmocercoides* spp., *Raillietnema* sp. from a Solomon Island leaf frog, and *Nemhelix bakeri*

from a European brown garden snail) formed a clade with members of the order Rhigonematida, particularly Ransomnematoidea, rather than other members of the order Ascaridida (Fig. 6A). Members of Rhigonematida are parasitic nematodes of diplopods. In a phyML tree based on the 28S rDNA, *C. tonkinensis* n. sp. and *C. pulcher* were representatives of Cosmocercoidea, forming a clade with members of Ascaridoidea, then with members of Rhigonematoidea, rather than Ransomnematoidea (Fig. 6B).

2.5 Discussion

Members of *Cosmocercoides* Wilkie 1930 and its related genera *Cosmocercella* Steiner 1924 and *Paracosmocercella* Hasegawa and Ikeda 2007 are characterized by possession of caudal 'rosette papillae' (papillae surrounded by punctations), not combined with 'plectanes' (cuticular supports around caudal papillae) as found in *Cosmocerca* Diesing 1861 (Hasegawa and Ikeda 2007; Chabaud 2009a). The genus *Cosmocercoides* currently contains 19 nominal species (Ramallo et al. 2007; Rizvi 2009; Ávila et al. 2010), recorded mainly from amphibian hosts. *Cosmocercoides variabilis* parasitizes not only amphibians but also a variety of reptiles, such as lizards, snakes, and turtles in North and Central America (Bursey et al. 2007), and *C. sauria* is parasitic to the gymnophthalmid lizard (*Iphisa elegans*) in western Brazil (Ávila et al. 2010). Exceptionally, *C. dukae* is a parasitic species of land snails and slugs (Vanderburgh and Anderson, 1986, 1987). *Cosmocercoides tonkinensis* n. sp. is the 20th species assigned to the genus and the first species from a reptile host in the Oriental region.

Vietnam and its surrounding countries have undoubtedly rich herpetofauna, currently comprising 177 and 368 known amphibian and reptile species, respectively (Nguyen QT 2006; Ananjeva et al. 2008; Nguyen VS et al. 2009; Wood et al. 2009, 2010). In this study, a single but new *Cosmocercoides* sp., i.e. *C. tonkinensis* n. sp., was found mainly in the northern region of Vietnam. However, it is highly possible that future parasitological surveys on the same lizard species in other regions and/or different lizard species will find the same nematode species or its congeners. Another possible outcome from future surveys is the parasitism of *C. tonkinensis* n. sp. in amphibian hosts. In Vietnam, only *C. multipapillata* has been recorded from amphibians, i.e. the dark-sided chorus frog (*Microhyla heymonsi*) (Goldberg and Bursey 2005). Since these 2 species show close morphological resemblances as mentioned earlier, careful differentiation is needed. Indeed, as *Cosmocercoides* spp. are small or tiny in body size and often show overlapping ranges of morphological features or

unstable characters affected by processing, reliable species identification of the genus requires not only intensive morphological examination but also molecular genetic characterization of parasites. In the present study, *C. dukae* and *C. tonkinensis* n. sp., collected from evolutionary distant host species (land snails or slugs vs. lizards) and distant geographical regions (North America vs. Southeast Asia), showed an almost identical 18S rDNA sequence. As morphological differentiation appears to have reached a limit of usefulness, determination of valid and feasible molecular markers for *Cosmocercoides* species differentiation is now a necessity. The first genetic characterization of long rDNA sequences (18S, 5.8S, 28S rDNA along with ITS-1 and ITS-2) of *C. tonkinensis* n. sp. from the lizard and *C. pulcher* from the amphibian in the present study might promise further genetic explorations of this taxonomic group. It is highly possible that *Cosmocercoides* spp. might co-evolve with their host species, which show robust biodiversity as recently recognized (Nguyen QT 2006; Nguyen VS et al. 2009; Wood et al. 2009, 2010). Therefore, efforts to accumulate the genetic data of recorded species are paramount to disclosing the real biodiversity of *Cosmocercoides* spp.

Molecular phylogenetic trees based on the 18S and 28S rDNA indicated close phylogenetic relationships of the Cosmocercoidea with the Rhigonematida, particularly Ransomnematoidea, rather than with the Ascaridida (Fig. 6). Rhigonematida contains nematodes parasitic to diplopods, and active research on such unusual nematodes is also progressing in Vietnam (Hunt and Spiridonov 2001; Hunt et al. 2002; Malysheva and Spiridonov 2010; Malysheva and Luc 2012). Compared with the orders Ascaridida and Rhigonematida, only a few rDNA sequences (either 18S or 28S rDNA) of members of the superfamily Cosmocercoidea are currently available. Collection of more Cosmocercoidea sequences is required for a more precise understanding of its taxonomic position.

CHAPTER 3

Morphological and molecular genetic diversity of *Strongyluris calotis* (Nematoda: Ascaridida: Heterakidae) in South East and East Asian lizards

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Chapter 3–1
Scanning electron microscopy of *Strongyluris calotis* (Nematoda: Ascaridida: Heterakidae) in the large intestine of agamid lizards in Asia

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3.1.1. Abstract

Strongyluris calotis is a heterakid nematode which dwells in the large intestine of agamid lizards from the Oriental region. Specimens collected from the Ryukyu tree lizard, *Japalura polygonata* (Agamidae), in the Ryukyu Islands, Okinawa, Japan; the Taiwan japalure, *Japalura swinhonis*, in the northern part of Taiwan; and the Emma Gray's forest lizard, *Calotes emma* (Agamidae), in Singapore, were – for the first time – subjected to an intensive scanning electron microscopic observation of the head and caudal portion to clarify the arrangement and number of cephalic and caudal papillae. The worms had three lips offset from the body and distinct cuticular flanges extended from the upper part of the internal surface of each lip. For both sexes, the dorsal lip had a pair of cephalic papillae and each subventral lip had a cephalic papilla, an external labial papilla, and an amphid. Male worms had a posteriorly directed precloacal sucker bearing three pairs of papillae ventrally on both lateral sides, two pairs of small adcloacal papillae on both lateral sides of the cloacal opening, and two pairs of ventrolateral papillae on both sides around the level of the cloaca. In addition, near the posterior end around the terminal spike, three pairs of small papillae and a pair of phasmids were noted. However, the first and second pairs of postcloacal papillae were fused to form a united structure. Female worms had a pair of phasmids on the lateral sides of the posterior tail, which has been recorded as a pair of papillae in previous studies. Therefore, male *S. calotis* worms had 10 pairs of caudal papillae and a pair of phasmids; however, two pairs of postcloacal papillae were completely fused to form a pair of united papilla structures.

3.1.2. Introduction

The genus *Strongyluris* Müller, 1894 is assigned to heterakid nematodes with lips offset from the body, notable cuticular flanges extending from each lip, a posteriorly directed precloacal sucker, two non-alate spicules of equal length and shape, and an obliquely truncate tail with a short terminal spike in male worms (Inglis 1957; Skrjabin et al. 1961; Chabaud 2009a). Multiple stout pedunculated papillae support the caudal cuticular expansion of male worms. The type species of the genus is *S. brevicaudata* Müller, 1894 and currently more than

30 species have been recorded from the large intestine of lizards and, rarely, amphibians. Bursey et al. (2003, 2013) chronicled 32 nominal *Strongyluris* spp. recorded worldwide, including six species from the Oriental zoogeographic region. Inglis (1957) specifically expressed his concern regarding whether every nominal species at that time, also included in the latest list by Bursey et al. (2013), could be differentiated from congeners based on descriptions by each research group, in which variable levels of morphological observations were conducted and recorded. In the present study, we employed scanning electron microscopy (SEM) to observe for the first time fine structures of the anterior and posterior ends of *S*trongyluris *calotis* Baylis et Daubney, 1923 from agamid lizards in Japan, Taiwan, and Singapore.

3.1.3. Materials and Methods

Japanese isolates of *S. calotis* were collected from Ryukyu tree lizards, *Japalura polygonata* (Hallowell 1861) (Agamidae), in the Ryukyu Islands, Okinawa Prefecture, Japan (see Hasegawa and Iwatsuki 1984). Other specimens were collected from Taiwan japalures, *Japalura swinhonis* (Günther, 1864), in the northern part of Taiwan (see Ota, 1991). These lizards from the Ryukyu Islands and Taiwan were collected by Prof. Hidetoshi Ota (formerly of the University of the Ryukyus and presently the University of Hyogo, Japan) and the third author (HH) between July 1981 and August 1986 (Hasegawa and Iwatsuki 1984; Ota 1991). Similarly, *S. calotis* specimens from an Emma Gray's forest lizard, *Calotes emma* (Agamidae), were collected by the fourth author (CHD) in Singapore in 2000.

Morphological observations using a light microscope were conducted as described in an earlier study (Chapter 2 in the present thesis). Measurements are in millimeters (mm), with the range followed by the mean in parentheses. Nematodes were deposited in the Meguro Parasitological Museum, Tokyo, Japan, under the specimen numbers MPM Coll. Nos. 21145-21160.

Individual male and female worms with different origins, stored in 70% ethanol or 10% neutral-buffered formalin solution, were cut into three longitudinally equal parts. The anterior and posterior one-third parts were used for SEM. They were washed three times in 0.2M Na_2HPO_4-NaH_2PO_4-buffered solution (PB), pH7.8, and immersed in 2.5% glutaraldehyde in PB overnight. The subsequent SEM processes were similar to those described previously (Chapter 2 in the present thesis).

3.1.4. Results

Strongyluris calotis specimens examined in the present study were small-sized nematodes, ca. 7–12 mm in length, and had tapering anterior ends and stout posterior ends with a small terminal spike in both sexes. No lateral alae were observed. The cephalic end had three lips offset from the body and distinct cuticular flanges extended from the upper part of the internal surface of each lip. The cephalic papillae and amphids on the lips of male and female worms were arranged similarly as follows: a pair of large-sized cephalic papillae on the dorsal lip, whereas a large-sized cephalic papilla, a small-sized external labial papilla, and an amphid were present on each subventral lip (Fig. 7). A small pharyngeal tooth was situated at the center of the inner wall of each lip. The esophagus consisted of a corpus and a tri-valved posterior bulb. Male worms had a posteriorly directed precloacal sucker, two non-alate spicules of equal length and shape, but no gubernaculum. The caudal papillae of male worms were arranged symmetrically (Fig. 8). Around an obliquely truncate tail of male worms, seven pairs of stout pedunculated papillae supported the caudal cuticular expansion: three pairs (precP-1 to 3 in Fig. 8), large, ventrally on the lateral sides of the precloacal sucker; two adcloacal pairs (adcP-1 and 2 in Fig. 8), smaller, at the levels of the anterior and posterior edges of the cloacal opening; and two pairs of ventrolateral papillae (vlcP-1 and 2 in Fig. 8) around the level of the cloaca or somewhat posteriorly. In addition, near the posterior end around the terminal spike, three pairs of small papillae (pocP- 1/2 and 3 in Fig. 8) and a pair of phasmids (Ph in Fig. 8) were observed. The first and second postcloacal papillae formed a structure of fused papillae (pocP-1/2) slightly anterolateral to the caudal spike and pocP-3 was lateral or somewhat dorsal to the terminal spike in all the male worms studied. In total, male worms had 10 pairs of caudal papillae and a pair of phasmids. In female worms, a pair of phasmids was seen on the lateral sides of the posterior tail (Fig. 9). The morphometric values of the worms chosen arbitrarily and examined in the present study are compared with those detailed in previous reports (Table 3).

3.1.5. Discussion

The specimens collected from the Ryukyu Islands and examined in the present study were similar to those previously examined by Hasegawa and Iwatsuki (1984). Originally described as '*Ascaridia japalurae*' (Yamaguti 1935), they identified the specimens as *Strongyluris japalurae* (Yamaguti 1935). The description by Yamaguti (1935) of the

arrangement of pedunculated papillae in the caudal portion of male worms is absolutely identical to that observed by light microscopy in the present study. Yamaguti (1935) counted 10 pairs of caudal papillae in total. These morphological features, particularly the arrangement and number of caudal papillae in male worms, correspond to the specific definition of *S. calotis* by Baylis and Daubney (1923), and thence Bursey et al. (2003) listed *A. japalurae* as a junior synonym of *S. calotis*. The number of caudal papillae of *S. calotis* differs among reports (Table 3). This inconsistency is due to the minute papillae assembled around the terminal spike that are frequently indiscernible by light microscopy. As shown by SEM in the present study, there were three pairs of small caudal papillae, two of which formed a structure of united papillae (pocP-1/2 in Fig. 8), and a pair of phasmids around the terminal spike in male worms. The phasmids resembled other caudal papillae under light microscopy, but were readily distinguished by SEM from their protrusion from a cavity and their tips being knob-shaped and larger than the terminus of the dendritic process of caudal papillae (Fig. 8). Similarly, a pair of phasmids was observed in the female tail by both light microscopy and SEM (Fig. 9). Also using SEM, Gibbons (1986) named such structures in the female tail of *S. brevicaudata* as 'papilla-like structures'. According to Bursey et al. (2013), with the addition of *S. amazonicus* by Santos et al. (2013), 33 nominal *Strongyluris* spp. have currently been recorded worldwide. They are divided into five groups of different zoogeographical distribution, with six species being recorded from the Oriental region, namely, *S. chamaeleonis*, *S. calotis*, *S. bengalensis*, *S. karawirensis*, *S. bufonis*, and *S. japalurae* (Baylis and Daubney 1922, 1923; Chakravorty 1936; Karve 1938; Yamaguti and Mitunaga 1943; Jiang and Lin 1980). Except for *S. bufonis*, all species were recorded from lizards. Based on morphological features at the level of light microscopy, such as body size, length of esophagus or its proportion to body length, spicule length or its proportion to male body length, number and arrangement of caudal papillae of male worms, vulval position, and egg size, these nominal species were characterized as independent species by combinations of several morphological differences (Baylis and Daubney 1922, 1923; Hsü and Hoeppli 1931; Chakravorty 1936; Karve 1938; Yamaguti 1935; Yamaguti and Mitunaga 1943; Soota and Chaturvedi 1971; Jiang and Lin 1980; Hasegawa and Iwatsuki 1984; Lakshmipyari et al. 2011). For reference, the morphometrics of these *Strongyluris* spp. with Oriental distribution are summarized in Table 4. In the present study, measurements of *S. calotis* specimens collected at three different localities (one in Taiwan and two on different islands of Okinawa, Japan) (Table 3) were considerably variable by their origin or arbitrary but artificial grouping. Furthermore, microscopic observation of the arrangement and number of caudal papillae in

the obliquely truncate posterior end of male worms is often difficult and can result in varying degrees of recording accuracy. Fundamentally, however, different species or different isolates of Oriental *Strongyluris* spp. had 10 or nine pairs of caudal papillae depending on different numbers of postcloacal caudal papillae around the terminal spike, i.e. three or two pairs, in male worms. Currently, it is unclear whether previous studies excluded a pair of phasmids from postcloacal caudal papillae. As mentioned above, in contrast to SEM, under light microscopy it is very difficult to differentiate the phasmids from small-sized caudal papillae near the end of male worms (Fig. 8). Furthermore, our SEM study also demonstrated that the anterior pairs of postcloacal papillae were fused and this morphological character was consistently detected in all the *S. calotis* specimens we examined. Baylis and Daubney (1923) described *S. calotis* concisely as a new species from the rectum of *Calotes nigrilabris* in Sri Lanka in 1923 and reported 10 pairs of caudal papillae in male worms - three at the sides of the precloacal sucker and seven postcloacal - without morphological drawings. This species has been recorded from a wide spectrum of lizards in the Oriental region from the Far East to Turkey including East Asia and India (Hasegawa and Iwatsuki 1984; Goldberg et al. 2003; Yildirimhan et al. 2006). It would be of great interest to examine *S. calotis* specimens from different hosts and geographical origins to determine whether the SEM morphological characters observed in this study are consistent in all of them.

Chapter 3–2

Morphological and molecular genetic diversity of *Strongyluris calotis* (Nematoda: Ascaridida: Heterakidae) in South East and East Asian lizards

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3.2.1. Abstract

Strongyluris calotis is a heterakid nematode in the large intestine of agamid lizards (Reptilia: Sauria: Agamidae) from the Oriental Region. The standard light microscopic definition of the species counts the "caudal papillae" as 10 pairs on male worms. However, previous work from our group using scanning electron microscopy (SEM) on the heterakid from agamid lizards in Japan, Taiwan, and Singapore revealed that this counting contained a pair of phasmids and that two pairs of postcloacal papillae were completely fused to form a pair of united papillae, thus resulting in "10 pairs". In the present study, we examined *S. calotis* specimens from the Emma Gray's forest lizard, *Calotes emma* (Agamidae), living in the plain forest at low altitude, and the Vietnam false bloodsucker, *Pseudocalotes brevipes* (Agamidae), living in the mountainous forest at high altitude in the northern part of Vietnam. Using SEM, the arrangement of caudal papillae in male worms from an Emma Gray's forest lizard was found to be comparable to classical *S. calotis* specimens from agamid lizards collected in Japan, Taiwan, and Singapore. However, male worms from Vietnam false bloodsuckers did not have a pair of united papillae but had 10 pairs of independent caudal papillae with a pair of phasmids. Molecular genetic analyses of the ribosomal RNA gene (rDNA) of worms of the classical *S. calotis* morphotype from Japan and Singapore and two *S. calotis* morphotypes from Vietnam demonstrated absolutely identical nucleotide sequences of partial 18S rDNA (at least 1764 base pairs (bp)) and 5.8S rDNA (158 bp). However, intraspecific differences were detected in other regions of the rDNA, related to the geographical distribution of hosts regardless of morphotype: 97.8–98.5 % identity (443–446 bp/453 bp) in the internal transcribed spacer (ITS)-1 region, 96.6– 98.0 % identity (425–431 bp/440 bp) in the ITS-2 region, and 99.6–99.7 % identity (1149–1151 bp/1154 bp) in the 28S rDNA. Thus, in the future, taxonomic relationships of *S. calotis* distributed widely in the Oriental Region as well as other nominal Oriental *Strongyluris* spp., currently six in number, need to be extensively explored based on molecular genetic analyses in addition to intensive morphological characterization.

3.2.2. Introduction

The genus *Strongyluris* Müller, 1894 is assigned to heterakid nematodes with lips offset from the body, notable cuticular flanges extending from each lip, a posteriorly directed precloacal sucker, and an obliquely truncate tail with a short terminal spike in male worms (Inglis 1957; Skrjabin et al. 1961; Chabaud 2009a). Multiple stout pedunculated papillae support the caudal cuticular expansion, sometimes referred to as the caudal alae, of male worms. The type species of the genus is *S. brevicaudata* Müller, 1894 and currently, a few dozen species have been recorded from the large intestine of lizards and, rarely, amphibians (Yamaguti and Mitunaga 1943). Bursey et al. (2003, 2013), by adding several species since Baker's report (1984), documented 32 nominal *Strongyluris* spp. recorded worldwide: six species from the Australian zoogeographic region; 12 species from the Ethiopian; four species from the Nearctic; four species from the Neotropical; and six species from the Oriental. Subsequently, a few new species such as *S. amazonicus* from the Neotropical have been added to the list (Santos et al. 2013). Inglis (1957) specifically expressed his concern regarding whether every nominal species at that time, also included in the latest list by Bursey et al. (2013), could be differentiated from congeners based on the morphological descriptions provided by each research group. As shown in our previous study (Chapter 3–1 of the present thesis), *S. calotis* specimens collected from various sources often showed different spectra of morphometric values, causing potential difficulties with species identification. Furthermore, the number of "caudal papillae" in male worms, one of the most important characters used for morphological species characterization of the genus *Strongyluris*, recorded in previous studies may have incorrectly included phasmids present in the caudal region of male worms (Chapter 3–1 of the present thesis). In addition, our previous study (Chapter 3–1 of the present thesis) using scanning electron microscopy (SEM) found a pair of united papilla structures formed from the complete fusion of two pairs of caudal papillae in male *S. calotis* worms collected in the southern part of Japan, Taiwan, and Singapore. This finding highlights the importance of reexamining previously described *Strongyluris* spp. for clear species differentiation using more advanced techniques in addition to light microscopy. In the present study, we collected *Strongyluris* worms from two types of agamid lizards; Vietnam false bloodsuckers, *Pseudocalotes brevipes* (Werner 1904), distributed limitedly in northern Vietnam and southern China, and Emma Gray's forest lizards, *Calotes emma* (Gray 1845), distributed widely in Vietnam. We compared not only the morphology of the worms but also their ribosomal RNA gene (rDNA) nucleotide sequences in order to clarify the taxonomic

relationship of morphologically differentiated worms.

3.2.3. Materials and methods

Animals and parasitological examination

Seven Vietnam false bloodsuckers (snout-to-vent length, 56– 68 mm) were collected by hand during the period from June 2012 to September 2013 from Xuan Son National Park, Phu Tho Province (21° 12′ 25.5″ N, 104° 50′ 26.2′ E) and Kim Hy Nature Reserve, Bac Kan Province (22° 11′05″ N, 106° 03′ 45.5″ E), Vietnam, at an altitude of 420 and 460 m above sea level, respectively. Six Emma Gray's forest lizards (snout-to-vent length, 44–58 mm) were similarly collected in September 2014 from Ba Be National Park, Bac Kan Province (22° 23′ N, 105° 57′ E), living in plain forests in the northern part of Vietnam at an altitude of 230 m above sea level. Parasitological examination of lizards and morphological observation of collected nematodes were performed as described previously (Chapter 2 of the present thesis). Drawings were made with the aid of a camera lucida, partly referring to SEM photographs of the selected worms. Measurements are in millimeters (mm), with the range followed by the mean in parentheses. Nematodes were deposited in the Vietnam National Museum of Nature, VAST, Hanoi, Vietnam, under specimen nos. VNMN-2014-001-003. Twelve Ryukyu tree lizards, *Japalura polygonata ishigakiensis* (Agamidae), were collected by hand on August 2 and 4, 2015 from Ishigaki Island, Okinawa, Japan (24° 26′ 14″ N, 124° 12′ 44″ E). All 12 lizards harbored *S. calotis* worms in the large intestine, 3–11 (geomean 5.6) in number/host. A portion of the collected worms from the Ryukyu tree lizards was used for morphological examination and genetic analysis. Similarly, a male *S. calotis* worm from an Emma Gray's forest lizard caught in Singapore in 2000 by Dr. C. H. Diong, National Institute of Education, Nanyang Technological University, was used for genetic analysis. Additionally, SEM photographs of a male worm of Singapore origin are shown in the present study as a representative of the classical *S. calotis* morphotype.

Scanning electron microscopy

Individual male and female worms, stored in 70 % ethanol, were cut into three longitudinally equal parts. The anterior and posterior 1/3 parts were used for SEM. Specimens were washed three times in 0.2 M Na_2HPO4-NaH_2PO4-buffered solution (PB), pH 7.8, then immersed in 2.5% glutaraldehyde in PB overnight. Subsequent processing was similar to that described previously (Chapter 2 of the present thesis).

DNA extraction, polymerase chain reaction, and sequencing

Parasite DNA extraction was performed using the middle 1/3 portion of individual worms (the two other parts were processed for SEM observation, see above). PCR amplification of overlapping DNA fragments of the 18S to 28S rDNA was performed in a 20-µl volume as described previously (Chapter 2 of the present thesis). Primers used for PCR amplification of parasite DNA are summarized in Table 5. As a forward primer annealing to the 5′-terminus of the 18S rDNA, F-47 (5′- CCCGATTGATTCTGTCGGC-3′) was initially used; however, no DNA amplification was obtained despite repeated trials with altered annealing temperatures. Consequently, three different forward primers, *Strongyluris* 18S_4F, 11F, and 15F (see Table 5), were designed referring to the 18S rDNA sequence of *Aspidodera* sp. SAN-2007 (DDBJ/EMBL/GenBank accession no. EF180070) from the nine-banded armadillo, *Dasypus novemcinctus*, caught in Costa Rica (Nadler et al. 2007). These primers successfully amplified the 5′ portion of 18S rDNA. The 3′-terminus of a reverse primer used for an internal transcribed spacer (ITS)-1 region of the rDNA fragment, NC13 (ITS1)/R, did not match the final rDNA sequence, indicating that this primer worked as a degenerate primer lacking BT at the 3′-terminus, i.e., 5′-GCTGCGTTCTTCATCGA-3′. The PCR cycling protocol for the amplification of each rDNA fragment was 94°C for 3min, then 40 cycles of 94°C for 45s, 64°C for 1min, and 72°C for 1 min, followed by a final extension at 72°C for 7 min. PCR products for sequencing were purified using a FastGene Gel/PCR Extraction Kit (Nippon Genetics, Tokyo, Japan). The amplicon was cloned into a plasmid vector, pTA2 (Target CloneTM; TOYOBO, Osaka, Japan), and transformed into *Escherichia coli* JM109 (TOYOBO) according to the manufacturer's instructions. Following propagation, plasmid DNA was extracted using a FastGene Plasmid Mini Kit (Nippon Genetics) and inserts from multiple independent clones were sequenced using universal M13 forward and reverse primers. Sequences were assembled manually with the aid of the CLUSTAL W multiple alignment program (Thompson et al. 1994). The newly obtained rDNA sequences of *S. calotis* in the present study were deposited in the DDBJ/ EMBL/GenBank database (accession nos. LC133186– LC133190).

Phylogenetic analysis

For phylogenetic analysis, the newly obtained rDNA sequences of *S. calotis* from agamid lizards caught in Vietnam, Singapore, and Japan were aligned using the CLUSTAL W multiple alignment program and demonstrated to contain no nucleotide insertions/deletions in the sequences compared. After removal of ambiguous nucleotide positions in any sequences,

maximum likelihood (ML) analysis was performed with the program PhyML (Guindon and Gascuel 2003; Dereeper et al. 2008) provided on the "phylogeny.fr" website (http://www.phylogeny.fr/). The probability of inferred branch was assessed by the approximate likelihood ratio test, an alternative to the non-parametric bootstrap estimation of branch support (Anisimova and Gascuel 2006).

3.2.4. Results

Parasite recovery

Seven Vietnam false bloodsuckers, three from Xuan Son National Park, Phu Tho Province and four from Kim Hy Nature Reserve, Bac Kan Province, were examined. All three lizards collected at the former locality and one at the latter locality were infected with *S. calotis* in the large intestine. Infected lizards harbored one to six worms/host, and one to four male and one or two female worms were collected from individual lizards. One of six Emma Gray's forest lizards from Ba Be National Park, Bac Kan Province was infected with *S. calotis* in the large intestine.

Morphological characterization of *S. calotis* isolates

The *Strongyluris* worms were medium-sized nematodes, 8–16 mm in length, and worms of either sex had tapering anterior ends and stout posterior ends with a small terminal spike (Fig. 10). Worms had three lips offset from the body and distinct cuticular flanges extended from the upper part of the internal surface of each lip (Fig. 11). An esophagus, following a pharynx, consisted of a corpus and a tri-valved posterior bulb. Male worms had a posteriorly directed precloacal sucker, two non-alate spicules of equal length and shape, but no gubernaculum. Around an obliquely truncate tail of male worms, numerous stout pedunculated papillae appeared to support the caudal cuticular expansion. These morphological characters were coincident with the definition of the genus *Strongyluris* (Inglis 1957; Skrjabin et al. 1961; Chabaud 2009a).

Either male or female worms collected from Vietnam false bloodsuckers and an Emma Gray's forest lizard had almost identical morphological features, close to *S. calotis* except for a minor but critical difference in the number and arrangement of caudal papillae in male worms (Table 6). They had three pairs of stout pedunculated papillae ventrally on both lateral sides of the precloacal sucker, two pairs of small adcloacal papillae on both lateral sides of the

cloacal opening, and two pairs of ventrolateral papillae on both sides around the level of the cloaca. In addition, near the posterior end around the terminal spike, four pairs and three pairs of small papillae were observed by light microscopy for specimens collected from Vietnam false bloodsuckers and an Emma Gray's forest lizard, respectively (Fig. 10). By SEM, it was demonstrated that four pairs or three pairs of small terminal papillae, observed by light microscopy, included a pair of phasmids (Fig. 12). This finding is in accordance with recent work by our group (Chapter 3–1 of the present thesis). Therefore, to be precise, male specimens in Vietnam false bloodsuckers and an Emma Gray's forest lizard had three or two pairs of caudal papillae around the terminal spike, respectively. Furthermore, all terminal papillae in the specimens from Vietnam false bloodsuckers were individual ones, whereas one of two pairs of terminal papillae in the specimens from an Emma Gray's forest lizard was a united papilla structure (Fig. 12). No other critical morphological differences between male worms of the two hosts were detected by SEM. Light microscopy showed that female worms of both sources had a pair of papillae at the lateral sides of the tail; however, in line with our earlier work (Chapter 3–1 of the present thesis), SEM revealed them to be a pair of phasmids.

Molecular genetic characterization of *S. calotis* isolates

The rDNA nucleotide sequences of three isolates of *S. calotis* from Vietnam, including the two morphotypes mentioned above, one isolate from Singapore, and one isolate from Japan were successfully sequenced. Nearly complete lengths of the 18S rDNA with 1760–1771 base pairs (bp), ITS-1 region with 453 bp, 5.8S rDNA with 158 bp, ITS-2 region with 440 bp, and partial 28S rDNA with at least 1154 bp, except for the isolate from Singapore, were obtained. The rDNA nucleotide sequences of two isolates of *S. calotis* from Vietnam false bloodsuckers at Xuan Son National Park, Phu Tho Province and Kim Hy Nature Reserve, Bac Kan Province (DDBJ/EMBL/GenBank accession nos. LC133189 and LC1331909) were absolutely identical to one another except for one nucleotide in the 18S rDNA (Table 7). The rDNA nucleotide sequences of two *S. calotis* isolates from Emma Gray's forest lizards in Vietnam and Singapore, 4284 and 3025 bp, respectively, were also absolutely identical to one another as far as they were able to be compared (DDBJ/ EMBL/GenBank accession nos. LC133186 and LC133187). The *S. calotis* isolate from a Ryukyu tree lizard, 4284 bp, displayed several nucleotide substitutions from the same morphotype of *S. calotis* from Emma Gray's forest lizards in Vietnam and Singapore as well as another morphotype from Vietnam false bloodsuckers in Vietnam (Table 7).

Different isolates of *S. calotis* showing two morphotypes had absolutely identical nucleotide sequences of 18S rDNA, except for one nucleotide substitution within the same morphotype, and 5.8S rDNA. However, they showed several intraspecific differences in other regions: 97.8– 98.5% identity (443–446 bp/453 bp) in the ITS-1 region, 96.6–98.0% identity (425–431 bp/440 bp) in the ITS-2 region, and 99.6–99.7% identity (1149–1151 bp/ 1154 bp) in the 28S rDNA. The relationships of the different isolates examined in the present study are illustrated in Fig. 13.

3.2.5. Discussion

The Vietnam false bloodsucker, *P. brevipes*, has a limited distribution in the Tonkin region of northern Vietnam and Guangxi region of southern China, adjacent to Vietnam (Ananjeva et al. 2007; Nguyen VS et al. 2009). This agamid lizard is found on trees or bushes in tropical mountain forests, generally at an altitude of more than 1000 m above sea level. In addition to *P. brevipes*, the country is also populated by *Pseudocalotes microlepis* (Boulenger 1887) found widely in Vietnam, southern China, Myanmar, Laos, Thailand, and India (Assam) as well as *Pseudocalotes ziegleri* (Hallermann et al. 2010) distributed only in the mountain rainforest of central Vietnam (Kon Tum Province) (Ananjeva et al. 2007; Hallermann et al. 2010). All these agamid lizards of the genus *Pseudocalotes* are rare, with most of them known from only a few specimens (Ananjeva et al. 2007; Hallermann et al. 2010). Consequently, to date, very few of their helminth faunas have been investigated.

According to Bursey et al. (2013), with the addition of *S. amazonicus* by Santos et al. (2013), 33 nominal *Strongyluris* spp. have currently been recorded worldwide. Specifically, six species have been described from the Oriental Region, namely, *S. chamaeleonis* (Baylis and Daubney 1922), *S. calotis* (Baylis and Daubney 1923), *S. bengalensis* (Chakravorty 1936), *S. karawirensis* (Karve 1938), *S. bufonis* (Yamaguti and Mitunaga 1943), and *S. japalurae* (Jiang and Lin 1980). Except for *S. bufonis*, the five other species were recorded from lizards. Baylis and Daubney (1923) described *S. calotis* concisely as a new species from the rectum of *Calotes nigrilabris* in Sri Lanka and reported 10 pairs of caudal papillae in male worms, seven postcloacal and three at the sides of the precloacal sucker without morphological drawings. As indicated to some extent by Soota and Chaturvedi (1971) who recorded *S. calotis* from a *Calotes* sp. in India, *S. chamaeleonis*, *S. bengalensis*, and *S. karawirensis*, all of which were described from *Calotes versicolor* in India (Baylis and Daubney 1922; Chakravorty 1936; Karve 1938), have the possibility to be congeners of *S. calotis*, although

the author(s) for each species based their differentiation on morphological criteria referring to morphometric values and arrangements of caudal papillae in male worms. Difficulties with the light microscopic observation of caudal papillae, particularly those in the tail area around the terminal spike, may be an explanation for the current taxonomic complications. Although the descriptions of different *Strongyluris* spp. referred to morphometric differences as one of the critical points for species differentiation (see Table 2), *S. calotis* specimens with different origins examined in our studies (Chapter 3–1 of the present thesis, and the present study) also showed such variation, sometimes with no overlapping of some morphometric values (Table 6). All previous records before the study described in Chapter 3–1 of the present thesis may have counted the number of caudal papillae without considering the presence of phasmids in the terminal region of male and female worms of *Strongyluris* spp. due to the difficulty of differentiating the phasmids from genuine caudal papillae by light microscopy. SEM observation of *S. calotis* specimens from different sources divided them into two morphotypes: classical *S. calotis* morphotype from agamid lizards at lower altitude with a pair of united papillae in the terminal area around the spike in male worms as observed by Chapter 3–1 of the present thesis and a new *S. calotis* morphotype from Vietnam false bloodsuckers living at high altitude in Vietnam without a pair of united papillae in the aforementioned part of male worms. As shown in the previous study (Chapter 3–1 of the present thesis), *S. calotis* specimens collected in Japan (*Japalura polygonata*), Taiwan (*Japalura swinhonis*), and Singapore (*Calotes emma*) consistently demonstrated the same number and arrangement of caudal papillae in male worms beyond their remarkable morphometric variations. All of them had nine pairs of caudal papillae (three pairs of precloacal ones; two pairs of adcloacal ones; two pairs of ventrolateral ones around the level of the cloaca; and two pairs of terminal ones) with a pair of phasmids, and the first pair of terminal papillae was a structure of fused papillae. Similarly, a male *S. calotis* specimen from *Calotes emma* in the plain forest at low altitude in Vietnam showed the same morphological characteristics.

Since PCR amplification of any rDNA fragments was not possible on the *S. calotis* specimens collected in Japan and Taiwan in the previous study (Chapter 3–1 of the present thesis), possibly due to a long duration of worm preservation for nearly 30 years, new specimens were collected from Ryukyu tree lizards in the southernmost part of Japan, close to Taiwan, for genetic analysis. Intriguingly, molecular genetic analyses based on the rDNA nucleotide sequences failed to divide the *S. calotis* isolates examined in the present study into two groups of different morphotypes. Genetic distances based on the rDNA nucleotide

sequences were almost equal among isolates from agamid lizards at low altitude in South East Asia (plain forest lizards in Vietnam and Singapore), isolates from agamid lizards at high altitude in Vietnam (mountainous forest lizards in Vietnam), and an isolate from plain forest lizards in Japan (Table 7 and Fig. 13). As briefly discussed above, the taxonomic status of *Strongyluris* spp. in Oriental lizards and possibly lizards worldwide remains highly complicated. Inglis (1957) described the taxonomic state of *Strongyluris* spp. in the world as "being complicated" by the description of virtually every sample collected as a new species so that almost the only specimens available for study are types of one kind or another. Today, technical difficulties with the microscopic observation of truncated posterior ends of male worms can be surmounted by using SEM. Additionally, as in the present study, DNA sequencing can assist the taxonomic differentiation of parasites. Sampling of *Strongyluris* spp. widely in the Oriental Region and consequent intensive SEM observation and DNA sequencing following routine light microscopic observation may resolve their current taxonomic complications.

CHAPTER 4

Meteterakis spp. (Nematoda: Ascaridida: Heterakidae) in the scale-bellied tree lizard (*Acanthosaura lepidogaster)* from Vietnam, with special reference to molecular phylogenetic relationships of multiple morphotypes

4.1. Abstract

Members of the genus *Meteterakis* (Ascaridida: Heterakoidea: Heterakidae), currently 25 nominal species, are intestinal parasites of Oriental amphibians and reptiles. Although two *Meteterakis* spp., *M. striatura* from a turtle and *M. japonica* from an accidental fish host, have been recorded in Vietnam, little is known about the species and distribution of the nematode in Vietnamese hosts. In the present work, we characterized morphologically and genetically *Meteterakis* worms collected from the large intestine of the scale-bellied tree lizard, *Acanthosaura lepidogaster* (Sauria: Agamidae), in Vietnam. Based on morphological criteria, the collected *Meteterakis* specimens were divided into three morphotypes: (A) worms with alate spicules of intermediate length (0.45–0.58 mm); (B) worms with non-alate spicules of shorter length (0.33–0.49 mm); and (C) worms with non-alate spicules of longer length (0.62–0.72 mm). Morphotype A was found in 10 of 32 lizards (31.3%) from North to South Vietnam, whereas morphotypes B and C were found in 10 lizards (31.3%) and four lizards (12.5%), respectively, from the northern part of Vietnam. Genetic analyses based on the ribosomal RNA gene, i.e. the 18S, 28S, and internal transcribed spacer regions, consistently divided the collected worms into two major populations with some noticeable genetic diversity within each group, and determined morphotypes B and C as the same population. Although an apparent discrepancy between the morphological and genetic characterizations made the taxonomical differentiation of *Meteterakis* populations in the scale-bellied tree lizard from Vietnam complicated, we identified tentatively the worms of morphotype A as *Meteterakis* sp. W, and worms of morphotypes B and C as *Meteterakis* sp. N. A wider collection of *Meteterakis* specimens from Asian lizards and amphibians, and intensive morphological as well as genetic analyses may offer a solution to the complicated taxonomy of *Meteterakis* spp.

4.2. Introdution

The genus *Meteterakis* (Ascaridida: Heterakoidea: Heterakidae) was erected by Karve (1930) based on well-developed caudal alae supported by stout papillae, small sessile papillae in the caudal region, and simple spicules of equal length in male worms, in addition to three lips not offset from the body (Chabaud 2009a). The type species is *Meteterakis govindi* Karve, 1930 recorded from the bufonids, *Duttaphrynus himalayanus* (syn. *Bufo himalayanus*) and *Duttaphrynus melanostictus*. Inglis (1957) revised the genus and recognized eight valid species. Baker (1984) listed 16 valid species from amphibians and reptiles with a discussion

of their geographical distribution in the Oriental region. Zhang and Zhang (2011) listed up to 23 nominal species, and in the last few years two new species from Oriental amphibians and reptiles have been added to the list (Junker et al. 2015; Purwaningsih et al. 2015). From Vietnam, four *Meteterakis* spp. have been recorded by Oshmarin and Demshin (1972), Moravec and Sey (1988), Nguyen TM (2002), and Nguyen TM and Bui (2007): i.e. *M. striatura* (Oshmarin et Demshin 1972) emended Zhang et Zhang 2011 (syn. *M. striaturus*) from the yellow pond turtle, *Clemmys mutica* (Sauropsida: Testudinea: Geoemydidae); *M. japonica* (Wilkie 1930) from the yellowcheek, *Elopichthys bambusa* (Actinopterygii: Cypriniformers: Cyprinidae), in the northern part of Vietnam; *M. varani* (Maplestone 1931) from the , *Varanus nebulosus* (Reptilia: Squamata: Varanidae), in the northern part of Vietnam, and *M. mabuyae* Chakravarty 1944 from the long-tailed sun skink, *Eutropis longicaudata* (syn. *Mabuya longicaudata*) (Repilia: Squamata: Scincidae) in the northern part of Vietnam. The recovery of *M. japonica* from a fish was explained as an accidental infection (Moravec and Sey 1988).

During a helminthological survey of the scale-bellied tree lizard, *Acanthosaura lepidogaster* (Cuvier, 1829) (Reptilia: Squamata: Agamidae), from South to North Vietnam, 23 of 32 individuals (71.9%) were found to harbor *Meteterakis* nematodes in the large intestine, along with *Cosmocercoides tonkinensis* in 10 of the 32 lizards (31.3%) (Chapter 2). Since the collected *Meteterakis* worms showed critical variations in their morphometric values, i.e. worm sizes and spicule lengths, intensive morphological observations by light and scanning electron microscopies as well as molecular genetic analyses were conducted in the present study. We found that the ribosomal RNA gene (rDNA)-based molecular phylogenetic relationships of multiple morphotypes of *Meteterakis* specimens from the Vietnamese scale-bellied tree lizards exposed a difficulty with species differentiation based solely on morphological criteria.

4.3. Materials and Methods

Animals examined and parasitological examination

Thirty-two scale-bellied tree lizards (snout-to-vent length: 60–95 mm) were collected by hand during the period February 2012 to February 2013 in the Trung Khanh Nature Reserve (22°40'N, 106°38'E), Tam Dao National Park (21°30'N, 105°36'E), Phu Yen (21°13'N, 104°40'E), Xuan Son National Park (21°12'N, 104°50'E), Tay Yen Tu National Park (21°11'N, 106°42'E), Pu Hu National Park (20°28'N, 104°55'E), Kon Ka Kinh National Park

(14°22'N, 108°20'E), and Vinh Cuu National Park (11°23'N, 107°03'E). The localities of the lizard collection are plotted on a map (Fig. 3). Parasitological and morphological examinations using light microscopy and scanning electron microscopy (SEM) were performed as described in our previous study (Chapter 2 of the present thesis). Measurements are in millimeters (mm) unless otherwise stated, with the range followed by the mean in parentheses. Nematode specimens were deposited in the Vietnam National Museum of Nature (VNMN) and Institute of Ecology and Biological Resources (IEBR), Vietnam Academy of Science and Technology (VAST), Hanoi, Vietnam. Three male and three female specimens of *Meteterakis amamiensis* Hasegawa, 1990 from the Ryukyu short-legged skink, *Ateuchosaurus pellopleurus* (Hallowell, 1861) (Repilia: Squamata: Scincidae), were provided by Prof. Hideo Hasegawa, Oita University, Japan, and examined in a similar manner for morphological and genetic characterization to the *Meteterakis* specimens collected from the scale-bellied tree lizards.

DNA extraction, polymerase chain reaction (PCR), and sequencing

DNA extraction, PCR, and sequencing were performed as described previously (Chapter 2), except for the used primer combinations which were virtually identical to those described in Table 1 (primer combinations for segment nos. 1, 3, 4, 6, and 8) of our previous study (Chapter 3.2 of the present thesis).

Phylogenetic analysis

For phylogenetic analysis, the newly obtained 18S to 28S rDNA sequences of the *Meteterakis* specimens examined in the present study (56 nucleotide sequences will be deposited at DDBJ/EMBL/GenBank databanks) and related sequences of ascarid nematodes retrieved from the DDBJ/EMBL/GenBank databases were aligned using the CLUSTAL W multiple alignment program (Thompson et al. 1994), with subsequent manual adjustment. The accession numbers of the sequences analyzed in the present study are given in the figure showing the phylogenetic trees. Regions judged to be poorly aligned and characters with a gap in any sequences were excluded from subsequent analyses; 1,739 characters, of which 38 were variable, remained for subsequent analysis for the 18S rDNA, 1,082 characters, of which 103 were variable, remained for subsequent analysis for the 28S rDNA, and 320 characters, of which 23 were variable, remained for subsequent analysis for the internal transcribed spacer 2 (ITS-2) region. Maximum likelihood (ML) analysis was performed with the program PhyML (Guindon and Gascuel 2003; Dereeper et al. 2008) as described in our previous works (Chapters 2 and 3.2 of the present thesis).

4.4. Results

Parasite recovery

Meteterakis spp. were found in the large intestine of 23 (71.9%) scale-bellied tree lizards collected from eight localities in the northern, central, and southern parts of Vietnam (Table 8). Total worm recovery including juvenile worms from an individual host ranged from 3 to 103 (12.1 geomean). Collected worms numbered 490 in total, comprising 339 adult worms (69.2%) and 151 juvenile worms (30.8%).

Morphological observation and morphotypes

Collected adult worms had a narrowed anterior end with three lips not offset from the body and no interlabia. In the very small, funnel-shaped buccal cavity three pharyngeal teeth were found, and the beginning part of the intestine was expanded. Male worms bore well-developed caudal alae supported by stout papillae with a wide distribution of small sessile papillae on the ventral and ventrolateral parts of the caudal area. Two spicules were equal in shape and length. These morphological features were coincident with the definition of the genus *Meteterakis* after Chabaud (2009a).

According to the body size and spicule morphology of male worms, the collected specimens were divided into three morphotypes: (A) worms with alate spicules of intermediate length (0.45–0.58 mm); (B) worms with non-alate spicules of shorter length (0.33–0.49 mm); and (C) worms with non-alate spicules of longer length (0.62–0.72 mm). The morphometric values for these three morphotypes are shown in Table 9. Three representative groups of morphotype A from northern, central, and southern parts of Vietnam (localities E, G, and H, respectively; see Table 8), as well as morphotypes B and C were compared. Numerous appreciable differences between values were observed among geographical isolates or different morphotypes when assessed irrespective of the taxonomical importance of each morphometric point. Apart from spicular characters, alate or non-alate, and morphometric differences, there were very few qualitative differences in morphology among isolates or morphotypes under light microscopy (Fig. 14). Narrow lateral alae were found in both male and female worms of all three morphotypes.

By SEM on multiple representative specimens of different isolates, we tried to find any qualitative differences in the head and caudal regions of the different morphotypes with particular attention to the number and arrangement of labial and caudal papillae (Figs. 15 and 16). The anterior end had three lips not offset from the body and no interlabia, as also

observed under light microscopy (see above). A small pharyngeal tooth was situated at the center of the inner wall of each lip. The cephalic papillae and amphids on the lip of male and female worms were arranged in a similar manner as follows: a pair of large-sized cephalic papillae on the dorsal lip, with a large-sized cephalic papilla, three small-sized external labial papillae, and an amphid present on each subventral lip. Fig. 15 shows the cephalic end of each morphotype with the most prominent pattern of papilla arrangement. All observed specimens of morphotypes A and C had three independent external labial papillae on each subventral lip, whereas morphotype B frequently had a united papilla structure of two of three external labial papillae, but not all specimens followed this papilla arrangement pattern. Although three external labial papillae were always present in the external area close to a large-sized cephalic papilla and an amphid, the positioning of the three external labial papillae was variable, even within each morphotype, thus showing inter-individual variations. As shown in Fig. 16, all morphotypes had three pairs of stout papillae, two around the precloacal sucker level and one around the cloacal level, supporting caudal alae, a pair of middle-sized papillae on the anterior rim of the cloacal opening, and two pairs of middle-sized papillae at adcloacal sites, with an additional six or seven pairs of small-sized sessile papillae. The arrangement of large- and middle-sized papillae was virtually consistent among morphotypes, but the occurrence of small-sized sessile papilla pairs was capricious, often lacking a papilla on one side. Generally, the following pattern was seen: a pair of papillae in the anterior area of the precloacal sucker, two or three pairs of papillae between the precloacal sucker and cloaca, one or two pairs of papillae in the adcloacal region, and one pair of papillae on the tail, close to a pair of phasmids near the caudal tip.

Molecular phylogenetic analyses

To clarify the molecular phylogenetic relationships among the different morphotypes, nucleotide sequencing of the rDNA was conducted using 37 worms of the three *Meteterakis* morphotypes collected from scale-bellied tree lizards in Vietnam. *Meteterakis amamiensis* from a Ryukyu short-legged skink on Amami-oshima Island, Japan, was also sequenced.

The Basic Local Alignment Search Tool (BLAST) at the DNA Data Bank of Japan (DDBJ) homepage (http://ddbj.nig.ac.jp/blast/) found the partial 18S rDNA nucleotide sequences of the *Meteterakis* samples to be close to those of nematodes of Heterakidae (Ascaridida:) such as *Strongyluris calotis* (DDBJ/EMBL/GenBank accession nos. LC133186–LC133190) at 97.6% (1715 bp/1757 bp) identity, *Heterakis* sp. 14690 isolate (AF083003) at 96.6% (1706 bp/1766 bp) identity, and *Heterakis gallinarum* (DQ503462) at 95.2% (1676

bp/1761 bp) identity, followed by ascarid nematodes of other families at identities of 94% or less. Fig. 17 shows the molecular phylogenetic relationships among the different *Meteterakis* morphotypes from scale-bellied tree lizards based on the 18S, 28S, and ITS-2 regions of the rDNA. The Vietnamese *Meteterakis* isolates formed a clade distinct from *M. amamiensis*, and further divided into several small groups belonging to either group with alate or non-alate spicules, i.e. morphotype A and morphotypes B and C, respectively. Thus, monophyletic *Meteterakis* species showed morphometric variations by collection chance even when we examined fully developed adult worms (cf. morphotypes B and C).

Species determination of *Meteterakis* spp. from scale-bellied tree lizards in Vietnam

Based on a taxonomically important difference, alate or non-alate spicules, and molecular phylogenetic grouping of morphotypes, we concluded that two *Meteterakis* spp. dwelled in the large intestine of scale-bellied tree lizards in Vietnam when we interpreted some genetic variations as being intraspecific (see Fig. 17). The genus *Meteterakis* currently contains 25 nominal species, with the number of species exhibiting gubernaculums like the present specimens amounting to 14 (Inglis 1958; Skrjabin et al. 1961; Adamson 1986; Bursey et al. 2005b; Zhang and Zhang 2011; Purwaningsih et al. 2015). Considering that the present species with morphotype A had alate spicules of equal length of 0.453–0.583 mm, the closest species were *M. baylisi* Inglis, 1958 with 0.420–0.450-mm long spicules, *M. guptai* Gupta et Naiyer, 1993 with 0.490-mm long spicules, *M. paucipapillosa* Wang, 1980 with 0.400–0.416-mm long spicules, *M. sinharajensis* Crusz et Ching, 1975 with 0.344–0.451-mm long spicules, and *M. japonica* Wilkie, 1930 with 0.460–0.690-mm long spicules. Except for *M. guptai* and *M. japonica* with 13 pairs of caudal papillae, the three remaining species have distinctly more (19–23) or fewer (six) pairs of caudal papillae than the present species with 12 or 13. Furthermore, due to several distinct characters of *M. guptai*, e.g. non-alate spicules, equatorial position of the vulva, and double-sized eggs (0.120–0.150 mm by 0.080–0.090 mm), morphotype A more closely resembled *M. japonica* reported from amphibians but not from lizards. At present, howerer, we cannot clearly determine the species due to some important variations of described characters of *M. japonica* (cf. Baker 1984 vs. Purwaningsih et al. 2015), and it is the best to await molecular analyses of the rDNA nucleotide sequence of *M. japonica* reported widely in the Oriental region (Baker 1984; Hasegawa 1989; Purwaningsih et al. 2015). When morphotypes B and C were similarly differentiated from currently known *Meteterakis* spp., morphotype C rather closely resembled *M. striatura*, but not perfectly identical. Similarly, it is necessary to carry out molecular analyses of the rDNA nucleotide

sequence of *M. striatura* and other related species. Therefore, we describe two species with alate or non-alate spicultes from scale-bellied lizards from Vietnam as *Meteterakis* spp. W and N at present, respectively.

Description

Meteterakis sp. W (Ascaridida: Heterakidae)

(Figs. 14A, B, 15A, A', 16A, A'; and Table 9 [morphotype A])

General: Similar to the description for *Meteterakis* sp. N below except for no united papilla structures of two small external labial papillae.

Male (14 specimens of morphotype A): Similar to the description of *Meteterakis* sp. N below except for alate spicules and morphometric values (Table 9).

Female (12 specimens of morphotype A): Similar to the description of *Meteterakis* sp. N below except for morphometric values (Table 9).

Taxonomic summary

Deposited specimens: VNMN-2016-004 and VNMN-2014-005 in VNMN, VAST, Vietnam.

Other materials: Voucher IEBR, VAST (110 specimens), Vietnam.

Type host: *Acanthosaura lepidogaster* (Cuvier, 1829).

Locality: Kon Ka Kinh, Gia Lai Province (14˚22'N, 108˚20'E); Trung Khanh, Cao Bang Province (22˚40'N, 106˚38'E); Tam Dao, Vinh Phuc Province (21˚30'N, 105˚36'E); Tay Yen Tu, Bac Giang Province (21˚11'N, 106˚42'E); Pu Hu, Thanh Hoa Province (20˚ 28'N, 104˚55'E); and Vinh Cuu, Dong Nai Province (11˚23'N, 107˚03'E), Vietnam.

Site of infection: Large intestine.

Prevalence: 10 (31.3%) of 32 lizards examined.

Intensity of infection: 2–23 adult worms (9.1 geomean).

Remarks: A detailed discussion can be found above. The morphological characters with taxonomical significance used for species differentiation are alate spicules of equal length, the presence of gubernaculums, and the total number and arrangement of caudal papillae. Although only a few morphological variations but several noticeable deviations in morphometric values were seen among different worm isolates from distant geographical sites ranging from northern to southern Vietnam, the nucleotide sequences of the rDNA showed intraspecific diversity to a certain extent (Fig. 17). It is important to identify the reason for such genetic variations. *Meteterakis japonica* was originally described from *Bufo bufo japonicus* (syn. *Rana japonica sense* Wilkie, 1930), and Yamaguti (1941) found the species in

Bufo vulgaris japonicus from Shiga, Japan. Additionally, the same species was recorded from bufonids in Ryukyu Archipelago, Taiwan, China (Baker 1984; Hasegawa 1989), and recently from *Duttaphrynus melanostictus* (syn. *Bufo melanostictus*), *Fejervarya cancrivora*, and *Fejervarya limnocharis* from Bogor, Central Java, Indonesia (Purwaningsih et al. 2015). Some *Meteterakis* spp. such as *M. singaporensis*, *M. amamiensis*, and *M. ishikawanae* are known to be shared by sympatric amphibian and lizard hosts (Hasegawa 1990, 1992; Bursey et al. 2015a), and *M. japonica* has also been recorded from lizards such as *Eumeces latiscutatus* and *Takydromus tachydromoides* in Japan (Purwaningsih et al. 2015). Genetic confirmation of the conspecificity of *Meteterakis* sp. W. from scale-bellied tree lizards in Vietnam with *M. japonica* isolates from bufonids in Japan, Taiwan, China, and Indonesia is required. In addition, a parasitological survey of *M. japonica* in Vietnamese amphibians is also desirable.

Meteterakis sp. N (Ascaridida: Heterakidae)

(Figs. 3–14, 15B, B', 15C, C', 16B, B', 16C, C'; and Table 9 [morphotypes B and C])

General: Slight sexual dimorphism, male worms slightly smaller than female worms. Cephalic end narrowed, bearing three lips without interlabia. In addition to two tiny internal labial papillae on each lip, dorsal lip with two large-sized cephalic papillae, and each subventral lip with one large-sized cephalic papilla, three small external labial papillae, and one amphid. On occasion, two of three small external labial papillae fused to form a united papilla structure, although the combination pattern of the two of three papillae seemed to be arbitrary. Very small, funnel-shaped buccal cavity with three pharyngeal teeth, each located at the center on the internal surface of each lip. Rather narrow lateral alae on both sides. Esophagus composed of pharynx, cylindrical corpus, and bulb. Glandular tissue at beginning part of bulb after corpus.

Male (six and seven specimens of morphotypes B and C, respectively): Male worms with precloacal sucker and caudal alae supported by three large and fleshy papilla pairs; two pairs in front or marginally anterior to the precloacal sucker and one pair at the cloaca. One pair of middle-sized papillae on the anterior rim of the cloacal opening, and two pairs of middle-sized papillae in the adcloacal region, with an additional six or seven pairs of small-sized sessile papillae: one pair of papillae in the frontal area of the precloacal sucker, two or three pairs of papillae between the precloacal sucker and cloaca, one or two pairs of papillae in the adcloacal region, and one pair of papillae on the tail, close to a pair of phasmids near the caudal tip. In addition, occasional lack of or addition of caudal sessile papillae on one side of

the ventral surface. In general, 12 or 13 caudal papillae. Caudal end tapering after cloaca with sharp point, and bent ventrally. Spicules equal in length and shape, well sclerotized, non-alate, curved ventrally, and posterior-end pointed. Gubernaculum present. Morphometric values are shown in Table 9.

Female (six and seven specimens of morphotypes B and C, respectively): Vulva pre-equatorial, at anterior 40–47% of worm length. Vulva slit-like without flaps, and thick-walled muscular vagina directed posteriorly. Posterior ovary not reaching level of rectum. Eggs elliptical with thick smooth eggshell, containing single non-divided cell. Tail conical, ending with sharp point. Morphometric values are shown in Table 9.

Taxonomic summary

Deposited specimens: VNMN-2016-001–VNMN-2016-003 in VNMN, VAST, Vietnam.

Other materials: Voucher IEBR (51 specimens of morphotype B; and 126 specimens of morphotype C).

Type host: *Acanthosaura lepidogaster* (Cuvier, 1829).

Type locality: Tam Dao, Vinh Phuc Province, Vietnam (21˚30’N, 105˚36’E)

Additional localities: Phu Yen, Son La Province (21˚13’N, 104˚40’E); and Xuan Son, Phu Tho Province (21˚12’N, 104˚50’E), Vietnam.

Site of infection: Large intestine.

Prevalence: 14 (43.8%) of 32 lizards examined.

Intensity of infection: 4–52 adult worms (ca. 11 geomean), excluding juvenile worms.

Remarks: A detailed discussion can be found above. The morphological characters with taxonomical significance used for differentiation of the present species from congeners are non-alate spicules of equal length, the presence of distinct gubernaculums, and the total number and arrangement of caudal papillae. As shown in the present study, at least two morphotypes of different sizes or morphometric values are easily isolated by arbitrary selection of examined worms by locality. Nucleotide sequencing of the rDNA appears to be critical for reliable species identification.

4.5. Discussion

The genus *Meteterakis* contains 25 nominal species from Oriental amphibians and reptiles (Inglis 1958; Skrjabin et al. 1961; Bursey et al. 2005b; Zhang and Zhang 2011; Junker

et al. 2015; Purwaningsih et al. 2015). As mentioned above, *M. japonica* from a yellow cheek and *M. striatura* from the yellow pond turtle have been recorded in Vietnam, although the former record is considered to be an accidental infection (Oshmarin and Demshin 1972; Moravec and Sey 1988). In addition, *M. varani* from the clouded monitor and *M. mabuyae* from the long-tailed sun skink were recorded in the northern part of Vietnam. Morphotype A of *Meteterakis* spp. examined in the present study, tentatively identified as *Meteterakis* sp. W, showed wide geographical distribution in Vietnam from the northern to southern parts of the country, and closely resembled *M. japonica*, particularly the species sensu Purwaningsih et al. (2015). Considering a wide geographical distribution of *M. japonica* from Japan to Indonesia in various bufonids, it is highly possible for the species to be distributed in Vietnam. Indeed, Moravec and Sey (1988) recorded the spcecies from an accidental fish host. Similarly, it is highly possible that *M. japonica* takes not only bufonids but also lizards as natural hosts, like occurrence of *M. amamiensis* and *M. ishikawanae* in both sympatric amphibian and lizard hosts (Hasegawa 1990, 1992). This speculation, however, needs confirmation by modern techniques such as nucleotide sequencing of the rDNA or other genes as well as parasitological surveys of the parasite in Vietnamese amphibians. In the present study, we obtained long sequences of the rDNA from 18S to 28S regions of *Meteterakis* sp. W from scale-bellied lizards in Vietnam. It is intriguing to clarify the molecular phylogenetic relationships among *M. japonica* isolates as well as its closest congeners collected from different hosts and/or distant localities widely in the Oriental region.

Two distinct morphotypes B and C of *Meteterakis* sp. N in the present study showed numerous morphometric differences including spicule length (0.33—0.49 mm vs. 0.62—0.72 mm). Then we considered a possibility of two different species for them at beginning. Molecular phylogenetic characterization of multiple isolates from different host individuals or different localities using the 18S rDNA, 28S rDNA and ITS region, however, demonstrated a conspecificity of morphotypes B and C (Fig. 17). Irrespective of morphotypes or localities of host collection, there were noticeable nucleotide variations within each morphotypes or *Meteterakis* sp. N, as seen in morphotype A (Fig. 17). This genetic diversity of a single species, which has been observed sometimes in lizard nematodes (see Chapter 3.2 in the present thesis) needs further investigation to explain the evolutionary background.

As demonstrated partially in the present study and our previous study on *Strongyluris calotis* (Chapter 3.2 in the present thesis), species description or identification of heterakid nematodes with numerous caudal papillae as well as multiple cephalic papillae are often difficult due to substantial variation of numbers and arrangements of papillae, particularly

when microscopic observation is applied to a limited number of worms for species characterization. We cited a special mention of Inglis (1957) in the previous study on *Strongyluris calotis* (Chapter 3.2 in the present thesis) to introduce the current and historical complicated status of taxonomy of lizard heterakids; 'The taxonomic state of *Strongyluris* spp. in the world was being complicated by the description of virtually every sample collected as a new species so that almost the only specimens available for study are types of one kind or another'. Although morphometric values for morphotype B and C in the present study were rather distinct, we could not find critical qualitative differences. This problem was given a brilliant solution by molecular genetic data which showed conspecificity of morphotypes B and C (Fig. 17). Therefore, not only at new species description but also records of known species, it is recommended to attach molecular genetic data to clarify the relationships among a large number of species often with minor interspecific differences in morphometric values, host categories, or geographical distribution. This approach may disclose an extent of intraspecific morphological variation which is important to make a species identification based on morphological criteria. At the same time, it is highly possible that molecular genetic analyses on sufficient numbers of specimens may disclose the presence of cryptic species or special geographical lineages within a species.

Chapter 5

A list of endoparasites of Vietnamese lizards recorded in the last 50 years (1966–2015)

This work described in the chapter has been published as follows:
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5.1. Abstract

At present, there is a limited knowledge of amphibian and reptile parasites in Vietnam. To date, 45 species of endoparasite in Vietnamese lizards have been recorded. These species consist of 11 cestode, 12 trematode, 18 nematode, one acanthocephalan and three pentastomid species from 10 host species. As Vietnam is one of the global hot spots for herpetofauna diversity (a recent report documented 385 reptiles and 181 amphibians in the country), it appears that only a fraction of the parasites of lizards in this richly biodiverse territory has been recorded. To facilitate the accurate taxonomical identification of parasites and clarify the taxonomic relationship of parasites from Vietnamese lizards with those from Oriental lizards or lizards of other geographical regions, parasites should be characterized both morphologically and phylogenetically.

5.2. Introduction

Vietnam is one of the global hot spots for reptilian and amphibian diversity. Nguyen VS and Ho (1996) recorded 258 reptiles and 82 amphibians as the herpetofauna of Vietnam. More recent active surveys on the herpetofauna in the country by Vietnamese herpetologists and collaborative overseas scientists have disclosed more and more species. Nguyen VS et al. (2009) recorded 368 reptiles and 177 amphibians, while Ziegler and Nguyen TQ (2010) reported 385 reptiles and 181 amphibians. The lizard group, such as the agamids, geckos and skinks, currently comprises a total of more than 120 species, of which at least 57 species were described during the period 1996 to 2010 (Nguyen QT 2011; Nguyen VS and Ho, 1996; Nguyen VS et al. 2005, 2009; Ziegler and Nguyen TQ, 2010). The major reptile families in Vietnam are Gekkonidae (42 species) and Scincidae (46 species).

In contrast to the active research on herpetofauna or lizard fauna, little is known about the parasites of lizards endemic in Vietnam. To date, parasitological studies have been conducted on only 10 host species, with 45 endoparasite species being found, of which 80.0% (36 species) were parasites of the spiny-tailed house gecko *Hemidactylus frenatus* (Schlegel, 1836), clouded monitor *Varanus nebulosus* (Gray, 1831) and water monitor *Varanus salvator* (Laurenti, 1768). Due to multiple records being published in Vietnamese or in domestic conference books in Vietnam, overseas researchers outside of Vietnam may experience difficulties accessing them. To address this, the present study lists the parasite records from Vietnamese lizards for the benefit of researchers interested in this topic.

5.3. Vietnamese lizards examined for their endoparasites

We surveyed conference records and scientific publications in domestic and international journals. According to these sources, parasitological surveys on Vietnamese lizards were often conducted at several places in the Red River Delta, where the Red River flows from Yunnan in southwest China through northern Vietnam to the Gulf of Tonkin, and mountainous provinces of central and southern regions of Vietnam as well as unknown places in the southern region (Fig. 18). We found 45 parasite species recorded from 10 host lizard species, and more than 360 host individuals were examined by Vietnamese researchers as well as their overseas collaborators (Table 10). These host lizards were classified into four families (Table 10 and Fig. 19): Varanidae (*Varanus nebulosus* and *Varanus salvator*); Gekkonidae (*Gekko badenii* and *Hemidactylus frenatus*); Scincidae (*Eutropis longicaudata*); and Agamidae (*Acanthosaura lepidogaster*, *Calotes emma*, *Calotes versicolor*, *Pseudocalotes brevipes* and *Leiolepis reevesii*).

5.4. Parasites recorded in Vietnamese lizards

As mentioned above, 45 species parasitic to lizards were recorded in Vietnam. These species comprised 11 cestodes, 12 trematodes, 18 nematodes, one acanthocephalan and three pentastomids (Le and Nguyen VL 1966; Ali et al. 1981; Nguyen TM 2002; Nguyen TL et al. 2003, 2005, 2008; Pham and Nguyen TL 2003, 2007; Nguyen TL and Pham 2005; Tran et al. 2005, 2007, 2015a,b, 2016; Nguyen TM and Bui 2007; Amin et al. 2008; Bui et al. 2009; Tran 2009; Nguyen TL and Ha 2010; Vlnová 2014). Among them, six species were described as new to science at the time of publication: *Abbreviata deschiensi* Le et Nguyen, 1966; *Pharyngodon duci* Tran et al., 2007; *Spauligodon vietnamensis* Tran et al., 2007; *Thelandros vietnamensis* Bui et al., 2009; *Cosmocercoides tonkinensis* Tran et al., 2015; and *Pseudoacanthocephalus nguyenthileae* Amin et al., 2008. In the following subsections, localities, when defined, are shown by the name of the province and the number plotted on the map (Fig. 18) in parentheses. The incidence (prevalence) and intensity are shown for parasite species where these data were provided, although it was found that this information was generally missing from the Vietnamese reports.

5.4.1. Cestoda

Eleven recorded species were classified into three families: Anoplocephalidae Cholodkowsky, 1902 (*Oochoristica* Lühe, 1898 – four spp.); Proteocephalidae La Rue, 1911 (*Acanthotaenia* von Linstow, 1903 – four spp. and *Kapsulotaenia* Freze, 1965 – one sp.); and Diphyllobothriidae Lühe, 1910 (*Duthiersia* Perrier, 1873 – one sp. and *Scyphocephalus* Riggenbach, 1898 – one sp.).

5.4.1.1. *Oochoristica chinensis* Jensen, Schmidt et Kuntz, 1983

Host and location: *Hemidactylus frenatus*, small intestine (Nguyen TL et al. 2005; Tran et al. 2005)

Locality: Yen Bai Province (5); additionally, Hanoi Province (14) [unpublished]

Incidence and intensity: 4.7% (7/149) with 1–5 worms/host

Comment: Anoplocephalid cestodes of the genus *Oochoristica*, ca. 80 species at present, are cosmopolitan in distribution and predominantly parasitize lizards, but also snakes, turtles and marsupials (Criscione and Font 2001). The present species was recorded from *Japalura swinhonis* (the type host) and *Eutropis longicaudata* (syn. *Mabuya longicaudata*) in Taiwan (Jensen et al. 1983; Norval et al. 2014). The morphological features of the isolate from common house geckos (*H. frenatus*) in Vietnam corresponded well with the original description (Jensen et al. 1983).

Since assumptions of strict host specificity and geographical isolation had apparently been used as criteria in determining species of this genus, Criscione and Font (2001) conducted an experimental infection of *Oochoristica javaensis* of lizard hosts distributed in a non-endemic region of the cestode and concluded that members of the *Oochoristica* may lack strict host specificity.

5.4.1.2. *Oochoristica tuberculata* (Rudolphi, 1819) Lühe, 1898

Syn. *Skrjabinochora sobolevi* Spasskii, 1948

Host and location: *Hemidactylus frenatus*, small intestine (Nguyen TL et al. 2005; Tran et al. 2005)

Locality: Yen Bai Province (5); additionally, Hanoi Province (14) [unpublished]

Incidence and intensity: 2.7% (4/149) with 1–3 worms/host

Comment: This cestode is the type species of the genus and is distributed widely in Eurasia and northern Africa (Palaearctic region) by parasitism of a variety of lizards (at least

31 species of 23 genera) as well as snakes (nine species of nine genera) (Yildirimhan et al. 2011; Dugarov et al. 2012). This species recorded from common house geckos in Vietnam had 28–30 testes per segment, different from the aforementioned *O. chinensis* with 17–22 testes per segment.

5.4.1.3. *Oochoristica* sp. 1

Host and location: *Hemidactylus frenatus*, small intestine (Nguyen TL et al. 2005; Tran et al. 2005)

Locality: Yen Bai Province (5)

Incidence and intensity: 1.3% (2/149) with 1–3 worms/host

Comment: This cestode was small in size (7–20 mm in length and 1.3 mm in width) and had 20–24 testes per segment and a small cirrus sac extending 16–20% of the width of the mature segment. The cirrus sacs of the two former species, *O. chinensis* and *O. tuberculata*, extended 25–30% and 18–20% of the segment width, respectively. Although morphological characters were recorded in detail, the exact taxonomic situation of this species was uncertain (Tran et al. 2005).

5.4.1.4. *Oochoristica* sp. 2

Host and location: *Hemidactylus frenatus*, small intestine (Nguyen TL et al. 2005; Tran et al. 2005)

Locality: Yen Bai Province (5)

Incidence and intensity: 2.0% (3/149) with no intensity information

Comment: This cestode was small in size (7–9 mm in length and 0.3 mm in width) and had 12–14 testes per segment and a cirrus sac extending 50–58% of the width of the mature segment. Although morphological characters were recorded in detail, the exact taxonomic situation of this species was uncertain (Tran et al. 2005).

In addition, Nguyen TL et al. (2005) recorded another *Oochoristica* sp. from *Eutropis longicaudata* at the same localities in northern Vietnam. This species was different from the four aforementioned *Oochoristica* spp. from *H. frenatus*. No detailed information is available.

5.4.1.5. *Acanthotaenia shipleyi* von Linstow, 1903

Host and location: *Varanus nebulosus*, small intestine (Tran 2009)

Locality: Southern Vietnam (not specified)

Incidence and intensity: 17.4% (4/23) with no intensity information

Comment: Members of the genus *Acanthotaenia* have a scolex with an apical muscular organ (piercing organ). The scolex and anterior part of the strobila are covered with a dense network of spines (Rego 1994). The uterus has numerous, irregular diverticula. *Acanthotaenia* spp. are parasitic to varanid reptiles in Africa, Australia and the Indo-Pacific region (Yamagiti 1954; Rego 1994), and *A. shipleyi* collected from *Varanus salvator* in Sri Lanka is the type species of the genus. Since the original description of the species was made using immature worms, Yamaguti (1952) redescribed it using mature cestodes collected from *Varanus salvator* on Sulawesi Island, formerly known as Celebes, Indonesia.

Recently, de Chambrier et al. (2015) conducted phylogenetic analyses of proteocephalid cestodes (110 taxa of 54 genera classified in all 13 currently recognized subfamilies, including *A. shipleyi*) using almost complete 28S ribosomal RNA gene (rDNA) nucleotide sequences. Their findings led them to propose a need to revise the systematics of the family based on phylogenetic achievements or newly defined morphological characters suitable for the division of subgroups.

5.4.1.6. *Acanthotaenia beddardi* (Woodland, 1925) Schmidt et Kuntz, 1974

Host and location: *Varanus nebulosus* and *Varanus salvator*, small intestine (Tran 2009)

Locality: Vietnam (not specified)

Incidence and intensity: 18.6% (8/43) with no intensity information

Comment: This species was originally described in *Varanus bengalensis* in India. It was defined as having 60–77 testes per segment and a uterus with 15–20 lateral branches on each side, whereas the aforementioned species, *A. shipleyi*, had 40–65 testes per segment and a uterus with 29–36 lateral branches.

5.4.1.7. *Acanthotaenia nilotica* Beddard, 1913

Host and location: *Varanus nebulosus* and *Varanus salvator*, small intestine (Tran 2009)

Locality: Vietnam (not specified)

Incidence and intensity: 27.9% (12/43) with no intensity information

Comment: This species was originally described in *Varanus niloticus* from Africa, having 75–92 testes per segment and a uterus with 17–25 lateral branches on each side.

5.4.1.8. *Acanthotaenia* sp.

Host and location: *Varanus nebulosus* and *Varanus salvator*, small intestine (Tran 2009)

Locality: Vietnam (not specified)

Incidence and intensity: 14.0% (6/43) with no intensity information

Comment: This species was characterized to have 150–195 testes per segment and a uterus with 25–52 lateral branches on each side, distinct from any known *Acanthotaenia* spp.

5.4.1.9. *Kapsulotaenia sandgroundi* (Carter, 1943) Freze, 1965

Syn. *Proteocephalus sandgroundi* Carter, 1943

Host and location: *Varanus salvator*, small intestine (Tran 2009)

Locality: Northern Vietnam (not specified)

Incidence and intensity: 15.0% (3/20) with no intensity information

Comment: The genera *Kapsulotaenia* and *Acanthotaenia* are closely related, although formation of membranous egg capsules only occurs in the former genus (Rego 1994). The type species of the genus is the present species, which was fully redescribed by de Chambrier (2006) on the basis of the type specimen from *Varanus komodoensis* on Komodo Island, Indonesia, and museum materials from varanid lizards on the Lesser Sunda Islands, Indonesia, and Australia.

5.4.1.10. *Duthiersia expansa* Perrier, 1873

Syns. *Duthiersia crassa* Woodland, 1938; *Duthiersia venusta* Woodland, 1938

Host and location: *Varanus nebulosus* and *Varanus salvator*, small intestine (Tran 2009)

Locality: Vietnam (not specified)

Incidence and intensity: 18.6% (8/43) with no intensity information

Comment: Members of the genus *Duthiersia* are small worms from varanid lizards, not exceeding 200 mm in length, and having a broad and fan-like scolex with bothrial margins frilled or crenulated (Bray et al. 1994). Woodland (1940) examined numerous specimens from varanids in Africa and Asia, and divided them into *D. fimbriata* (Diesing, 1854) and *D. expansa* Perrier, 1873, respectively, with the suppression of *D. elegans* Perrier, 1873, *D. robusta* Woodland, 1938, and *D. latissima* Woodland, 1938, as junior synonyms of *D. fimbriata*, and similarly *D. crassa* Woodland, 1938, and *D. venusta* Woodland, 1938, as junior synonyms of *D. expansa*. Woodland (1940) indicated that the critical differences between these two species, or African and Asian forms, were few except for the shapes of scoleces and the absence or presence of posterior pore openings of the bothrial grooves. Current taxonomy follows his division. Along with the aforementioned *D. expansa* specimens, Tran (2009) reported the collection of '*D. fimbriata*' from Vietnamese varanids at an

incidence of 16.3% (7/43). Since the basis for the separation between these two species was not shown in the work (Tran 2009), the present study omits this record.

The Asian form, i.e. *D. expansa*, is the type species of the genus. It is commonly found in a variety of Asian *Varanus* spp. (*V. bengalensis*, *V. flavescens*, *V. komodoensis*, *V. marmoratus*, *V. nebulosus*, *V. nuchalis*, *V. salvator* and *V. salvadorii*) or *Iguana* sp. and *Cyclura stejnegeri* (Iguanidae) in Indonesia, Thailand, Philippines, China, Malaysia, India, Sri Lanka, Pakistan and Afghanistan (Yamaguti 1954).

5.4.1.11. *Scyphocephalus bisulcatus* Riggenbach, 1898

Syns. *Scyphocephalus secundus* Tubangui, 1968; *Scyphocephalus longus* Sawada et Kugi, 1973

Host and location: *Varanus salvator*, small intestine (Vlnová 2014)

Locality: Nghe An Province (27)

Incidence and intensity: No information

Comment: The genus *Scyphocephalus* is characterized by a special scolex with rudimentary bothria and an invaginated anterior end forming a sucking organ (Bray et al. 1994). This cestode is the type species of the genus, described based on worms from *Varanus salvator* in Java, Indonesia (Riggenbach 1898). As detailed above, *S. secundus* from *Varanus salvator* on Leyte Island, Philippines (Tubangui 1938) was synonymized by Vlnová (2014). '*Scyphocephalus jadhavi*' described from the same host species in Andhra Pradesh, India (Kalyankar and Nanware 2010) appears to be a species of a distinct genus.

5.4.2. Trematoda

Twelve recorded species were classified into 10 families: Plagiorchidae Lühe, 1901 (*Plagiorchis* Lühe, 1899 – one sp.); Encyclometridae Mehra, 1931 (*Encyclometra* Baylis et Cannon, 1924 – one sp.); Philophthalmidae Looss, 1899 (*Singhiatrema* Simha, 1954 – one sp.); Dicrocoeliidae Odhner, 1911 (*Euparadistomum* Tubangui, 1931 – one sp. and *Paradistomum* Kossak, 1910 – one sp.); Phaneropsolidae Mehra, 1935 (*Parabascus* Looss, 1907 – one sp. and *Postorchigenes* Tubangui, 1928 – one sp.); Diplodiscidae Cohn, 1904 (*Diplodiscus* Diesing, 1836 – one sp.); Mesocoeliidae Dollfus, 1929 (*Mesocoelium* Odhner, 1910 – one sp.); Meristocotylidae Fischthal et Kuntz, 1964 (*Meristocotyle* Fischthal et Kuntz, 1964 – one sp.); Heterophyidae Leiper, 1909 (*Haplorchis* Looss, 1899 – one sp.); and Echinostomatidae Dietz, 1909 (*Testisacculus* Bhalerao, 1927 – one sp.).

5.4.2.1. *Plagiorchis molini* Lent et Freitas, 1946

Host and location: *Hemidactylus frenatus*, intestine (Pham and Nguyen TL 2007)

Locality: Quang Tri Province (30)

Incidence and intensity: 3.0% (1/33) with 3 worms/host

Comment: This plagiorchid species is often found in lacertid lizards such as sand lizards (*Lacerta agilis*) and common wall lizards (*Podarcis muralis*) distributed widely in Europe (Lewin 1992a, b). Okulewicz et al. (2015) found *P. molini* in a Chinese water dragon (*Physignathus cocincinus*) imported into Poland, suggesting that this species is also distributed in China and Southeast Asia. *Plagiorchis elegans* (Rudolphi, 1802) Braun, 1902, has also been reported from lacertid lizards (Yildirimhan et al. 2011).

5.4.2.2. *Encyclometra colubrimurorum* (Rudolphi, 1819) Dollfus, 1929

Host and location: *Varanus nebulosus* and *Varanus salvator*, oesophagus and intestine (Pham and Nguyen 2003)

Locality: Hanoi Province (14) and southern Vietnam (not specified)

Incidence and intensity: No information

Comment: Members of the genus *Encyclometra* are trematodes that dwell in the oesophagus, stomach and intestine of snakes in Eurasia (Tkach 2008). Gupta and Mehrotra (1977) differentiated three valid species: *E. colubrimurorum* (testes tandem or obliquely tandem, with equal intestinal caeca); *E. bungara* Srivastava et Ghosh, 1968 (testes almost symmetrically placed); and *E. asymmetrica* Wallace, 1936 (testes tandem or obliquely tandem, with very unequal intestinal caeca). *Encyclometra japonica* Yoshida et Ozaki, 1929, and *E. vitellata* Gupta, 1954, were synonymized with *E. colubrimurorum* and *E. asymmetrica*, respectively (Gupta and Mehrotra 1977). Metacercariae of *E. colubrimurorum* are often found in amphibians (Amin et al. 2012). In Vietnam, this encyclometrid species was found in snakes (*Xenopeltis unicolor* and *Xenochrophis piscator*), as reported in various snakes (*Ptyas mucosus*, *Natrix natrix* and *Natrix piscator*) from other Eurasian areas (Ivanov and Semenova 2000).

5.4.2.3. *Singhiatrema vietnamensis* Curran et al., 2001

Host and location: *Varanus nebulosus* and *Varanus salvator*, oesophagus and intestine (Pham and Nguyen 2003)

Locality: Hanoi Province (14) and southern Vietnam (not specified)

Incidence and intensity: No information

Comment: This species was originally described from the small intestine of Chinese water snakes (*Enhydris chinensis*) and rice paddy snakes (*Enhydris plumbea*) in Vietnam (Curran et al. 2001). It also parasitizes other snakes such as the Taiwan cobra (*Naja atra*), Chinese ratsnake (*Ptyas korros*) and banded krait (*Bungarus fasciatus*). Members of the genus have pyriform bodies with a head-collar with a row of spines, interrupted dorsally (Kanev et al. 2005). A well-developed ventral sucker is located centrally and the intestinal caeca end at the posterior margin of the ventral sucker.

5.4.2.4. *Euparadistomum varani* Tubangui, 1931

Host and location: *Varanus salvator*, gall bladder (Pham and Nguyen 2003)

Locality: Hanoi Province (14)

Incidence and intensity: No information

Comment: This discoid dicrocoeliid species with almost full occupation of the body by uterine coils and two testes situated symmetrically at anterolateral positions to the acetabulum was originally described in *Varanus salvator* from the Philippines, then other places in Southeast Asia, Pacific Islands and Madagascar (Capron et al. 1961; Fischthal and Kuntz, 1964). In Vietnam, this species was found in the Asian water monitor (*V. salvator*) in 2003 (Pham and Nguyen 2003), and more recently in the Asian house shrew (*Suncus murinus*) (Ngo et al. 2010).

5.4.2.5. *Paradistomum orientalis* (Narain et Das, 1929) Bhalerao, 1936

Syns. *Dicrocoelium orientale* Narain et Das, 1929; *Para- distomoides orientale* (Narain et Das, 1929) Yamaguti, 1958

Host and location: *Eutropis longicaudata* and *Hemidactylus frenatus*, gall bladder (Nguyen TL and Pham 2005; Nguyen TL et al. 2005)

Locality: Hanoi Province (14) and Yen Bai Province (5)

Incidence and intensity: No information

Comment: This oval dicrocoeliid species is a common trematode in the gall bladder of various lizards including *Calotes versicolor* and *Hemidactylus flaviviridis* in India. Examining *C. versicolor* in India, Arora and colleagues (Arora and Agarwal 1960; Arora et al. 1962) found a high prevalence (67/74) of the species in the gall bladder, and from their studies on the intraspecific variations of the specimens concluded several described species to be junior synonyms of *P. orientalis*. Studying the monthly population dynamics of *P. orientalis* in *C.*

versicolor in India, Madhavi et al. (1998) reported that the frequency distribution of the fluke in the host followed the overdispersion pattern and that crowding effects serve as a major regulatory force for maintaining the equilibrium of parasite densities throughout the year. Killick and Beverley-Burton (1982) made a taxonomical evaluation of 21 nominal *Paradistomum* spp. described from Southeast Asian lizards.

5.4.2.6. *Parabascus lepidotus* Looss, 1907

Host and location: *Hemidactylus frenatus*, intestine (Nguyen TL and Pham 2005; Nguyen TL et al. 2005)

Locality: Hanoi Province (14) and Yen Bai Province (5)

Incidence and intensity: No information

Comment: This trematode is the type species of the genus, originally described from *Pipistrellus kuhlii* (syn. *Vesperugo kuhlii*; Chiroptera: Vespertilionidae) in Cairo, Egypt (Lotz and Font 2008). This Kuhl's pipistrelle bat is distributed widely around Mediterranean regions (southern Europe and northern Africa) to India, and there are records of this trematode in the regions with the host distribution.

5.4.2.7. *Postorchigenes ovatus* Tubangui, 1928

Syn. *Parabascus ovatus* (Tubangui, 1928) sensu Nguyen et Pham, 2005

Host and location: *Hemidactylus frenatus*, intestine (Oshmarin and Demshin 1972; Nguyen TL and Pham 2005; Nguyen TL et al. 2005)

Locality: Yen Bai Province (5)

Incidence and intensity: No information

Comment: This oval trematode is the type species of the genus, originally described from *Hemidactylus frenatus* in the Philippines (Yamaguti 1958; Lotz and Font 2008). This species is one of the very common trematodes of geckos in Southeast Asian countries such as Indonesia, Thailand, Vietnam and Philippines (Killick and Beverley-Burton 1982; Matsuo and Oku 2002; Barton 2015).

5.4.2.8. *Diplodiscus mehrai* Pande, 1937

Host and location: *Varanus salvator*, intestine (Pham and Nguyen 2003)

Locality: Hanoi Province (14)

Incidence and intensity: No information

Comment: This species was originally described from *Euphlyctis cyanophlyctis* (syn. *Rana cyanophlyctis*) in India (Yamaguti 1958), followed by records in various frogs in India (Imkongwapang et al. 2014). Similarly in Vietnam, this trematode was found in various amphibians including *Fejervarya limnocharis* (syn. *Rana limnocharis*), *Duttaphrynus melanostictus* (syn. *Bufo melanostictus*), *Hylarana nigrovittata*, *Quasipaa verrucospinosa* and *Paramesotriton deloustali* (Sey 1985; Nguyen et al. 2008). The family Diplodiscidae is a small group of paramphistomoids found predominantly in amphibians, but also in reptiles and fish, and characterized by a single testis and a ventroterminal sucker (Jones 2005).

5.4.2.9. *Mesocoelium brevicaecum* Ochi, 1930

Syn. *Mesocoelium pearsei* sensu Pham et Nguyen, 2003

Host and location: *Varanus nebulosus* and *Varanus salvator*, small intestine (Nguyen TL and Pham 2005; Pham and Nguyen TL 2003)

Locality: Hanoi Province (14) and southern Vietnam (not specified)

Incidence and intensity: No information

Comment: Pham and Nguyen (2003) collected a small-sized oval mesocoeliid species from the small intestine of varanid lizards in Vietnam and identified it as *Mesocoelium pearsei* Goto et Ozaki, 1930. Subsequently, however, Nguyen and Ha (2010) reidentified it as *M. brevicaecum*. This species was originally described from the small intestine of various amphibians in Japan, such as *Bufo japonicus* (syn. *Bufo vulgaris japonicus*), *Pelophylax nigromaculatus* (syn. *Rana nigromaculata*), *Glandirana rugosa* (syn. *Rana rugosa*), *Lithobates catesbeianus* (syn. *Rana catesbeiana*), *Elaphe quadrivirgata* and *Plestiodon latiscutatus* (syn. *Eumeces latiscutatus*) (Ochi 1930). Similarly, the trematode was found in *Duttaphrynus melanostictus* and *Hylarana guentheri* (syn. *Rana guentheri*) from Vietnam and *D. melanostictus* from Taiwan (Dronen et al. 2012). As too many species in the genus *Mesocoelium* have been described, Dronen et al. (2012) recently undertook their evaluation based on intensive morphological criteria and consequently proposed synonymies of multiple species.

5.4.2.10. *Meristocotyle provitellaria* Liu et al., 2002

Syn. *Meristocotyle* sp. sensu Pham et Nguyen, 2003

Host and location: *Varanus salvator*, small intestine (Pham and Nguyen TL 2003)

Locality: Hanoi Province (14)

Incidence and intensity: No information

Comment: Fischthal and Kuntz (1964) erected a new genus for their new species *Meristocotyle varani* from *Varanus salvator* in the Philippines. Trematodes of this genus are characterized by the unusual bipartite nature of the ventral sucker; in other words, a horizontally divided ventral sucker with two lumina. Later, Liu et al. (2002) described the second species, *M. provitellaria*, from the same host species in China. The morphological characteristics of *Meristocotyle* sp. from *V. salvator* in Vietnam provided by Pham and Nguyen (2003) coincided well with those of *M. provitellaria* detailed by Liu et al. (2002), whereas they were less coincident with those of *M. varani* reported by Fischthal and Kuntz (1964). These two studies from the Philippines and China documented that worms were collected from the lungs in addition to the intestine.

5.4.2.11. *Haplorchis pumilio* Looss, 1896

Host and location: *Varanus nebulosus* and *Varanus salvator*, small intestine (Pham and Nguyen TL 2003)

Locality: Hanoi Province (14) and southern Vietnam (not specified)

Incidence and intensity: No information

Comment: In Southeast Asia including Vietnam, human infections with fishborne zoonotic intestinal trematodes have been increasingly reported in the last two decades, with one of the major causes being *Haplorchis pumilio* (Trung Dung et al. 2007). This species is common not only in humans but also in mammals, birds and varanid lizards through the consumption of raw freshwater fish (Sommerville 1982; Lan Anh et al. 2009). The original description was made using specimens from white pelicans (*Pelecanus onocrotalus*) in Egypt (Yamaguti 1958).

5.4.2.12. *Artyfechinostomum sufrartyfex* Lane, 1915

Syns. *Testisacculus indicus* Bhalerao, 1931; *Artyfechinostomum varanum* Simha et Deshpande, 1964; *Testisacculus indicus* sensu Pham et Nguyen, 2003

Host and location: *Varanus nebulosus*, small intestine (Pham and Nguyen TL 2003)

Locality: Southern Vietnam (not specified)

Incidence and intensity: No information

Comment: Echinostomatid trematodes of the genus *Artyfechinostomum* Lane, 1915 (syn. *Testisacculus* Bhalerao, 1927) are characterized by a spinose tegument with very large scale-like spines comparable in size to collar-spines, dorsal collar-spines in double rows, and deeply branched testes (Kostadinova 2005). The species was originally described using specimens

from the duodenum of the Indian spiny-tailed lizard *Saara hardwickii* (syn. *Uromastyx hardwickii*) in India (Bhalerao 1931; Yamaguti 1958). Simha and Deshpande (1964) collected a dozen specimens from the intestine of a single *Varanus bengalensis* and described a new species, namely *Artyfechinostomum varanum*. Bhardwaj (1963) collected and examined two specimens from *Varanus bengalensis* in Jabalpur, India, and erected a new genus and species, *Pseudoartyfechinostomum larueiformis*. Premvati and Pande (1974) synonymized all described species under the genera *Artyfechinostomum*, *Neoartyfechinostomum* and *Pseudoartyfechinostomum*, and retained only *A. malayanum* (Leiper, 1911) Mendheim, 1943. Kostadinova (2005) essentially followed their revision, but elected *A. sufrartyfex* Lane, 1915 (syn. *A. malayanum*) as the type species of the genus. Based on these interpretations, we decided to replace the name of the parasites '*Testisacculus indicus*' collected by Pham and Nguyen (2003) with *A. sufrartyfex* in this article.

5.4.3. Nematoda

Eighteen recorded species were classified into 12 families: Cosmocercidae Travassos, 1925 (*Cosmocercoides* Wilkie, 1930 – one sp.); Strongyloididae Chitwood et McIntosh, 1934 (*Strongyloides* Grassi, 1879 – one sp.); Diaphanocephalidae Travassos, 1920 (*Kalicephalus* Molin, 1861 – one sp.); Molineidae (Skrjabin et Schulz, 1937, subfam.) Durette-Desset et Chabaud, 1977 (*Oswaldocruzia* Travassos, 1917 – two spp.); Herpetostrongylidae (Skrjabin et Schulz, 1937, subfam.) Durette-Desset et Chabaud, 1981 (*Herpetostrongylus* Baylis, 1931 – one sp.); Ascaridida, Ascaridoidea (*Raillietascaris* Sprent, 1985 – one sp.); Gnathostomatidae Railliet, 1895 (*Tanqua* von Linstow, 1897– one sp.); Heterakidae Railliet et Henry, 1914 (*Meteterakis* Karve, 1930 – two spp. and *Strongyluris* Müller, 1894 – one sp.); Diplotriaenidae (Skrjabin, 1916, subfam.) Anderson, 1958 (*Hastospiculum* Skrjabin, 1923 – one sp.); Onchocercidae Leiper, 1911 (*Piratuboides* Bain et Sulahian, 1974 – one sp.); Pharyngodonidae Travassos, 1919 (*Spauligodon* Skrjabin, Schikhobalova et Lagodovskaja, 1960 – two spp.; *Pharyngodon* Diesing, 1861– one sp.; and *Thelandros* Wedl, 1862 – one sp.); and Physalopteridae Railliet, 1893 (*Abbreviata* Travassos, 1919 – one sp.).

5.4.3.1. *Cosmocercoides tonkinensis* Tran et al., 2015

Host and location: *Acanthosaura lepidogaster*, large intestine (Tran et al. 2015a)

Locality: Cao Bang (8), Son La (4), Bac Giang (17), Thanh Hoa (26) Provinces

Incidence and intensity: 31.3% (10/32) with 1–29 (geomean 4.1) worms/host

Comment: Currently, ca. 20 species of the genus with caudal rosette papillae, not combined with plectanes, have been described mainly in amphibians worldwide. The morphology of this species was well characterized using both light and scanning electron microscopies. The phylogenetic relationships with related species with available genetic data (*Cosmocercoides pulcher* and *Cosmocercoides dukae*) were also reported (Tran et al. 2015a). This is the third species from lizard hosts after *C. variabilis* in North America and *C. sauria* in Brazil (Goldberg et al. 1995; Ávila et al. 2010; Bursey and Brooks.2011).

5.4.3.2. *Strongyloides mirzai* Singh, 1954

Host and location: *Varanus nebulosus*, intestine (Nguyen TM 2002)

Locality: Vietnam (not specified)

Incidence and intensity: No information

Comment: This species was originally described using specimens from Indian sand boas (*Eryx johnii*) and Oriental ratsnakes (*Ptyas mucosa*) in India (Singh 1954). Likewise in Vietnam, the species was found in a variety of snakes such as Chinese cobra (*Naja atra*), many-banded krait (*Bungarus multicinctus*), banded krait (*Bungarus fasciatus*) and Chinese ratsnake (*Ptyas korros*) (Nguyen TL et. 2008).

5.4.3.3. *Kalicephalus* sp.

Syn. *Kalicephalus macrovulvus* sensu Nguyen, 2002, nec Caballero, 1954

Host and location: *Varanus nebulosus*, oesophagus (Nguyen TM 2002)

Locality: Vietnam (not specified)

Incidence and intensity: No information

Comment: Nguyen TM (2002) recorded *Kalicephalus* nematodes from the oesophagus of clouded monitors (*Varanus nebulosus*) in Vietnam as *K. macrovulvus* Caballero, 1954, originally isolated from *Agkistrodon bilineatus* in Guatemala. Schad (1962) reclassified the species as *K. inermis macrovulvus* distributed in the Neotropical region. Zhang et al. (2011) reported two *Kalicephalus* spp., i.e. *K. guangdongensis* and *K. schadi fotedari*, from the intestine of *Varanus salvator* from Guangdong Wildlife Rescue Centre, Guangdong Province, South China, although these two species resembled each other except for spicule length and other morphometric values, and length and position of the externodorsal ray. As the measurements and drawings provided by Nguyen TM (2002) are not sufficient to determine the species, in this article we describe the species from clouded monitors in Vietnam as *Kalicephalus* sp. Re-examination of the specimens in the future should yield a more precise

identification. In addition, *Kalicephalus (Kalicephalus) costatus indicus* Ortlepp, 1923, from *Varanus bengalensis* in India, *K. (K.) megacephalus* Schad, 1962, from *Varanus indicus* in India and *V. salvator* in the Philippines, and *K. (K.) schadi* Ogden, 1966, from *V. bengalensis* in India or Sri Lanka (London Zoo) have been recorded (Baker 1987).

Furthermore, a variety of *Kalicephalus* spp. has also been recorded from snakes in Southeast and South Asia (Maplestone 1931; Schad 1959; Yamaguti 1961; Purwaningsih et al. 2011; Kuzmin et al. 2013; Liu et al. 2016). Le and Pham (1968), and Oshmarin and Demshin (1972) recorded the following *Kalicephalus* spp. from snakes in Vietnam: *K. alatospiculus* from Chinese ratsnakes *Ptyas korros* (syn. *Zamenis korros*), *K. viperae chungkingensis* from Indian cobras *Naja naja*, *K. indicus* from Chinese ratsnakes and radiated ratsnakes *Coelognathus radiata* (syn. *Elaphe radiata*), *K. bungari* (syn. *K. najae* after Schad 1962 and Baker 1987) from Indian cobras, *K. natricis* from Sri Lankan keelbacks *Xenochrophis asperrimus* (syn. *Natrix piscator*) and *Kalicephalus* sp. from banded kraits *Bungarus fasciatus*. *Kalicephalus natricis* Yamaguti**,** 1935, originally from *Rhabdophis tigrinus* (syn. *Natrix tigrina*) and *Elaphe quadrivirgata* in Japan, is considered to be a 'species inquirenda', since multiple species, probably *K. costatus indicus*, *K. brachycephalus*, *K. sinensis* or *K. viperae chungkingensis*, were described under the name '*K. natricis*' (Schad 1962; Baker 1987).

5.4.3.4. *Oswaldocruzia* sp. 1

Syn. *Oswaldocruzia agamae* sensu Nguyen et Bui, 2007

Host and location: *Hemidactylus frenatus* and *Eutropis longicaudata*, intestine (Nguyen TL et al. 2005; Nguyen TM and Bui 2007)

Locality: Hanoi Province (14) and Yen Bai Province (5)

Incidence and intensity: No information

Comment: Nominal species of the genus *Oswaldocruzia* are currently more than 80 in number (Ben Slimane et al. 1996; Santos et al. 2008; Guerrero 2013). Ben Slimane et al. (1996) attempted to subdivide the genus into five groups based on the disposition of the caudal bursal rays, the morphology of the synlophe and the anatomy of the spicules: 1) Oriento-Ethiopian species, 10 spp.; 2) Neo-Ethiopian species, 11 spp.; 3) Holarctic species, 24 spp.; 4) Continental Neotropical species, 21 spp.; and 5) Caribbean Neotropical species, 8 spp. From Vietnam, *O. mitunagai* Durette-Desset, Nasher et Ben Slimane, 1992, and *O. hoepplii* Hsü, 1935, both from *D. melanostictus* and Ranidae, were noted in the identification key of this comprehensive taxonomic study by Ben Slimane et al. (1996). *Oswaldocruzia hoepplii* sensu Moravec et Sey, 1985, nec Hsü, 1935, was considered to be a junior synonym

of *O. mitunagai* (the renamed species of *O. hoepplii* sensu Yamaguti et Mitunaga, 1943, nec Hsü, 1935). Following the systematics of Ben Slimane et al. (1996), *O. agamae* Sandground, 1930 is a parasite of agamid lizards distributed in Liberia. As the measurements and drawings provided by Nguyen TM and Bui (2007) are not sufficient to determine the species, in this article we describe the species from geckos in Vietnam as *Oswaldocruzia* sp. Re-examination of the specimens in the future should yield a more precise identification.

5.4.3.5. *Oswaldocruzia* sp. 2

Host and location: *Varanus salvator* and *Varanus nebulosus*, intestine (Nguyen TM 2002)

Locality: Vietnam (not specified)

Incidence and intensity: No information

Comment: This species was differentiated from the former species based on multiple morphological features, e.g. different development of caudal bursa and rays, different morphology of spicules and different egg dimensions (0.07–0.10 mm by 0.05–0.06 mm vs. 0.06–0.07 mm by 0.03–0.04 mm, respectively).

5.4.3.6. '*Herpetostrongylus varani*' sensu Nguyen, 2002

Host and location: *Varanus nebulosus*, intestine (Nguyen TM 2002)

Locality: Vietnam (not specified)

Incidence and intensity: No information

Comment: Although the specific name '*Herpetostrongylus varani*' assigned by Nguyen TM (2002) to the herpetostrongylid nematodes from clouded monitors in Vietnam is retained in this article, an exact specific identification is required in the future. At this time, we are unable to ascertain the exact taxonomy of the present specimens due to Nguyen's measurements and drawings (Nguyen TM 2002) being insufficient for species determination. For reasons outlined in the next paragraph, it is particularly important to evaluate its possible classification in the genus *Vaucherus* Durette-Desset, 1980.

Herpetostrongylus varani Baylis, 1931, was originally recorded from Gould's monitors (*Varanus gouldii*) in Townsville, North Queensland, Australia (Baylis 1931). At the same time, another species, *Herpetostrongylus pythonis* Baylis, 1931, was described from *Morelia spilota* (syn. *Python spilotes*) in Australia (Baylis 1931), and later recorded in *Varanus salvator* from Palawan, Philippines (Schmidt and Kuntz 1972). Durette-Desset (1980) erected a new genus, *Vaucherus*, for three herpetostrongylid nematode species from Indian and Asian

varanid hosts: *V. vaucheri* Durette-Desset, 1980 from *Varanus rudicollis* in Kuala Lumpur, Malaysia; *V. leiperi* (Sharief, 1957) Durette-Desset, 1980 from *Varanus indicus* in Hyderabad, India; and *V. indicus* (Deshmukh, 1969) Durette-Desset, 1980 from *Varanus indicus* in Aurangabad, India. Durette-Desset et al. (1994) differentiated the genus *Vaucherus* from the genus *Herpetostrongylus* based on differences in bursal ray arrangements; 2-2-1 type with hypertrophied ray 2, and 1-3-1 type with rays 2 and 3 more strongly developed than 5 and 6, respectively.

5.4.3.7. *Raillietascaris varani* (Baylis et Daubney, 1922) Sprent, 1985

Syns. *Amplicaecum varani* Baylis et Daubney, 1922; *Amplicaecum monitor* Khera, 1954; *Amplicaecum iguaneae* Wahid, 1961

Host and location: *Varanus salvator* and *Varanus nebulosus*, stomach and intestine (Nguyen TM 2002)

Locality: Vietnam (not specified)

Incidence and intensity: No information

Comment: This species was originally described as *Amplicaecum varani* using specimens from the intestine of *Varanus salvator* in Calcutta, India, and several different species from Indian varanids were recorded in the decades that followed (Yamaguti 1961). Sprent (1985) erected a new genus, *Raillietascaris*, and unified such species from varanids as junior synonyms of *R. varani* (Baylis et Daubney, 1922). This species was also recorded from *Varanus rudicollis* in Borneo, Indonesia, and imported Chinese water dragons in Poland (Schad 1959; De and Dey 1992; Okulewicz et al. 2015).

5.4.3.8. *Tanqua tiara* (von Linstow, 1879) Blanchard, 1904

Host and location: *Varanus salvator*, stomach (Nguyen TM 2002)

Locality: Vietnam (not specified)

Incidence and intensity: No information

Comment: This species was originally described using specimens from the stomach of varanid lizards in South Africa. It was subsequently isolated from *Varanus salvator* in Sumatra, *Varanus gouldii* in Australia or New Guinea, *Varanus bengalensis* in Sri Lanka and *Varanus niloticus* in the White Nile (Baylis 1916; Yamaguti 1961; Chabaud 2009b). Gibbons and Keymer (1991) redescribed the species in detail, along with a list of previous records of *T. tiara* from varanids in Africa through to Asia. Phylogenetic analyses of geographical isolates distributed widely would be very interesting.

5.4.3.9. *Meteterakis varani* (Maplestone, 1931) Skrjabin et al., 1961

Host and location: *Varanus nebulosus*, intestine (Nguyen TM 2002)

Locality: Vietnam (not specified)

Incidence and intensity: No information

Comment: The genus *Meteterakis* Karve, 1930, currently contains 25 nominal species from Oriental amphibians and reptiles. In Vietnam, *Meteterakis striatura* (Oshmarin et Demshin, 1972) from yellow pond turtle *Mauremys mutica* (syn. *Clemmys mutica*) and *Meteterakis japonica* (Wilkie, 1930) from a yellowcheek (*Elopichthys bambusa*) have been recorded (Oshmarin and Demshin 1972; Moravec and Sey 1988), although the latter record was considered to be an accidental infection (Moravec and Sey 1988). *Meteterakis varani* was the third *Meteterakis* species recorded in Vietnam.

5.4.3.10. *Meteterakis mabuyae* Chakravarty, 1944

Host and location: *Eutropis longicaudata*, intestine (Nguyen TL et al. 2005; Nguyen TM and Bui 2007)

Locality: Hanoi Province (14) and Yen Bai Province (5)

Incidence and intensity: No information

Comment: This was the fourth species recorded in Vietnam after *M. striatura*, *M. japonica* and *M. varani*. In addition, at least two *Meteterakis* spp. were detected in the large intestine of scale-bellied tree lizards, *Acanthosaura lepidogaster*, in Vietnam. Specific identification of these species is currently in progress (unpublished).

5.4.3.11. *Strongyluris calotis* Baylis et Daubney, 1923

Syns. *Ascaridia japalurae* Yamaguti et Mitunaga, 1935; *Strongyluris brevicaudata* sensu Hsü Hsü Hsü et Hoeppli, 1931, nec Müller, 1894

Host and location: *Pseudocalotes brevipes* and *Calotes emma*, large intestine (Tran et al. 2016)

Locality: Phu Tho Province (12) and Bac Kan Province (7)

Incidence and intensity: 57.1% (4/7) with 1–6 worms/host (*P. brevipes*); 16.7% (1/6) with no intensity information (*C. emma*)

Comment: This species is distributed widely in agamid lizards from the Oriental region. Tran et al. (2016) confirmed for the first time the distribution of the species in Vietnam. Furthermore, their morphological studies of two isolates from *P. brevipes* and *C. emma* in

Vietnam demonstrated differences in numbers and arrangements of caudal papillae. However, in combination with genetic characterization, their conspecificity was shown (Tran et al. 2015b, 2016). There are at least four nominal *Strongyluris* spp. in agamid lizards in the Oriental region: *S. chamaeleonis* Baylis et Daubney, 1922; *S. bengalensis* Chakravorty, 1936; *S. karawirensis* Karve, 1938; and *S. japalurae* Jiang et Lin, 1980. As there are few critical morphological differences among these described species, they need to be genetically characterized to confirm the validity of their taxonomy.

5.4.3.12. *Hastospiculum varani* Skrjabin, 1923

Host and location: *Varanus nebulosus*, body cavity (Nguyen TM 2002)

Locality: Vietnam (not specified)

Incidence and intensity: No information

Comment: There are five *Hastospiculum* spp. recorded from varanid lizards (Yamaguti 1961; Bolette 1998): *H. varani* Skrjabin, 1923, from *Varanus griseus* in Turkistan and *Varanus indicus* in India; *H. bipinnatum* von Linstow, 1899, from *Varanus griseus* (syn. *Psammosaurus griseus*) in northeastern Africa; *H. macrophallos* Parona, 1889, or its junior synonym *H. spinigerum* Chandler, 1929 from *Varanus* spp. in Myanmar, India and Russia; *H. gouldi* Yorke et Maplestone, 1926 from *Varanus gouldii* in Australia; and *H. spiralis* Bolette, 1998 from *Varanus indicus* in Indonesia. As the record in Vietnam was based on a single male specimen from the host (Nguyen TM 2002), more specimens need to be examined for an accurate identification of the species.

5.4.3.13. *Piratuboides varanicola* (Mackerras, 1962) Bain et Sulahian, 1974

Syn. *Piratuba varanicola* Mackerras, 1962

Host and location: *Varanus nebulosus*, lungs (Nguyen TM 2002)

Locality: Vietnam (not specified)

Incidence and intensity: No information

Comment: Mackerras (1962) described two onchocercid filariae of the subfamily Oswaldofilariinae in Australian varanid lizards and newly named them as *Piratuba queenslandensis* and *Piratuba varanicola*. Bain and Sulahian (1974) moved them from the genus *Piratuba* (equal spicules in size and shape, a short and simple ovejector, and numerous caudal papillae) to a new genus, *Piratuboides*, characterized as having subequal spicules, a long but simple ovejector, and a smooth female tail or one with small terminal elevations. The type species of the genus is *Piratuboides zeae* (Bain, 1974) from skinks of Scincidae in

Central America, and *Piratuboides huambensis* was described from blue-tailed skink *Trachylepis quinquetaeniata* (syn. *Mabuya quinquetaeniata*) in Angola (Petit et al. 1983).

5.4.3.14. *Spauligodon vietnamensis* Tran et al. 2007

Host and location: *Gekko badenii* (syn. *Gekko ulikovskii*), large intestine (Tran et al. 2007)

Locality: Gia Lai Province (36) and Tay Ninh Province (46)

Incidence and intensity: 90.0% (9/10) with no intensity information (Gia Lai Province)

Comment: Currently, 51 species have been described worldwide in the genus *Spauligodon* Skrjabin, Schikhobalova et Lagodovskaja, 1960 (Bursey et al. 2014). *Spauligodon vietnamensis* recorded in golden geckos from Vietnam was the 44th species assigned to the genus (Tran et al. 2007).

Golden geckos (*Gekko badenii*) have a limited distribution in the high mountains of central and southern Vietnam, i.e. Tay Ninh, Kon Tum and Gia Lai Provinces (Darevsky and Orlov 1994; Nguyen VS et al. 2009). As two scientific names for golden geckos, *G. badenii* and *G. ulikovskii*, were published independently in different scientific journals on 15 May and 15 June, 1994, respectively, the former scientific name has priority.

5.4.3.15. *Skrjabinodon azerbajdzanicus* (Sharpio, 1974) Bursey et Goldberg, 1999

Syn. *Spauligodon azerbajdzanicus* Sharpio, 1974

Host and location: *Hemidactylus frenatus*, intestine (Nguyen TL et al. 2005; Nguyen TM and Bui 2007)

Locality: Hanoi Province (14) and Yen Bai Province (5)

Incidence and intensity: No information

Comment: The species was originally described from the green-bellied lizard *Darevskia chlorogaster* (syn. *Lacerta chlorogaster*) (Sauria: Lacertidae) in Azerbaijan. Referring to the absence of caudal alae in the description of '*Spauligodon azerbajdzanicus*' by Sharpio (1974), Bursey and Goldberg (2005) reassigned the species as *Skrjabinodon azerbajdzanicus*.

5.4.3.16. *Pharyngodon duci* Tran et al., 2007

Host and location: *Gekko badenii*, large intestine (Tran et al. 2007)

Locality: Gia Lai Province (36) and Tay Ninh Province (46)

Incidence and intensity: 90.0% (9/10) with no intensity information (Gia Lai Province)

Comment: As with the genera *Spauligodon* Skrjabin, Schikhobalova et Lagodovskaja, 1960, and *Skrjabinodon* Inglis, 1968 (Petter and Quentin 2009), the genus *Pharyngodon* Diesing, 1861, is confined to reptile and amphibian hosts. Although members of these three genera resemble each other, major differences lie in the presence (*Pharyngodon* and *Spauligodon*) or absence (*Skrjabinodon*) of caudal alae. Caudal alae of *Pharyngodon* spp. are supported by all three pairs of genital papillae, whereas those of *Spauligodon* spp. are supported by the two anterior pairs only. The genus *Pharyngodon* currently contains 37 species, of which five were recorded from amphibians (Bursey and Goldberg 2005; Bursey et al. 2008; Fenner et al. 2008). *Pharyngodon duci* recorded in golden geckos from Vietnam was the 35th species assigned to the genus (Tran et al. 2007).

5.4.3.17. *Thelandros vietnamensis* Bui et al., 2009

Host and location: *Leiolepis reevesii*, intestine (Bui et al. 2009)

Locality: Ha Tinh Province (28)

Incidence and intensity: 85.0% (17/20) with an average of 25.8 worms/host

Comment: The genus *Thelandros* is closely related to the aforementioned pharyngodonid genera. Parasites of this genus have three, sometimes four, pairs of genital papillae clearly separated into an anterior group (two pairs) around the cloaca and one posterior pair. There is often a fringed membrane covering the cloaca [98]. Petter and Quentin (2009) synonymized *Parapharyngodon* Chatterji, 1933, with *Thelandros* Wedl, 1862, but Bursey and Goldberg (2005) disagreed with this view based on several morphological differences. According to Bursey and Goldberg (2005), 31 species are currently assigned to the genus *Thelandros*, with only two being described from the Oriental region. In the case of the genus *Parapharyngodon*, 41 species are assigned, with five being described from the Oriental region. After Bursey and Goldberg's 2005 article (2005), *T. vietnamensis* recorded in Reeves' butterfly lizard (*L. reevesii*) from Vietnam has become the 32nd (and 3rd Oriental) species assigned to the genus (Bui et al. 2009).

5.4.3.18. *Abbreviata deschiensi* Le et Nguyen, 1966

Host and location: *Calotes versicolor*, stomach (Le and Nguyen VL 1966)

Locality: Binh Thuan Province (43)

Incidence and intensity: No information

Comment: The physalopterid genus *Abbreviata* Travassos, 1920 has an internolateral tooth and externolateral tooth, and two double pairs of submedian teeth on each pseudolabium

(Chabaud 2009b). Multiple *Abbreviata* spp. have been recorded in varanid lizards and snakes (Jones 1978, 1979, 1988, 2007, 2010, 2013).

5.4.4. Acanthocephala

Nguyen VH (2015) recently provided a list of 76 acanthocephalan species recorded in Vietnam up to the year 2015. This list comprised 13 spp. from freshwater fish, 21 spp. from marine fish, three spp. from amphibians, five spp. from reptiles, 29 spp. from birds and five spp. from mammals. One recorded species in Vietnamese lizards was classified in the family Echinorhynchidae Cobbold, 1876 (*Pseudoacanthocephalus* Petrochenko, 1956).

5.4.4.1. *Pseudoacanthocephalus nguyenthileae* Amin et al., 2008

Syn. *Acanthocephalus* sp. sensu Nguyen et al., 2005

Host and location: *Hemidactylus frenatus*, small intestine (Nguyen TL et al. 2005; Amin et al. 2008)

Locality: Bac Kan Province (7)

Incidence and intensity: 4.0% (1/25) with 2 worms/host

Comment: This species was dedicated to Prof. Nguyen Thi Le, a parasitologist of IEBR, VAST (Amin et al. 2008). It was also found in amphibians and other reptiles in northern Vietnam, i.e. *Hylarana guentheri*, *Hylarana taipehensis*, *Duttaphrynus melanostictus*, *Quasipaa verrucospinosa* (syn. *Paa verrucospinosa*), *Polypedates mutus* and *Naja atra* (Amin et al. 2008; Nguyen VH 2015). Amin et al. (2008) provided an identification key for 11 valid *Pseudoacanthocephalus* spp. in the world.

5.4.5. Arthropoda

The subclass Pentastomida Diesing, 1836 (phylum Arthropoda: subphylum Crustacea: class Maxillopoda Dahl, 1956) is commonly known as tongue worms, parasitizing the respiratory tracts of vertebrates (Riley et al. 1988; Almeida et al. 2008). It is divided into four orders: Cephalobaenida Heymons, 1935 (one family); Porocephalida Heymons, 1935 (four families); Raillietiellida Almeida et Christoffersen, 1999 (one family); and Reighardiida Almeida et Christoffersen, 1999 (one family). The worms have a segmented body covered by a chitinous cuticle and the anterior end bears five appendages, i.e. one mouth and two pairs of hooks for attachment to the host.

Three recorded pentastomid species from Vietnamese lizards were classified in Raillietiellidae Sambon, 1922 (*Raillietiella* Sambon, 1910).

5.4.5.1. *Raillietiella frenatus* Ali, Riley et Self, 1981

Host and location: *Hemidactylus frenatus*, lungs (Ali et al. 1981; Nguyen et al. 2005)

Locality: Hanoi (14), Yen Bai (5), Tuyen Quang (6), Bac Kan (7) Provinces and southern Vietnam (not specified)

Incidence and intensity: 30.9% (46/149) with 1–14 worms/host

Comment: Members of the genus *Raillietiella* are parasites in the respiratory tract of carnivorous lizards. This species was recorded from *H. frenatus* in Malaysia, Thailand, Vietnam, Indonesia, Philippines and Taiwan, *Japalura swinhonis* and *Eutropis longicaudata* in Taiwan (Ali and Riley 1983; Ali et al. 1981; Matsuo and Oku 2002), and *Hemidactylus platyurus* (syn. *Cosymbotus platyurus*) and *Gehyra mutilata* in Indonesia (Ali and Riley 1983; Matsuo and Oku 2002). Barton (2007) reported *R. frenatus* from invasive Asian house geckos (*H. frenatus*) as well as native geckos, *Gehyra australis*, in northern Australia, suggesting the possible spread of alien parasites through introduced hosts. Furthermore, Kelehear et al. (2014) collected *R. frenatus* not only from invasive Asian house geckos (*H. frenatus*) but also from invasive cane toads (*Rhinella marina*) and native tree frogs (*Litoria caerulea*) in tropical Australia. Riley et al. (1988) and Goldberg and Bursey (1996) reported a similar problem, i.e. the invasion of alien parasites through introduced hosts, in Texas and Hawaii, respectively.

Kelehear et al. (2011) emphasized the importance of molecular analyses of pentastomes in addition to morphological characterization for valid descriptions of new species, because often the same species adopts different morphological phenotypes of taxonomic importance in different host species.

5.4.5.2. *Raillietiella orientalis* (Hett, 1915) Sambon, 1922

Host and location: *Varanus salvator* and *Varanus nebulosus*, lungs (Nguyen TL et al. 2003)

Locality: Vietnam (not specified)

Incidence and intensity: 12.8% (2/20 *V. salvator* and 3/19 *V. nebulosus*) with no intensity information

Comment: This species has a wide spectrum of snake hosts of Colubridae, Elapidae, Viperidae and Boidae in Southeast Asia, India, Philippines, Taiwan, Japan and China (Ali et al. 1982, 1985; Christoffersen and de Assis 2013). Kelehear et al. (2014) reported high

prevalences of this Asian pentastomid species in wild snakes native to the Australian tropics such as *Tropidonophis mairii* (Colubridae), *Acanthophis praelongus* (Elapidae), *Demansia vestigiata* (Elapidae) and *Liasis fuscus* (Pythonidae). They considered these records as a recent translocation of alien parasites via an unknown pathway. In Vietnam, *R. orientalis* causes outbreaks of serious infection in farmed snakes such as *Naja naja* and *Ptyas mucosus*. Dang (2000) conducted epidemiological surveys of *R. orientalis* in Indian cobras (*Naja naja*) and Asian common toads (*Duttaphrynus melanostictus*) in the field of Vietnam and found infections at an incidence of 39.6% (44/111) with an average intensity of 6.2 worms/snake (range 1–50) and 0.4% (2/500) with 3 worms/toad, respectively. Furthermore, he demonstrated a direct life cycle of this pentastomid species by an experimental infection. Nguyen TL et al. (2003) recorded the species from two varanid species in Vietnam; however, no detailed description was given. In addition, they reported the recovery of possibly another pentastomid species in these two varanid species.

5.4.5.3. *Raillietiella affinis* Bovien, 1927

Host and location: *Eutropis longicaudata*, lungs (Nguyen TL et al. 2003)

Locality: Hanoi (14), Yen Bai (5), Tuyen Quang (6), Bac Kan (7) Provinces

Incidence and intensity: 30.9% (46/149) with 1–14 worms/host

Comment: This species was first collected from the lungs of *Gekko gecko* (syn. *Gekko verticillatus*) in Java, Indonesia, then noted in geckos and skinks from Egypt, Sudan or Hawaii (Christoffersen and de Assis 2013). As an invasive species, Dervin et al. (2014) reported *R. affinis* from the Madagascar giant day gecko, *Phelsuma grandis*. Human cases of *R. affinis* infection are also known.

In Vietnam, Nguyen TL et al. (2005) recorded a parasitism of the same gecko species with possibly another *Raillietiella* sp., different from *R. frenatus* and *R. affinis*.

5.5. Discussion

In this article, we present a list of 45 species of endoparasite of Vietnamese lizards. Specifically, 11 cestode, 12 trematode, 18 nematode, one acanthocephalan and three pentastomid species have been recorded from 10 host species. As shown in Table 1, the majority of endoparasite species (55.6%, i.e. 25/45) was recorded from two varanid lizards, *V. nebulosus* and *V. salvator*. These lizard species are widely endemic in Vietnam as well as other Southeast and South Asian countries such as Myanmar, Laos, Thailand, Cambodia,

Malaysia and Indonesia for the former species, and India, Sri Lanka, Bangladesh, South China, Myanmar, Laos, Thailand, Cambodia, Malaysia, Singapore, Indonesia and Philippines for the latter species (Nguyen VS et al. 2009). Table 10 also shows that 14 of the 45 endoparasite species (31.1%) were detected in two gecko species, *Hemidactylus frenatus* and *Eutropis longicaudata* (149 and 38 geckos examined, respectively). The remaining six endoparasite species (13.3%) were nematodes described from five agamid species and one gecko species. The reports on these six endoparasite species include five new species descriptions, concentrating on one or two targeted parasite(s). Therefore, it would appear that rather than these lizard host species having only a few endoparasites, more parasites remain to be recorded from them. Indeed, we collected at least two *Meteterakis* spp. from scale-bellied tree lizards, but due to difficulties with taxonomic differentiation, specific identification has yet to be completed.

Except for two species, *Cosmocercoides tonkinensis* and *Strongyluris calotis* from agamid lizards, no molecular analyses accompanied the taxonomical characterization of collected parasites (Tran et al. 2015a, 2016). *Strongyluris calotis* collected from two different agamid species showed two morphotypes with different numbers or arrangements of caudal papillae, which are believed to be of taxonomic importance to separate *Strongyluris* species (Tran et al. 2015b, 2016). When the geographical and/or ecological isolation of host species are distinct and multiple morphotypes of parasites from them are noted, molecular characterization of parasites can support our specific differentiation or leave invaluable clues to other scientists for future research. As mentioned above, Kelehear et al. (2011) emphasized the taxonomic importance of molecular analyses of pentastomes in addition to morphological characterization for accurate species differentiation. Furthermore, recent worldwide spreads of invasive parasites accompanying hosts beyond geographical borders make molecular characterization of isolated parasites a key technology in understanding their exact taxonomic situation, i.e. invasive species or native species.

At the beginning of this review, we highlighted the rich herpetofauna diversity of Vietnam (385 reptiles and 181 amphibians). However, only a fraction of the parasites of reptiles has been recorded in this richly biodiverse territory. Additionally, most of the specimens recorded in the past are no longer available. Parasitological surveys, if actually possible, on multiple lizard hosts in a territory with rich herpetofauna diversity may disclose the ecological relationships among different categories of host lizards in Vietnam or clarify the taxonomic relationship of parasites from Vietnamese lizards with those from lizards of

other neighbouring or remote regions. In this sense, it is again recommended that parasites are characterized both morphologically and genetically.

Research on parasite diversity in Vietnam, as having been conducted as large-scale surveys in the country, exclusively used a classical descriptional approach on parasites based on morphological criteria. With this approach, it is often difficult to evaluate morphological variation, as having been experienced in many categories of parasites. Consequently, more recent parasitological research has applied molecular genetic technologies to surveys and parasite characterization, enabling synonymization of morphological variants or detection of cryptic species with an identical morphological manifestation. Furthermore, with the latest taxonomic approaches, we can determine the phylogenetic position of observed parasites or evolutional relationships with related taxa. Usability of these advanced molecular approaches is dependent on the calibre of the background genetic data of the targeted species as well as related species. In contrast to parasites of medical and veterinary importance, the depository of molecular genetic data of lizard or amphibian parasites is sparse at present. General interest in the biodiversity of local nature or worldwide spread of invasive parasites via translocation of vertebrates beyond natural borders may enhance our particular understanding of all organisms including parasites. We are still a long way from disclosing the full repertoire of endoparasite fauna of lizards from Vietnam.

GENERAL DISCUSSION

In Vietnam, multiple large-scale surveys of parasites have been carried out in different regions of the country in the last 50 or more years. Up to now, Vietnamese scientists listed 373 trematode species, 185 cestode species, more than 400 nematode species, 72 acanthocephalan species, and 3 pentastomid species and 116 species of Arachnida in mammals, birds, reptiles, and amphibians (Phan TV et al. 1977, Nguyen TL et al. 1996a, b, 2003, 2005; Nguyen TK 2003; Tran 2009; Nguyen TL and Ha. 2010; Nguyen VH 2015). However, parasites of reptiles and amphibians have been mostly ignored with few regions where researches have been conducted. Particularly, no or little information has been obtained on parasites of the lizard species which are so rarely distributed in nature of Vietnam. A limited number of reports have been published so far on helminths or helminth fauna of reptiles and amphibians in Vietnam, and since 1996 efforts to record reptile and/or amphibian parasites by Vietnamese researchers or collaborative foreign researchers have gained some results (Amin et al. 2008; Bui et al. 2009; Nguyen TM 2002; Nguyen TL et al. 2003, 2005, 2008; Pham and Nguyen 2003, 2007; Tran et al. 2005, 2007, 2015a, b, 2016; Tran 2009; Nguyen VH 2015). These researches disclosed very high prevalences of parasites, particularly nematodes, in lizards: *Varanus nebulosus* and *Varanus salvator* (100%), *Gekko badenii* (syn. *G. ulikovskii*) (98%) and *Acanthosaura lepidogaster* (85%). The scientists recorded a total of 45 parasitic species belonging to 34 genera of 27 families in 10 lizard species, including 6 species new to science at those times (*Abbreviata deschiensi; Pharyngodon duci; Spauligodon vietnamensis; Thelandros vietnamensis; Cosmocercoides tonkinensis,* and *Pseudoacanthocephalus nguyenthileae*) (Le and Nguyen 1966; Tran et al. 2007, 2015a; Amin et al. 2008; Bui et al. 2009).

Researches on parasite diversity in Vietnam, as having been conducted as large-scale surveys in the country, used exclusively a classical descriptional approach on parasites based on morphological criteria. In this approach often it is difficult to evaluate morphological variation as having been experienced in many categories of parasites. Then in modern researches a majority of parasitological researches applied molecular genetic methodologies to such parasitological surveys and parasite characterization, enabling synonimization of morphological variants or detection of cryptic species with an identical morphological manifestation. Furthermore, by modern taxonomic approaches, we can know the phylogenetic position of the observed parasites or evolutional relationships with related taxa. Usability of modern molecular approaches is dependent on the condition of background genetic data of the

targeted species as well as related species. Stock of molecular genetic data of lizard or amphibian parasites is sparse at present, in contrast to parasites of medical and veterinary importance. In my thesis studies, I approached nematodes parasitic to Vietnamese lizards by both classical and modern parasitological methodologies, disclosing some important points as follows: 1) Parasites from lizards are sometimes poorly characterized using convientional light microscopy, and description of species depended on the geographical distribution or host species; 2) Differentiation of parasitic nematodes with caudal papillae tended to make more species than nematode taxa without caudal papillae, and there are little interspecific genetic difference in the genus; 3) There are sometimes noticeable intraspecific genetic variations regardless of geographical distibution; and 4) Molecular genetic analyses disclose conspecificity of distinct morphotypes and the base of appearance of distinct morphotypes cannot be elucidated.

In this thesis, we focused on nematodes of three categories (Pharyngodonidae Travassos 1919, Cosmocercoidae (Railliet 1916) Travassos 1925, and Heterakidae Railliet et Henry 1912) in Vietnamese lizards to clarify their taxonimical statuses with an aid of phylogenetic characterizations based on the ribosomal RNA gene (rDNA). Although morphological characters of taxonomic importance have been refined to identify the species or make a border between different species during a long history of parasite descriptions, often the range of useful criteria varied by researchers or by observed parasites. In this sence, nucleotide sequencing provides objective criteria to assess taxonomical importance of proposed morphological characters.

In Chapter 1, I charaterized two nematode species of Pharyngodonidae (Oxyuroidea) collected from golden geckos, *G. badenii* (Sauria: Gekkonidae), which are currently known only from the highland in the central part of Vietnam. Geckos had abundant oxyurid nematods in the large intestine with a high prevalence (9 out of 10 geckos), and based on morphological criteria I identified two new species, i.e., *P. duci* and *S. vietnamensis* as 35th and 44th species assigned for each genus, respectively. A large number of immature oxyurids dwelled in the large instestine as well, indicating active and continuous infection with these two oxyurid species occurred among the golden geckos. Records of two new species from an isolated lizard host population stimulate the understanding of their phylogenetic relationships with congeners, and this point should be pursued in future works. In the same central region of Vietnam, a new species, i.e., *Thelandros vietnamensis* Bui, Bursey et Goldberg 2009, was recorded in the Reeves' butterfly lizard, *Leiolepis reevesii* Gray 1931 (Squamata: Agamidae).

In Chapter 2, I characterized morphologically and phylogenetically a new *Consmocercoides* sp., i.e., *C. tonkinensis*, in the scale-bellied tree lizard, *A. lepidogaster* (Squamata: Agamidae), from the northern and central parts of Vietnam. Currently 19 nomial *Cosmocercoides* spp. have been recorded, mainly from amphibian host, and only two species, *C. variabilis* in North America and *C. sauria* in South America, have been recorded from lizards. Therefore, this new species is just the third species to be recorded from a reptilian host. When considering the taxonomic position of this species, the phylogenetic relationship with congeners recorded from amphibian hosts is highly intriguing. The 18S rDNA of the new species is almost identical to that of a unique congener *C. dukae* from land snails and slugs in North America. Similarly, between the present new species and *C. pulcher* from a toad (*Bufo japonicus*) in Japan, few nucleotide changes were noticed in the 18S to 28S rDNA including the internal transcribed spacer (ITS) regions. It is needed to collect more specimens of diverse *Cosmocercoides* spp. to elucidate the significance of appreciated morphological criteria with reference to phylogenetic datasets. More genetic data of the genus are necessary to know appropriately the genus and its species diversity. From the family Cosmocercidae, additional two species, *Aplectana macintoshii* and *Cosmocerca ornate,* have been recorded in Vietnam from Mao-Son frog, *Hylarana maosonensis* (Anura: Ranidae) (Bursey and Goldberg 2011), and the former species was also recorded from the specklebelly keelback snake (*Rhabdophis chrysargus*) in Vietnam (Moravec and Sey 1985).

The family Heterakidae is divided into three subfamilies (Spinicaudinae, Meteterakinae, and Heterakinae) and contains 11 genera. Roughly saying, the first two species, Spinicaudinae (6 genera) and Meteterakinae (2 genera), contain ascarid parasites of reptiles and amphibians, and Heterakinae (3 genera) contains parasites of birds (Chabaud 2009a). Little is known on heterakid nematodes in Vietnamese lizards, although 4 *Meteterakis* spp. were recorded from Vietnamese reptiles: *M. varani* from the clouded monitor *Varanus nebulosus*; *M. mabuyae* from the long-tailed sun skink *Eutropis longicaudata* (syn. *Mabuya longicaudata*); *M. striatura* from the yellow pond turtle *Clemmys mutica*; and *M. cophotis* from the elongated tortoise *Indotestudo elongate* (Oshmarin and Demshin 1972). In addition, *M. japonica* was found in a freshwater fish, yellowcheek (*Elopicthys bambusa*) as an accidental infection (Moravec and Sey 1988). Parasites of the genus *Stronguluris* have not been recorded in Vietnam.

In Chapter 3, I observed and compared the morphology of *Strongyluris calotis*, a heterakid nematode with cuticular flanges extend from the inner surface of lips, in the large

intestine of agamid lizards (*Japalura* spp. and *Calotes emma*) from the Oriental region (Japan, Taiwan and Singapore) by light microcopy and SEM. It was for the first time to clarify the accurate arrangement of cephalic and caudal papillae in *Strongyluris* nematodes worldwide, including currently 32 nominal species. Furthermore, although *S. calotis* was described as having 10 pairs of caudal papillae in the previous works including the original species description, we noticed a pair of united papilla structure and a pair of phasmids in this count of caudal papillae. When I collected and examined *S. calotis* specimens from *C. emma* living in the plain forest at low altitude, and *Pseudocalotes brevipes* living in the mountainous forest at high altitude in the northern part of Vietnam, the arrangement of caudal papillae in male worms from the former lizard was found to be comparable to classical *S. calotis* specimens from Japan, Taiwan and Singapore, but male worms from the latter lizards did not have a pair of united papillae but had 10 pairs of independent caudal papillae with a pair of phasmids. Molecular genetic analyses of the rDNA of worms of the classical *S. calotis* morphotype from Japan and Singapore and two *S. calotis* morphotypes from Vietnam demonstrated absolutely identical nucleotide sequences of 18S rDNA and 5.8S rDNA with moderate nucleotide diversity in the ITS regions (96.6–98.5 %) and 28S rDNA (99.6–99.7 %). These results indicate the usefulness of molecular genetic analyses to know the relationships among multiple isolates of different origins, particularly when the isolate shows extraordinary morphological characters (in our cases, numbers and arrangements of caudal papillae and other morphometric values).

In Chpeter 4, I characterized morphologically and genetically *Meteterakis* worms collected from the large intestine of scale-bellied tree lizards in Vietnam. The prevalence of *Meteterakis* worms in the lizards was high (23 positive lizards out of 32 examined lizards; 71.9%). Based on morphological criteria, collected *Meteterakis* specimens were divided into three morphotypes: (A) worms with alate spicules of intermediate length (0.45—0.58 mm); (B) worms with non-alate spicules of shorter length (0.33—0.49 mm); and (C) worms with non-alate spicules of longer length (0.62—0.72 mm). Morphotype A was found in 10 of 32 lizards (31.3%) from North to South Vietnam, whereas morphotypes B and C were found in 10 lizards (31.3%) and 4 lizards (12.5%), respectively, from the northern part of Vietnam. Genetic analyses based on the rDNA, i.e. the 18S, 28S, and ITS regions, consistently divided the collected worms into two major populations with some noticeable genetic diversity within each group, and determined the morphotypes B and C as a same population. An apparent discrepancy between morphological and genetic characterizations made the taxonomical

differentiation of *Meteterakis* populations in the scale-bellied tree lizard from Vietnam complicated. To solve such taxonomic complications, more collection of *Meteterakis* specimens widely from Asian lizards and amphibians, and intensive morphological as well as genetic analyses are necessary. Particularly when considering remarkable intraspecific variation in numbers and arrangements of caudal papillae are suggested in *Meteterakis* spp. (Purwaningsih et al. 2015), phylogenetic analyses of a wide spectrum of the nematodes may be fruitful to understand the significance of such variations and specific borders.

In Chapter 5, I list up parasite species recorded from Vietnamese lizards, which include a total of 45 parasite species (11 cestode species, 12 trematode species, 18 nematode species, 1 acanthocephalan species, and 3 pentastomida species) of 34 genera in 27 families. These parasites were recorded only from 10 lizard species in Vietnam. Therefore, we can easily suppose that we know an absolutely limited number of parasitic helminths in lizards distributed in Vietnam. More efforts should be devoted to our understanding of the real diversity of parasitic helminths in Vietnamese lizards.

In my thesis study, I characterized intensively the morphology and genetic background of lizard nematodes in Vietnam with extensive literature searches. As exemplified in Chapers 3 and 4, accurate understaning of the taxonomic status of Vietnamese parasites needs sampling of congeneous samples from amphibian hosts, or parasites from neighboring countries or widely in Asia or other regions. As a conclusion, I would like to emphasize the importance of research collaboration among researchers focusing on parasites of special hosts of different categories (e.g., reptiles and amphibians), as well as international research collaboration to compare the parasites at similar criteria. Considering scarcity of basic genetic data of lizard parasites, the genetic datasets on some species of Cosmocercoidae and Heterakidae which were obtained in my thesis study, would be useful widely for researchers who are interested in parasites of lizards or other vertebrates.

REFERENCE

1. Adamson, M. L. 1986. *Meteterakis vaucheri* n. sp. (Nematoda: Heterakoidea) from *Varanus grayi* (Varanidae) in the Philippines. Canadian Journal of Zoology 64: 814–817.
2. Ali, J. H., Riley, J. 1983. Experimental life-cycle studies of *Raillietiella gehyrae* Bovien, 1927 and *Raillietiella frenatus* Ali, Riley & Self, 1981: pentastomid parasites of geckos utilizing insects as intermediate hosts. Parasitology 86: 147–160.
3. Ali, J. H., Riley, J., Self, J. T. 1981. A revision of the taxonomy of the blunt-hooked *Raillietiella,* pentastomid parasites of African, South-East-Asian and Indonesian lizards, with a description of a new species. Systematic Parasitology 3: 193–207.
4. Ali, J. H., Riley, J., Self, J. T. 1982. A revision of the taxonomy of *Raillietiella boulengeri* (Vaney & Sambon, 1910) Sambon 1910, *R. orientalis* (Hett, 1915) Sambon, 1922 and *R. agcoi* Tubangui & Masiluñgan, 1956 (Pentastomida: Cephalobaenida). Systematic Parasitology 4: 285–310.
5. Ali, J. H., Riley, J., Self, J. T. 1985. A review of the taxonomy and systematics of the pentastomid genus *Raillietiella* Sambon, 1910 with a description of a new species. Systematic Parasitology 7: 111–123.
6. Almeida, W. O., Christoffersen, M. L., Amorim, D. S., Amorim, D. S., Eloy, E. C. C. 2008. Morphological support for the phylogenetic positioning of Pentastomida and related fossils. Biotemas 21: 81–90.
7. Amin, O. M., Nguyen, V. H., Heckmann, R. A. 2008. New and already known acanthocephalans from amphibians and reptiles in Vietnam, with keys to species of *Pseudoacanthocephalus* Petrochenko, 1956 (Echinorhynchidae) and *Sphaerechinorhynchus* Johnston and Deland, 1929 (Plagiorhychidae). Journal of Parasitology 94: 181–189.
8. Amin, O. M., Düşen, S., Oğuz, M. C. 2012. Review of the helminth parasites of Turkish anurans (Amphibia). Scientia Parasitologica 13: 1–16.
9. Ananjeva, N. B., Orlov, N. L., Truong, N. Q. 2007. Agamid lizards (Agamidae, Acrodonta, Sauria, Reptilia) of Vietnam. Mitteilungen aus dem Museum fur Naturkunde in Berlin. Zoologische Reihe 83 (Supplement): 13–21.

10. Ananjeva, N. B., Orlov, N. L., Kalyabina-Hauf, S. A. 2008. Species of *Acanthosaura* Gray, 1831 (Agamidae: Sauria. Reptilia) of Vietnam: results of molecular and morphological study. Biology Bulletin 35: 178–186.

11. Anderson, R. C., Chabaud, A. G., Willmott, S. 2009. Keys to the nematode parasites of vertebrates; archival volume. CAB International, Oxfordshire, UK, 463 pp.

12. Anisimova, M., Gascuel, O. 2006. Approximate likelihood-ratio test for branches: a fast, accurate, and powerful alternative. Systematic Biology 55: 539–552.

13. Arora, S., Agarwal, S. M. 1960. Studies on some intra-specific variations in *Paradistomum orientalis* Narain and Das collected from the gall-bladder of *Calotes versicolor* Daud: Part I. (Dicrocoeliidae: Trematoda). Bulletin of the Zoological Society of India 3: 43–52.

14. Arora, S., Agarwal, M. M., Agarwal, S. M. 1962. Studies on some intra-specific variations in *Paradistomum orientalis* Narain and Das collected from the liver and intestine of *Calotes versicolor* Daud: Part II. (Dicrocoeliidae: Trematoda). Indian Journal of Helminthology 14: 5–15.

15. Ávila, R. W., Strüssmann, C., Da Silva, R. J. 2010. A new species of *Cosmocercoides* (Nematoda: Cosmocercidae) from a gymnophthalmid lizard of western Brazil. Journal of Parasitology 96: 558–560.

16. Bain, O., Sulahian, A. 1974. Trois nouvelles Filaires du Oswaldofilaria chez des Lézards sud-américains; essai de classification des Oswaldofilariinae. Bulletin du Muséum national d'histoire naturelle. 3e série 156: 827–841.

17. Baker, M. R. 1984. The systematics and zoogeography of Spinicaudinae and Meteterakinae (Heterakoidea: Nematoda) parasitic in reptiles and amphibians. Systematic Parasitology 6: 275–287.

18. Baker, M. R. 1987. Synopsis of the Nematoda parasitic in amphibians and reptiles. Memorial University of Newfoundland, Occasional Papers in Biology 11: 1–325.

19. Barton, D. P. 2007. Pentastomid parasites of the introduced Asian house gecko, *Hemidactylus frenatus* (Gekkonidae), in Australia. Comparative Parasitology 74: 254–259.

20. Barton, D. P. 2015. Helminth parasites of the introduced Asian house gecko (*Hemidactylus frenatus*) (Gekkonidae), in the northern territory, Australia. Northern Territory Naturalist 26: 44–55.
21. Baylis, H. A. 1916. XIX.—The nematode genus *Tanqua*, R. Blanchard. Annal and Magazine of Natural History, series *8* 17: 223–232.
22. Baylis, H. A. 1931. Two more new Trichostrongylid nematodes from Queensland. Annal and Magazine of Natural History, series *10* 7: 500–507.
23. Baylis, H. A., Daubney, R. 1922. Report on the parasitic nematodes in the collection of the Zoological Society of India. Memoirs of the Indian Museum 7: 263–347.
24. Baylis, H. A., Daubney, R. 1923. Preliminary descriptions of three new parasitic nematodes. Annals and Magazine of Natural History, Series *9* 11: 333-335.
25. Ben Slimane, B., Chabaud, A. G., Durette-Desset, M.-C. 1996. Les Nématodes Trichostrongylina parasites d'Amphibiens et de Reptiles: problèmes taxonomiques, phylétiques et biogéographiques. Systematic Parasitology 35: 179–206.
26. Bhalerao, G. D. 1931. Two new trematodes from reptiles: *Paryphostomum indicum* n. sp. and *Stunkardia dilymphosa* n. g., n. sp. Parasitology 23: 99–108.
27. Bhardwaj, O. N. 1963. Morphological studies and taxonomic discussion of a new trematode *Pseudoartyfechinostomum larueiformis* n. g., n. sp. (Trematoda: Echinostomatidae). Proceedings of the National Academy of Sciences, India; Section B: Biological Sciences 33: 303–313.
28. Bolette, D. P. 1998. *Hastrospiculum spiralis* n. sp. (Nematoda: Diplotriaenoidea: Dicheilonematinae), from an Indonesian mangrove monitor, *Varanus indicus* (Reptilia: Sauria: Varanidae). Journal of Parasitology 84: 366–369.
29. Bray, R. A., Jones, A., Andersen, K. I. 1994. Order Pseudophyllidea Carus, 1863. pp.205–247. In: Keys to the Cestode Parasites of Vertebrates (Khalil, L. F., Jones, A. and Bray, R. A. eds.), CAB International, Oxfordshire, UK.
30. Bui, T. D., Bursey, C. R., Goldberg, S. R. 2009. A new species of *Thelandros* (Nematoda, Oxyuroidea, Pharyngodonidae) in *Leiolepis reevesii* (Sauria, Agamidae) from Vietnam. Acta Parasitologica 54, 151–153.
31. Bursey, C. R., Brooks, D. R. 2011. Nematode parasites of Costa Rican snakes (Serpentes) with description of a new species of *Abbreviata* (Physalopteridae). Comparative Parasitology 78: 333–358.

32. Bursey, C. R., Goldberg, S. R. 1996. *Pharyngodon lepidodactylus* sp. n. (Nematoda: Pharyngodonidae) from the mourning gecko, *Lepidodactylus lugubris* (Lacertilia: Gekkonidae), from Hawaii. Journal of the Helminthological Society of Washington 63: 51–55.
33. Bursey, C. R., Goldberg, S. R. 1999a. *Pharyngodon oceanicus* sp. n. (Nematoda: Pharyngodonidae) from the oceanic gecko, *Gehyra oceanica* (Sauria: Gekkonidae) of the Pacific Islands. Journal of the Helminthological Society of Washington 66: 37–40.
34. Bursey, C. R., Goldberg, S. R. 1999b. *Spauligodon ovifilus* n. sp. (Nematoda: Pharyngodonidae) and other helminths from *Diplodactylus stenodactylus* (Reptilia: Gekkonidae) from Australia. Journal of Parasitology 85: 898–902.
35. Bursey, C. R., Goldberg, S. R. 2005. Two new species of Pharyngodonidae (Nematoda: Oxyuroidea) and other nematodes in *Agama caudospina* (Squamata: Agamidae) from Kenya, Africa. Journal of Parasitology 91: 591–599.
36. Bursey, C. R., Goldberg, S. R. 2011. Helminths of the Mao-Son Frog, *Hylarana maosonensis* (Anura: Ranidae), from Vietnam. Comparative Parasitology 78: 373–374.
37. Bursey, C. R., Goldberg, S. R., Telford, S. R., Jr. 2003. *Strongyluris panamaensis* n. sp. (Nematoda: Heterakidae) and other helminthes from the lizard, *Anolis biporcatus* (Sauria: Polychrotidae), from Panama. Journal of Parasitology 89: 118–123.
38. Bursey, C. R., Goldberg, S. R., Kraus, F. 2005a. New species of *Spauligodon* (Nematoda: Pharyngodonidae) in *Lepidodactylus novaeguineae* (Sauria: Gekkonidae) from Papua New Guinea. Journal of Parasitology 91: 324–328.
39. Bursey, C. R., Goldberg, S. R., Kraus, F. 2005b. Endoparasites in *Sphenomorphus jobiensis* (Sauria: Scincidae) from Papua New Guinea with description of three new species. Journal of Parasitology 91: 1385–1394.
40. Bursey, C. R., Goldberg, S. R., Telford, S. R., Jr. 2007. Gastrointestinal helminths of 14 species of lizards from Panama with descriptions of 5 new species. Comparative Parasitology 74: 108–140.
41. Bursey, C. R., Goldberg, S. R., Kraus, F. 2008. A new species of *Pharyngodon* (Nematoda, Pharyngodonidae) and other helminths in *Cyrtodactylus louisiadensis* (Sauria, Gekkonidae) from Papua New Guinea. Acta Parasitologica 53: 41–45.
42. Bursey, C. R., Goldberg, S. R. Telford, S. R., Jr. 2013. A new species of *Strongyluris* (Nematoda: Heterakidae) from the lizard, *Lophognathus temporalis* (Sauria: Agamidae) from Papua New Guinea. Journal of parasitology 99: 1024–1027.

43. Bursey, C. R., Goldberg, S. R., Grismer, L. L. 2014. A new species of *Spauligodon* (Nematoda: Oxyuroidea: Pharyngodonidae) in *Cyrtodactylus bintangrendah* (Sauria: Gekkonidae) from peninsular Malaysia. Journal of Parasitology 100: 317–322.
44. Bursey, C. R., Goldber,g S. R., Grismer, L. L. 2015a. New species of *Oswaldofilaria* (Nematoda; Filarioidea: Onchocercidae) and other helminths in *Acanthosaura cardamomensis* (Sauria; Agamidae) from Indochina Peninsula. Acta Parasitologica 60: 112–115.
45. Bursey, C. R., Goldber,g S. R., Grimer, L. L. 2015b. New species of *Cosmocercoides* (Nematoda: Cosmocercidae) and other helminths in *Gonocephalus liogaster* (Squamata; Agamidae) from Peninsular Malaysia. Acta Parasitologica 60: 631–637.
46. Bursey, C. R., Goldberg, S. R., Cameron, D. S., Rafe, M. B. 2015c. A new species of *Cosmocerca* (Nematoda: Cosmocercidae) and other helminths in *Cyrtodactylus gubaot* (Squamata: Gekkonidae) from the Philippines. Acta Parasitologica 60: 675–681.
47. Capron, A., Deblock, S., Brygoo, E. R. 1961. Miscellanea helminthological Madagascariensis. Trématodes de caméléons de Madagascar. Archives de l'Institut Pasteur de Madagascar 29: 1–71.
48. Chabaud, A. G. 2009a. Ascaridida: Cosmocercoidea, Seuratoidea and Heterakoidea. In: AndersonRC, Chabaud AG, Willmott S (eds) Keys to the nematode parasites of vertebrates; archival volume. CAB International, Oxfordshire, UK, 248–308 pp.
49. Chabaud, A. G. 2009b. Spirurida: Camallanoidea, Dracunculoidea, Gnathostomatoidea, Physalopteroidea, Rictularoidea and Thelazioidea. pp.334–360. In: Keys to the nematode parasites of vertebrates; archival volume (Anderson, R. C., Chabaud, A. G. and Willmott, S. eds.), CAB International, Oxfordshire, UK.
50. Chakravorty, G. K. 1936. A new nematode *Strongyluris bengalensis* n. sp. with a note on the genus. Zeitschrift für Parasitenkunde 8: 542–545.
51. Christoffersen, M. L., De Assis, J. E. 2013. A systematic monograph of the recent Pentastomida, with a compilation of their host. Zoologische Mededelingen, Leiden 87: 1–206.
52. CITES Scientific Authority of Vietnam. 2008. Report on the review of Vietnam's wildlife trade policy. Hanoi, 70 pp.
53. Criscione, C. D., Font, W. F. 2001. Development and specificity of *Oochoristica javaensis* (Eucestoda: Cyclophyllidea: Anoplocephalidae: Linstowiinae). Comparative Parasitology 68: 149–155.
54. Curran, S. S., Overstreet, R. M., Dang, T. T., Nguyen T. L. 2001. *Singhiatrema*

vietnamensis sp. n. (Digenea: Ommatobrephidae) and *Szidatia taiwanensis* (Fischthal and Kuntz, 1975) comb. n. (Digenea: Cyathocotylidae) from colubrid snakes in Vietnam. Comparative Parasitology 68: 219–227.

55. Dang, T. T. 2000. Ecological and biological characteristics of *Raillietiella orientalis* (Hett, 1915) Sambon, 1922 (Pentastomida) parasiting in snakes. Journal of Biology, Hanoi 22: 27–32. (in Vietnamese with English summary)
56. Darevsky, I. S., Orlov, N. L. 1994. Eine bemerkenswerte neue, grobwüchsige Art der Gattung Gekko; *Gekko ulikovskii* sp. n. aus Zentral Vietnam. Salamandra 30: 71–75.
57. De, N. C., Dey, J. 1992. A scanning electron microscope study of *Raillietascaris varani* (Baylis et Daubney, 1922) (Nematoda: Ascaridioidea). Folia Parasitologica 39: 145–151.
58. de Chambrier, A. 2006. Redescription of *Kapsulotaenia sandgroundi* (Carter, 1943) (Eucestoda: Proteocephalidea: Acanthotaeniinae), a parasite of *Varanus komodoensis* (Varanoidea: Varanidae) from Indonesia. Systematic Parsitology 63: 83–93.
59. de Chambrier A, Waeschenbach, A., Fisseha, M., Scholz, T., Mariaux, J. 2015. A large 28S rDNA-based phylogeny confirms the limitations of established morphological characters for classification of proteocephalidean tapeworms (Platyhelminthes, Cestoda). ZooKeys 500: 25–59.
60. Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J. F., Guindon, S., Lefort, V., Lescot, M., Claverie, J. M., Gascuel, O. 2008. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Research 36: 465–469.
61. Dervin, S., Sanchez, M., Bursey, C. R., Goldberg, S. R. 2014. First record of *Raillietiella affinis* Bovien, 1927 (Arthropoda: Raellietiellidae) as an endoparasite of the Madagascan giant day gecko, *Phelsuma grandis* Gray, 1870 introduced at Reunion Island. Herpetology Notes 7: 779–781.
62. Diesing, K. M. 1861. Revsion der Nematoden. In: Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften. Mathematisch-naturwissenschaftliche Classe. 42 (Suppl.): 595–738.
63. Dronen, N. O., Calhoun, D. M., Simcik, S. R. 2012. *Mesocoelium* Odhner, 1901 (Digenea: Mesocoelidae) revisited; a revision of the family and re-evaluation of species composition in the genus. *Zootaxa* 3387: 1–96.
64. Dugarov, Zh. N., Baldanova, D. R., Khamnueva, T. R. 2012. Tapeworm *Oochoristica tuberculata* (Rudolphi, 1819)—Parasite of the lizard *Eremias argus* Peters, 1869 in Zabaikalie. Parazitologiia 46: 463–471. (in Russian with English summary)

65. Durette-Desset, M.-C. 1980. Nouveaux Nématodes Trichostrongyloïdes parasites de Sauriens en Malaisie et à Bornéo. Bulletin du Muséum national d'Histoire naturelle, 4 ème, série, 2, section A 3: 697–706.
66. Durette-Desset, M.-C., Ben Slimane, B., Cassone, J., Barton, D. P., Chabaud, A. G. 1994. *Johnpearsonia* gen. nov. and Johnpearsoniinae subf. nov. (Molineoidea, Nematoda) from *Bufo marinus*, with comments on the primitive Trichostrongyle parasites of amphibians and reptiles. Parasite 1: 153–160.
67. Fenner, A. L., Smales, L. R., Bull, C. M. 2008. *Pharyngodon wandillahensis* n. sp. (Nematoda: Pharyngodonidae) from the endangered pygmy bluetongue lizard *Tiliqua adelaidensis* Peters, 1863 (Sauria: Scincidae), South Australia, Australia. Comparative Parasitology 75: 69–75.
68. Fischthal, J. H., Kuntz, R. E. 1964. A monogenetic and seven digenetic trematodes of amphibians and reptiles from Palawan Island, Philippines. Proceedings of the Helminthological Society of Washington 31: 230–240.
69. Fischthal, J. H., Kuntz, R. E. 1965. Digenetic trematodes of amphibians and reptiles from North Borneo (Malaysia). Proceedings of the Helminthological Society of Washington 32: 124–136.
70. Gibbons, L. M. 1986. SEM Guide to the Morphology of Nematode Parasites of Vertebrates. CAB International, Farnham Royal, Slough, U.K., 199 pp.
71. Gibbons, L. M., Keymer I. F. 1991. Redescription of *Tanqua tiara* (Nematoda, Gnathostomidae), and associated lesions in the stomach of the Nile monitor lizard (*Varanus niloticus*). Zoologica Scripta 20: 7–14.
72. Goldberg, S. R., Bursey, C. R. 1996. New helminth records for the mourning gecko, *Lepidodactylus lugubris* (Gekkonidae) from Hawaii. Occasional Papers of the Bernice Pauahi *Bishop* Museum of Polynesian Ethnology and Natural History 49: 54–56.
73. Goldberg, S. R., Bursey, C. R. 2005. *Microhyla heymonsi* (Dark-sided Chorus Frog). Endoparasites. Herpetological Review 36: 303.
74. Goldberg, S. R., Bursey, C. R. and McAllister, C. T. 1995. Gastrointestinal helminths of nine species of *Sceloporus* lizards (Phrynosomatidae) from Texas. Journal of the Helminthological Society of Washington 62: 188–196.
75. Goldberg, S. R., Bursey, C. R., Telford, S. R. 2003. Metazoan endoparasites of 11 species of lizards from Pakistan. Comparative Parasitology 70: 46–54.
76. Guerrero, R. 2013. Two new species of *Oswaldocruzia* (Nematoda: Trichostrongylina: Molineoidea) parasites of the cane toad *Rhinella marina* (Amphibia: Anura) from Peru.

Acta Parasitologica 58: 30–36.

77. Guindon, S., Gascuel, O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52: 696–704.
78. Gupta, N. K., Mehrotra, V. 1977. Redescription of *Encyclometra columbrimurorum* (Rudolphi, 1819) with remarks on the synonymy of *E. vitellata* Gupta, 1954. Folia Parasitologica 24: 179–182.
79. Hallermann, J., Truong, N. Q., Orlov, N., Ananjeva, N. 2010. A new species of the genus *Pseudocalotes* (Squamata: Agamidae) from Vietnam. Russian Journal of Herpetology 17: 31–40
80. Hasegawa, H. 1984. Helminth fauna of five Okinawan amphibian species. Biological Magazine, Okinawa 22: 11–22. (in Japanese with English summary).
81. Hasegawa, H. 1989. Nematodes of Okinawan amphibians and their host-parasite relationship. In: Matsui M, Hikida T, Goris RC (eds.), Current herpetology in East Asia. Herpetological Society of Japan, Kyoto, Japan, pp. 205–217.
82. Hasegawa, H. 1990. Helminths collected from amphibians and reptiles on Amami-oshima island. Memoirs of the National Science Museum, Tokyo 23: 83–92.
83. Hasegawa, H. 1992. Parasitic helminthes collected from amphibians and reptiles on Kume-jima Island, Okinawa, Japan. Biological Magazine Okinawa 30: 7–13.
84. Hasegawa, H., Ikeda, Y. 2007. *Paracosmocercella rosettae* n. gen. et n. sp. (Nematoda: Cosmocercoidea: Cosmocercidae) collected from the Japanese tree frog, *Hyla japonica* (Anura: Hylidae), in Japan. Journal of Parasitology 93: 884–888.
85. Hasegawa, H., Iwatsuki, N. 1984. Helminth fauna of the tree lizard, *Japarula polygonata* in Okinawa Prefecture, Japan. Akamata 2: 18–26. (in Japanese with English summary).
86. Hsü, H. F. 1933. On some parasitic nematodes collected in China. Parasitology 24: 512–541.
87. Hsü, H. F., Hoeppli, R. 1931. Parasitic nematodes mostly from snakes collected in China. National Medical Journal of China 17: 567–588.
88. Hunt, D. J., Spiridonov, S. E. 2001. A new genus of Carnoyidae (Nematoda: Rhigonematida) with description of *Cattiena trachelomegali* gen. n., sp. n. and *C. trigoniuli* gen. n., sp. n., parasites of spirobolid (Diplopoda) from Vietnam. Nematology 3: 559–571.
89. Hunt, D. J., Luc, P. V., Spiridonov, S. E. 2002. Bizarre Ichthyocephalidae (Nematoda: Rhigonematida) from Vietnam with description of *Ichthyocephaloides comatus* sp. n.,

Xystrongnathus priscus gen. n., sp. n. and *X. phrissus* gen. n., sp. n. Nematology 4: 829–843.

90. Imkongwapang, R., Jyrwa, D. B., Lal, P., Tandon, V. 2014. A checklist of helminth parasite fauna in anuran Amphibia (frogs) of Nagaland, Northeast India. Journal of Parasitic Diseases 38: 85–100.
91. Inglis, W. G. 1957. A review of the nematode superfamily Heterakoidea. Annals and Magazine of Natural History (Series 12) 10: 905–912.
92. Inglis, W. G. 1958. A revision of the nematode genus *Meteterakis* Karve, 1930. Parasitology 48: 9–31.
93. IUCN. 2010. IUCN Red List of Threatened Species. Downloaded from the IUCN website [http://www.iucnredlist.org/] (Checked on May 1, 2016)
94. Ivanov, V. M., Semenova, N. N. 2000. Species composition and ecological peculiarities of trematodes from reptiles in the Volga delta. Parasitologia 34: 228–233. (in Russian with English summary)
95. Jensen, L. A., Schmidt, G. D., Kuntz, R. E. 1983. A survey of cestodes from Borneo, Palawan, and Taiwan, with special reference to three new species. Proceedings of the Helminthological Society of Washington 50: 117–134.
96. Jiang, M., Lin, J. 1980. A study of the nematodes in the lizards, *Japalura swinhonis formosensis* and *Hemidactylus*. Biological Bulletin Department of Biology College of Science Tunghai University 53: 1–30.
97. Jones, A. 2005. Family Diplodiscidae Cohn, 1904. pp.319-324. In: Keys to the Trematoda, Volume 2 (Jones, A., Bray, R. A. and Gibson, D. I. eds.), CABI Publishing and The Natural History Museum, London, U.K.
98. Jones, H. I. 1978. *Abbreviata* (Nematoda: Physalopte- roidae) from Western Australian snakes. Australian Journal of of Zoology 26:789–807.
99. Jones, H. I. 1979. Gastrointestinal nematodes, including three new species, from Australian and Papua New Guinean pythons. Proceedings of the Helminthological Society of Washington 46: 1–14.
100. Jones, H. I. 1988. Nematodes from nine species of *Varanus* (Reptilia) from tropical northern Australia, with particular reference to the genus *Abbreviata* (Physalopteridae). Australian Journal of of Zoology 36: 691–708.
101. Jones, H. I. 2007. Helminth parasites of the lace monitor *Varanus varius* (Reptilia: Varanidae) in Eastern Australia. Transactions of the Royal Society of South Australia 132: 160–166.

102. Jones, H. I. 2010. Dwarf monitor lizards (Varanidae: *Varanus*, *Odatria*, s. gen.) as definitive and paratenic hosts for physalopteran nematodes. *Aust. J. Zool.* 58: 69–75.

103. Jones, H. I. 2013. Gastrointestinal nematodes from three species of Australian leaf-tailed geckos (Reptilia: *Saltuarius* spp.), with descriptions of new species of *Skrjabinodon* (Oxyuroidea: Pharyngodonidae) and *Hedruris* (Habronematoidea: Hedruruidae). Comparative Parasitology 80: 47–59.

104. Junker, K., Mariaux, J., Measey, G. J., Mutafchiev, Y. 2015. *Meteterakis saotomensis* n. sp. (Nematoda: Heterakidae) from *Schistometopum thomense* (Bocage) (Gymnophiona: Dermophiidae) on São Tomé Island. Systematic Parasitology 92: 131–139.

105. Kalyankar, K. V., Nanware, S. S. 2010. Studies on diphyllidean cestode genus *Scyphocephalus* (Riggenbach, 1898) (Family-diphyllobothriidae Luhe, 1910) from *Varanus salvator* with description of a new species. Asian Journal of Animal Science 5: 33–35.

106. Kanev, I., Radev, V., Fried, B. 2005. Family Philophthalmidae. pp.87-97. In: Keys to the Trematoda, Volume 2 (Jones, A., Bray, R. A. and Gibson, D. I. eds.), CABI Publishing and The Natural History Museum, London, U.K.

107. Karve, J. N. 1930. Some parasitic nematodes of frogs and toads. Annals of Tropical Medicine and Parasitology 24: 481–491.

108. Karve, J. N. 1938. Some nematode parasites of Lizards. In: Livro jubilar do Professor Lauro Travassos. Editado para commenmorar o 25 anniversario de suas actividades scientificas (1913-1938), Rio de Janeiro, Brazil, pp. 251–258.

109. Karve J.N. 1944. On a small collection of parasitic nematodes from Anura. Proceedings of the Indian Academy of Science, section B, 19: 71–77.

110. Kelehear, C., Spratt, D. M., Dubey, S., Brown, G. P., Shine, R. 2011. Using combined morphological, allometric and molecular approaches to identify species of the genus *Raillietiella* (Pentastomida). PLoS ONE 6: e24936 [DOI: 10.1371/journal.pone.0024936]

111. Kelehear, C., Spratt, D. M., O'Meally, D., Shine, R. 2014. Pentastomids of wild snakes in the Australian tropics. International journal *for* parasitology. Parasites *and* wildlife 3: 20–31.

112. Khera, S. 1958. On a new species of *Cosmocercoides*. Indian Journal of Helminthology 10: 6–12.

113. Killick, L. M., Beverley-Burton, M. 1982. Observations on digeneans from lizards (Sauria) in Indonesia (*Paradistomum geckonum*, *Mesocoelium sociale*, and

Postorchigenes ovatus) with a revision of *Paradistomum* Kossack, 1910 (Dicrocoeliidae). *Can. J. Zool.* 60: 2093–2106.

114. Kostadinova, A. 2005. Family Echinostomatidae Looss, 1899. pp.9-64. In: Keys to the Trematoda, Volume 2 (Jones, A., Bray, R. A. and Gibson, D. I. eds.), CAB International and Natural History Museum, London, UK.
115. Kuzmin, Y., Kinsella, J. M., Tkach, V. V., Bush, S. E. 2013. New species of *Kalicephalus* (Nematoda: Diaphanocephalidae) from a snake, *Oxyrhabdium leporinum*, on Luzon Island, Philippines. Comparative Parasitology 80: 240–246.
116. Lakshmipyari, W., Gambhir, R. K., Tarnita, T. 2011. A new species of the genus *Strongyluris* Müller 1894, from the garden lizard, *Calotes versicolor* (Daudin) from Manipur, India. Uttar Pradesh Journal of Zoology 31: 383–388.
117. Lan Anh, N. T., Phuong, N. T., Johansen, M. V., Murrell, K. D., Phan, T. V., Dalsgaard, A., Luong, T. T., Thamsborg, S. M. 2009. Prevalence and risks for fishborne zoonotic trematode infections in domestic animals in a highly endemic area of North Vietnam. Acta Tropica 112: 198–203.
118. Le, V. H., Nguyen, V. L. 1966. A new Physaloptera, *Abbreviata deschiensi* n. sp., parasite of reptiles, *Calotes versicolor* (Daud.), in South Vietnam. Bulletin de la Société de pathologie exotique 59: 991–996.
119. Le, V. H., Pham, N. K. 1968. Les kalicéphales, *parasites* des *vipères*, Agkístrodon rhodostoma B. du Sud-Vietnam. Bulletin de la Société de pathologie exotique 61: 661–672.
120. Lewin, J. 1992a. Parasites of the sand lizard (*Lacerta agilis* L.) in Poland. Acta Parasitologica 37: 19–24.
121. Lewin, J. 1992b. Parasites of *Lacerta vivipara* Jacquin, 1787 in Poland. Acta Parasitologica 37: 79–82.
122. Liu, W., Li, Q.-K., Shih, H.-H., Qiu, Z.-Z. 2002. *Meristocotyle provitellaria* sp. nov. (Digenea: Meristocotylidae) from *Varanus salvator* in China. Zoological Studies 41: 283–287.
123. Liu, W., Wang, T., Liu, T. B., Tan, L., Lv, C. C., Liu, Y. 2016. The occurrence of the strongylid nematodes *Kalicephalus brachycephalus*, *K. bungari* and *K. indicus* in snake species from Hunan Province, China. Journal of Helminthology (in press) [Epub ahead of print: May 18 2016].

124. Lotz, J. M., Font, W. F. 2008. Family Phaneropsolidae Mehra, 1935. pp.545–562. In: Keys to the Trematoda, Volume 3 (Bray, R. A., Gibson, D. I. and Jones, A. eds.), CAB International and Natural History Museum, London, UK.

125. Mackerras, M. J. 1962. Filarial parasites (Nematoda: Filarioidea) of Australian animals. Australian Journal of of Zoology 10: 400–457.

126. Madhavi R., Nirmala E., Subbalakshmi C. 1998. A population study of the dicrocoeliid trematode *Paradistomum orientalis* in the garden lizard *Calotes versicolor*. Journal of Zoology, London 244: 489–496.

127. Makouloutou P., Setsuda A., Yokoyama M., Tsuji T., Saita E., Torii H., Kaneshiro Y., Sasaki M., Maeda K., Une Y., Hasegawa H., Sato H. 2013. Genetic variation of *Gongylonema pulchrum* from wild animals and cattle in Japan based on ribosomal RNA and mitochondrial cytochrome *c* oxidase subunit I genes. Journal of Helminthology 87: 326–335.

128. Malysheva, S. V., Luc, P. V. 2012. *Cattiena fansipanis* n. sp. (Nematoda: Rhigonematida: Carnoyidae) from a millipede (Myriapoda: Diplopoda: Spirobolida) in North Vietnam. Systematic Parasitology 81: 135–146.

129. Malysheva, S. V., Spiridonov, S. E. 2010. Four new species of *Heth* Cobb, 1898 (Rhigonematida: Hethidae) from Vietnamese diplopods. Nematology 12: 567–585.

130. Maplestone, P. A. 1931. Parasitic nematodes obtained from animal dying in the Calcutta Zoologicl Gardens: the genus *Kalicephalus* in Indian reptiles (6). Bulletin of the Zoological Survey *of* India 33: 104–125.

131. Matsuo, K., Oku, Y. 2002. Endoparasites of three species of house geckoes in Lampung, Indonesia. Journal of Helminthology 76: 53–57.

132. Moravec, F., Sey, O. 1985. Some nematode parasites of frogs (*Rana* spp.) from North Vietnam. Parasitologia Hungarica 18: 63–77.

133. Moravec, F., Sey, O. 1988. Nematodes of freshwater fishes from North Vietnam. Part 3. Cosmocercoidea, Seuratoidea, Atractoidea, Heterahoidea and Ascaridoidea. Věstník Československé společnosti zoologické 52: 250–265.

134. Nadler, S. A., Carreno, R. A., Mejia-Madrid, H., Ullberg, J., Pagan, C., Houston, R., Hugot, J. P. 2007. Molecular phylogeny of clade III nematodes reveals multiple origins of tissue parasitism. Parasitology 134: 1421–1442.

135. Ngo, H. D., Ermolenko, A. V., Gerasev, P. I. 2010. On the fauna of trematodes from the family Dicrocoeliidae (Plagiorchidida) parasitizing animals in Vietnam. Parazitologiia 44: 515–524. (in Russian with English summary)

136. Nguyen, Q. T. 2006. Herpetological collaboration in Vietnam. In: Herpetologia Bonnensis II. (Vences, M., Köhler, J., Ziegler, T., Böhme, W. eds.), Proceedings of the 13th Congress of the Societas Europaea Herpetologica, p. 233–240.

137. Nguyen, Q. T. 2011. Systematics, ecology and conservation of the lizard fauna in northeastern Vietnam, with special focus on the genera *Pseudocalotes* (Agamidae), *Goniurosaurus* (Eublepharidae), *Sphenomorphus* and *Tropidophorus* (Scincidae) from this country. – Ph. D. thesis, Univ. Bonn.

138. Nguyen Q. T., Schmitz, A., Böhme, W. 2010. Gekko ulikovskii Darevsky & Orlov, 1994: a junior synonym of Gekko badenii Szczerbak & Nekrasova, 199. Bonn zoological Bulletin 57: 15–17.

139. Nguyen, T. K. 2003. Cestode parasite in human and mammals in Viet Nam. Fauna of Vietnam, Vol. 3. Science and Technology Publishing House, Hanoi, 280 pp. (in Vietnamese)

140. Nguyen, T. L., Ha, D. N. 2010. Trematoda parasite in vertebrate of Vietnam. Science and Technology Publishing House, Hanoi, 288 pp. (in Vietnamese with English summary)

141. Nguyen, T. L., Pham, N. D. 2005. Three trematode species parasitic in two reptile species living closed to human in Vietnam. Journal of Biology, Hanoi 37: 17–19. (in Vietnamese with English summary)

142. Nguyen, T. L., Pham, V. L., Ha, D. N., Nguyen V. D., Nguyen T. M. 1996a. Helminths of domestic animals in Vietnam. Science and Technology Publishing House, Hanoi, 296 pp. (in Vietnamese).

143. Nguyen, T. L., Nguyen, T. K., Pham, V. L., Ha, D. N., Nguyen T. M. 1996b. Helminths ò poultry in Vietnam. Science and Technology Publishing House, Hanoi, 192 pp. (in Vietnamese).

144. Nguyen, T. L., Nguyen, T. M., Pham, N. D., Tran, B. T., Pham, V. L. 2003. Diversity on parasites of two monitor species (*Varanus nebulosus* and *Varanus salvator*) in Vietnam. pp. 157–159. In: Proceedings of the 2nd National Conference in Biology, Agriculture and Medicine, Hue University, July 25–26. 2003. (in Vietnamese with English summary)

145. Nguyen, T. L., Nguyen, T. M., Pham, N. D., Tran, B. T., Dang, T. T. 2005. Biodiversity of parasites in some reptile species closed to human. pp.215–217. In: Proceedings of the 2nd National Conference in Life Sciences, Hanoi, October 26, 2005. (in Vietnamese with English summary)

146. Nguyen, T. L., Nguyen, T. M., Tran, B. T., Bui, T. D. 2008. [Preliminary study on parasites in reptiles from northern part of central Vietnam.] Journal of Biology, Hanoi 30: 18–22. (in Vietnamese with English summary)
147. Nguyen, T. M. 2002. Parasitic nematodes in giant lizard from Vietnam. Journal of Biology, Hanoi 24: 133–141. (in Vietnamese with English summary)
148. Nguyen T. M., Bui, T.D. 2007. Parasitic nematodes of house gecko, *Hemydactylus frenatus* Schlegel et Bibron, 1836 and skink, *Maybuya longycaudata* (Hallowell, 1857) in Vietnam. pp.179–183. In: Proceedings of the 2nd National Scientific Conference on Ecology and Biological Resources, Hanoi, October 26, 2007. (in Vietnamese with English summary)
149. Nguyen, V. H. 2015. An updated list of acanthocephalans (Acanthocephala) from animals in Vietnam. *J. Biol. Hanoi* 37: 384–394. (in Vietnamese with English summary)
150. Nguyen, V. S., Ho, T. C. 1996. A checklist of reptiles and amphibians of Vietnam. Science and Technology Publishing House, Hanoi, 264 pp. (in Vietnamese)
151. Nguyen, V. S., Ho, T. C., Nguyen, Q. T. 2005. A checklist of reptiles and amphibians of Vietnam. Science and Technology Publishing House, Hanoi, 180 pp. (in Vietnamese)
152. Nguyen, V. S., Ho, T. C., Nguyen, Q. T. 2009. Herpetofauna of Vietnam. Edition Chimaira. Chimaira Buchhandelsgesellschaft mbH, Frankfurt am Main, Germany, 768 pp.
153. Norval, G., Goldberg, S. R., Bursey, C. R., Mao, J.-J., Slater, K. 2014. Internal parasites of lizards from Taiwan. Herpetological Conservation *and* Biology 9: 484–494.
154. Ochi, S. 1930. [A new trematode, *Mesocoelium brevicaecum* n. sp., from a Japanese snail intermediate host, with special reference to its life-cycle]. Okayama Medical Journal 42: 388–402. (in Japanese with German summary).
155. Okulewicz, A., Kaźmierczak, M., Hildebrand, J., Adamczyk, M. 2015. Endoparasites of lizards (Lacertilia) from captive breeding and trade networks. Helminthologia 52: 34–40.
156. Oshmarin, P. G., Demshin, N. I. 1972. The helminths of domestic and some wild animals of the Vietnam Democratic Republic. Trudy Biologicheskogo-Pochvennogo Instituta Vladivostok 11: 5–115 (In Russian).
157. Ota, H. 1991. Taxonomic redefinition of *Japalura swinhonis* Günther (Agamidae: Squamata), with a description of a new subspecies of *J. polygonata* from Taiwan. Herpetologica 47: 280–294.
158. Palmieri, J. R., Sullivan, J. T. 1977. *Stunkardia minuta* sp. n. (Trematoda: Paramphistomidae) from the Malayan box-tortoise *Cuora amboinensis.* Journal of Helminthology 51: 121–124.

159. Petit, G., Bain, O., Gomes, A. F. ~~and~~ Toubatier, L. 1983. *Piratuboides huambensis* n. sp., Filaire Oswaldofilariinae parasite de Lézard en Afrique australe. Bulletin du Muséum national d'Histoire naturelle, Paris, 4 ème 5: 743–747.

160. Petter, A. J, Quentin, J.-C. 2009. Oxyurida. In: *Keys to the Nematode Parasites of Vertebrates: Archival Volume*, Anderson, R. C., Chabaud, A. G. and Willmott, S. CAB International, Oxfordshire, Oxford, U.K., pp.218–247.

161. Pham, N. D., Nguyen, T. L. 2003. Trematodes parasiting in giant lizards in Vietnam. Journal of Biology, Hanoi 25: 5–10.

162. Pham, N. D., Nguyen, T. L. 2007. Discovery of *Plagiorchis (Plagiorchis) milini* Lent et Freitas, 1946 (Trematoda: Plagiorchiidae) from house gecko (*Hemidactylus frenatus* Schelegel at Bibron, 1836) in Vietnam. pp.38–39. In: Proceedings of the 2007 National Conference on Life Sciences, Quy Nhon University, August 10, 2007. (in Vietnamese with English summary)

163. Phan, T. V., Nguyen, T. L., Nguyen, T. K. 1977. Helminths parasite in Vietnamese mammals. Science and Technology Publishing House, Hanoi, 362 pp. (in Vietnamese)

164. Premvati, G., Pande, V. 1974. On *Artyfechinostomum malayanum* (Leiper, 1911) Mendheim, 1943 (Trematoda: Echinostomatidae) with synonymy of allied species and genera. Journal of the Helminthological Society of Washington 41: 151–160.

165. Purwaningsih, E., Mumpuni. 2011. New host and locality records of snake intestinal nematode *Kalicephalus* spp. in Indonesia. Asian Pacific Journal of Tropical Biomedicine 1: 121–123.

166. Purwaningsih, E., Dewi, K., Hasegawa, H. 2015. Nematodes of amphibians from Java, Indonesia, with a description of new species, *Meteterakis wonosoboensis* n. sp. (Nematoda: Heterakoidea). Zootaxa 3974: 507–516.

167. Ramallo, G., Bursey, C. R., Goldberg, S. R. 2002. *Spauligodon loboi* n. sp. (Nematoa: Pharyngodonidae) parasite of *Liollaemus* spp. (Iguania: Liolaemidae) from northwestern Argentina. Journal of Parasitology 88: 370–374.

168. Ramallo, G., Bursey, C. R., Goldberg, S. R. 2007. Two new species of cosmocercids (Ascaridida) in the toad *Chaunus arenarum* (Anura: Bufonidae) from Argentina. Journal of Parasitology 93: 910–916.

169. Read, C. P., Amerin, Y. U. 1953. North American nematodes of the genus *Pharyngodon* Diesing (Oxyuridae). Journal of Parasitology 39: 365–370.

170. Rego, A. A. 1994. Order Proteocephalidea Mola, 1928. pp.257–293. In: Keys to the Cestode Parasites of Vertebrates (Khalil, L. F., Jones, A. and Bray, R. A. eds.), CAB

International, Oxfordshire, UK.

171. Riggenbach, E. 1898. *Scyphocephalus bisulcatus* n. g. n. sp., ein neuer Reptiliencestode. Zoologische Jahrbücher. Abteilung für Systematik, Geographie und Biologie der Tiere 12: 145–153.

172. Riley, J., McAllister, C. T., Freed, P. S. 1988. *Raillietiella taegueselfi* n. sp. (Pentastomida: Cephalobaenida) from the Mediterranean gecko, *Hemidactylus turcicus* (Sauria: Gekkonidae), in Texas. Journal of Parasitology 74: 481–486.

173. Rizvi, A. N. 2009. Two new species of amphibian nematodes from Bhadra Wildlife Sanctuary, Western Ghats, India. Zootaxa 2013: 58–68.

174. Saehoong, P., Wongsawad, C. 1997. Helminths in house lizards (Reptilia: Gekkonidae). Southest Asian Journal of Tropical Medicne and Public Health 28: 184–189.

175. Santos, J. N., Giese, E. G., Maldonado, A., Jr., Lanfredi, R. M. 2008. A new species of *Oswaldocruzia* (Molineidae: Nematoda) in *Chaunus marinus* (Amphibian: Bufonidae) (Linneaus, 1758) from Brazil. Journal of Parasitology 94: 264–268.

176. Santos, J. N., Melo, F. T. V., Nazaré, L. C., Furtado, A. P., Giese, E. G. 2013. *Strongyluris amazonicus* n. sp. (Nematoda: Heterakidae): A parasite of *Tropidurus oreadicus* from the Brazilian Amazon. ACTA Tropica 128: 96–102.

177. Schad G. A. 1959. Nematodes from varanid lizards of Borneo. A description of *Abbreviata borneensis* n. sp. (Nematoda: Physalopteridae) and records of two other species. Canadian Journal of Zoology 37: 71–74.

178. Schad, G. A. 1962. Studies on the genus *Kalicephalus* (Nematoda: Diaphanocephalidae). II. A taxonomic revision of the genus *Kalicephalus* Molin, 1861. Canadian Journal of Zoology, 40: 1035–1165.

179. Schmidt, G. D., Kuntz, R. E. 1972. Nematode parasites of Oceanic. XIX. Report on a collection from Philippine reptiles. Transactions of the American Microscopical Society 91: 63–66.

180. Scholz, T., Ditrich, O. 1991. Some trematodes of reptiles from Laos. Folia. Parasitologica 38: 309–318.

181. Sey, O. 1985. Amphistomes of Vietnamese vertebrates (Trematoda: Amphistomida). Parasitologia Hungarica 18: 17–24.

182. Sharpio, V. P. 1974. *Spauligodon azerbajdzanicus* sp. n. (Nematoda, Pharyngodonidae) —a parasite of *Lacerta chlorogaster* Boul. Vestnik Zoologii 3: 82–83. (in Russian with English summary)

183. Simha, S. S., Deshpande, P. 1964. On a new species of *Artyfechinostomum* Lane, 1915,

from the intestine of the monitor lizard, *Varanus bengalensis* in Hyderabad-India. Zeitschrift fur Parasitenkunde 24: 23–26.

184. Singh, S. N. 1954. Studies on the morphology and life-history of *Strongyloides mirzai* n. sp. from snakes in India. Journal of Helminthology 28: 25–34.

185. Skrjabin, K. I., Shikhobalova, N. P., Lagodovskaya, E. A. 1961. Essentials of Nematodology (Ed. K. I. Skrjabin): Vol. X Oxyurata of Animals and Man, Academy of Sciences of the USSR, Helminthological Laboratory, Moscow, translated in English from Russian by R. Lavoott and edited by O. Theodor, 1974, Keter Publishing House Jerusalem, Jerusalem, Israel, 460 pp.

186. Smythe, A. B., Sanderson, M. J., Nadler, S. A. 2006. Nematode small subunit phylogeny correlates with alignment parameters. Systematic Biology 55: 972–992.

187. Sommerville, C. 1982. The life history of *Haporchis pumilio* (Looss, 1896) from cultured tilapias. Journal of Fish Diseases 5: 233–241.

188. Soota, T. D., Chaturvedi, Y. 1971. Notes on some nematodes from the unnamed collections of the zoological survey of India. Proceedings of the Zoological Society (Calcutta) 24: 13–24.

189. Sprent, J. F. A. 1985. Ascaridoid nematodes of amphibians and reptiles: *Raillietascaris* n. g. Annales de Parasitologie Humaine et Comparee (Paris) 60: 601–611.

190. Sterling, E. J., Hurley, M. M., Le, D. M. 2006. Vietnam: a natural history. Yale University Press, New Haven and London, 423 pp.

191. Thompson, J. D., Higgins, D. G., Gibson, T. J. 1994. CLUSTALW: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Research 22: 4673–4680.

192. Tkach, V. V. 2008. Family Encyclometridae Mehra, 1931. pp.349-352. In: Keys to the Trematoda, Volume 3 (Bray, R. A., Gibson, D. I. and Jones, A. eds.), CAB International and Natural History Museum, London, UK

193. Tkach V. V., Lisitsyna O. I., Crossley J. L., Tran T. B., Bush, S. E. 2013. Morphological and molecular differentiation of two new species of *Pseudoacanthocephalus* Petrochenko, 1958 (Acanthocephala: Echinorhynchidae) from amphibians and reptiles in the Philippines, with identification keys for the genus. Systematic Parasitology 85: 11–26.

194. Tran, B. T. 2009. Study of parasitic cestodes in some reptiles from Northern Vietnam. pp.41–45. In: Proceedings of the 3rd National Scientific Conference on Ecology and Biological Resources, Hanoi, October 22, 2009. (in Vietnamese with English summary)

195. Tran, B. T., Nguyen, T. L., Nguyen, V. H. 2005. Cestode parasite in house gecko (*Hemydactylus renatus*). pp.412–414. In: Proceedings of the 2005 National Conference on Life Sciences, Hanoi Medical University, November 03, 2005. (in Vietnamese with English summary)

196. Tran, B. T., Bursey, C. R., Goldberg, S. R. 2007. Two new species of Pharyngodonidae (Nematoda, Oxyuroidea) in *Gekko ulikovskii* from Vietnam. Acta Parasitologica 52: 363–367.

197. Tran, B. T., Sato, H. and Luc, P. V. 2015a. A new *Cosmocercoides* species (Nematoda: Cosmocercidae), *C. tonkinensis* n. sp., in the scale-bellied tree lizard (*Acanthosaura lepidogaster*) from Vietnam. Acta Parasitologica 60: 407–416.

198. Tran, B. T., Sato, H., Hasegawa, H., Diong, C. H., Luc, P. V. 2015b. Scanning electron microscopy of *Strongyluris calotis* (Nematoda: Ascaridida: Heterakidae) in the large intestine of agamid lizards in Asia. Japanese Journal of Veterinary Parasitology 14:13–21.

199. Tran, B. T., Ong, A. V., Luc, P. V., Sato, H. 2016. Morphological and molecular genetic diversity of *Strongyluris calotis* (Nematoda: Ascaridida: Heterakidae) in South East and East Asian lizards. Parasitology Research 115: 2807–2816.

200. Trung Dung, D., Van De, N., Waikagul, J., Dalsgaard, A., Chai, J. Y., Sohn, W. M., Murrell, K. D. 2007. Fishborne zoonotic intestinal trematodes, Vietnam. Emerging Infectious Diseases 13: 1828–1833.

201. Tubangui, M. A. 1938. Pseudophyllidean cestodes occurring in the Philippines. Livro Jubilar Lauro Travassos 1938: 489–494.

202. Vanderburgh, D. J., Anderson, R. C. 1986. The relationship between nematodes of the genus *Cosmocercoides* Wilkie, 1930 (Nematoda: Cosmocercoidea) in toads (*Bufo americanus*) and slugs (*Deroceras leave*). Canadian Journal of Zoology 65: 1650–1661.

203. Vanderburgh, D. J., Anderson, R. C. 1987. Seasonal changes in prevalence and intensity of *Cosmocercoides dukae* (Nematoda: Cosmocercoidea) in *Deroceras* leave (Mollusca). Canadian Journal of Zoology 65: 1662–1665.

204. Vlnová, I. 2014. Revize diphyllobothriidních tasemnic plazů (Eucestoda: Solenophoridae). Jihočeská univerzita v Českých Budějovicích, Přírodovědecká fakulta. Master thesis, 61 pp.

205. Wilkie, J. S. 1930. Some parasitic nematodes from Japan amphibia. Annals and Magazine of Natural History (Series 10) 6: 606–614.

206. Wood, P. L., Jr., Grismer, J. L., Grismer, L. L., Norhayati, A., Chan, K. O., Bauer, A. M.

2009. Two new montane species of *Acanthosaura* Gray, 1831 (Squamata: Agamidae) from Peninsular Malaysia. Zootaxa 2012: 28–46.

207. Wood, P. L., Jr., Grismer, L. L., Grismer, J. L., Neang, T., Chav, T., Holden, J. 2010. A new cryptic species of *Acanthosaura* Gray, 1831 (Squamata: Agamidae) from Thailand and Cambodia. Zootaxa 2488: 22–38.
208. Woodland, W. N. F. 1940. A revision of African and Asiatic forms of the genus *Duthiersia* (Cestoda). Proceedings of the Royal Society of London B Biological Sciences 110: 207-218.
209. Yamaguti, S. 1935. Studies on the helminth fauna of Japan. Part 11. Reptilian Nematodes. Japanese Journal of Zoology 6: 393–402.
210. Yamaguti, S. 1941. Studies on the helminth fauna of Japan. Part 34. Amphibian nematodes, II. Japanese Journal of Zoology 9: 397–408.
211. Yamaguti, S. 1952. Parasitic worms mainly from Celebes. Part 7. Cestodes of reptiles. Acta medica Okayama 8: 1–12.
212. Yamaguti, S. 1954. Systema Helminthum, Volume II. The cestodes of vertebrates. Interscience Publishers, Inc., New York, U.S.A., 860 pp.
213. Yamaguti, S. 1958. Systema Helminthum, Volume I. The digenetic trematodes of vertebrates. Interscience Publishers, Inc., New York, U.S.A., 1575 pp.
214. Yamaguti, S. 1961. Systema Helminthum, Volume III. The nematodes of vertebrates. Interscience Publishers, Inc., New York, U.S.A., 1261 pp.
215. Yamaguti, S. Mitunaga, I. 1943. Intestinal helminths from *Bufo melanostictus* of Formosa. Transactions of the Natural History Society of Formosa 33: 142–154.
216. Yildirimhan, H. S., Goldberg, S. R., Bursey, C. R. 2006. Helminth parasites of the *Caucasian agama*, *Laudakia caucasia*, and the roughtail rock agama, *Laudakia stellio* (Squamata: Agamidae), from Turkey. Comparative Parasitology 73: 257–262.
217. Yildirimhan, H. S., Bursey, C. R., Altunel, F. N. 2011. Helminth parasites of the Balkan green lizard, *Lacerta trilineata* Bedriaga 1886, from Bursa, Turkey. Turkish Journal of Zoology 35: 519–535.
218. Zhang, S., Zhang, L. 2011. A new species of *Meteterakis* Karve, 1939 (Nematoda: Heterakoidea) from *Indotestudo elongata* (Blyth) in China with a key to the species of *Meteterakis*. Zootaxa 2869: 63–68.
219. Zhang, H., Zhang, S., Zhang, L. 2011. Two species of the genus *Kalicephalus* Molin, 1861 (Nematoda, Diaphanocephaloidea) from the water monitor, *Varanus salvator* (Laurenti, 1768) in Guangdong Province, China. Acta Parasitologica 56: 48–53.

220. Ziegler, T., Nguyen, T. Q. 2010. New discoveries of amphibians and reptiles from Vietnam. Bonn Zoological Bulletin 57: 137–147.

Table 1. Recovery of *Cosmocercoides tonkinensis* n. sp. from scale-bellied tree lizards in Vietnam

	Locality	Date of collection	Number of lizards examined	Number of parasitized lizards	Intensity
A	Trung Khanh, Cao Bang Province (22°40' N, 106°38' E)	30 April 2012	6	2	1 & 8
B	Tam Dao National Park, Vinh Phuc Province (21°30' N, 105°36' E)	26 November 2012	3	0	0
C	Phu Yen, Son La Province (21°13' N, 104°40' E)	20 February 2013	2	1	2
D	Xuan Son National Park, Phu Tho Province (21°12' N, 104°50' E)	29 June & 4 July 2012	4	0	0
E	Tay Yen Tu, Bac Giang Province (21°11' N, 106°42' E)	9 July 2012	2	2	1 & 2
F	Pu Hu, Thanh Hoa Province (20°28' N, 104°55' E)	11 & 18 June 2012	11	5	2, 6, 8, 15 & 29
G	Kon Ka Kinh National Park, Gia Lai Province (14°22' N, 108°20' E)	29 February 2012	2	0	0
H	Vinh Cuu National Park, Dong Nai Province (11°23' N, 107°03' E)	25 January 2013	2	0	0
Total			32	10 (31.3%)	geomean 4.1

Table 2. Morphological comparison of *Cosmocercoides tonkinensis* n. sp. with related species

Species	*C. tonkinensis* n. sp.	*C. multipapillata*	*C. bufonis*	*C. pulcher*	*C. pulcher*	*C. pulcher*	*C. pulcher*	*C. pulcher*
Host	Lizard (*Acanthosaura lepidogaster*)	Toad (*Bufo melanostictus*)	Toad (*Bufo himalayanum*)	Frog (*Rana japonica*); Toad (*Bufo japonicus*)	Toad (*Bufo japonicus*)	Toad (*Bufo japonicus*)	Toad (*Bufo gargarizans*)	Toad (*Bufo japonicus*)
Locality	Vietnam	India	India	Japan	China	Japan	Japan	Japan
References	The present study	Khera (1958)	Karve (1944)	Wilkie (1930)	Hsü (1933)	Yamaguti (1941)	Hasegawa (1984)	The present study
Male	(n=3)	—	—	—	(n=2)	—	—	(n=2)
Body length	4.2 - 5.1	5.8 - 6.4	4.6 - 4.9	6.9	7.0 / 7.6	7.8 - 8.8	5.5 - 7.1	8.9 / 10.6
Max. width	0.34 - 0.37	0.36 - 0.39	0.35 - 0.43	0.43	0.41 / 0.50	0.35 - 0.40	0.28 - 0.37	0.58 / 0.60
Esophagus length	0.79 - 0.87	0.93 - 0.96	0.58 - 0.66	1.2	1.09 / 1.10	1.18 - 1.38	0.84 - 0.93	1.65 / 1.60
Excretory pore from anterior end	0.44 - 0.55	0.57 - 0.61	0.36 - 0.37	—	0.7	0.72 - 0.85	0.57 - 0.66	1.03 / 0.98
Spicule length	0.222 - 0.256	0.20 - 0.24	0.10 - 0.25	0.247	0.310 / 0.320	0.24 - 0.29	0.24 - 0.27	0.322 / 0.328
Gubernaculum length	0.113 - 0.122	0.13 - 0.14	0.120 - 0.145	0.143	0.160 / 0.180	0.14 - 0.16	0.14 - 0.15	0.184 / 0.186
Number of caudal rosettes	16 - 17 pairs	15 - 18 pairs	13 - 17 pairs	17 pairs	17 - 18 pairs	17 - 18 pairs	—	17 - 18 pairs
Caudal papillae: Rosette* / Simple*	23-25 : 2 : 6 / 0 : 0 : 10	21-27 : 2 : 8 / many	18-26 : 2 : 6 / present : 0 : 20	28 : 4 : 2 / many : 0 : 14	27 : 2 : 6 / many : 0 : 14	—	—	29 : 4 : 2 / many : 0 : 14
Lateral alae	Present	Present	Present	—	—	—	Present	Present
Somatic papillae	Present	Present	Absent	—	Present	—	Present	Present
Tail length	0.21 - 0.23	0.25 - 0.28	0.20 - 0.24	0.22	0.23 - 0.27	0.25 - 0.30	0.21 - 0.26	0.34 / 0.36
Female	(n=3)	—	—	—	(n=5)	—	—	(n=2)
Body length	5.3 - 5.5	6.9 - 7.1	5.2 - 6.3	9.0	7.9 - 11.0	8.9 - 9.7	6.4 - 7.7	12.8 / 14.5
Max. width	0.32 - 0.42	0.40 - 0.42	0.45 - 0.55	0.54	0.51 - 0.61	0.40 - 0.45	0.35 - 0.43	0.82 / 0.90
Esophagus length	0.82 - 0.91	1.0 - 1.1	0.85 - 1.06	1.4	1.22 - 1.45	1.3 - 1.5	0.77 - 0.95	1.84 / 1.77
Excretory pore from anterior end	0.51 - 0.54	0.63 - 0.65	0.50 - 0.68	1.0	0.74 - 0.88	0.75 - 0.85	0.46 - 0.67	1.21 / 1.32
Vulva position (anterior part : posterior part)	1.27 - 1.36 : 1	ca. 1.2 - 1.4 : 1	ca. 1.34 : 1	1.25 : 1	1.18 - 1.37 : 1	1.26 - 1.40 : 1	ca. 1.2 - 1.3 : 1	1.27 - 1.39 : 1
Tail length	0.26 - 0.30	0.22 - 0.25	0.23 - 0.26	0.38	0.35 - 0.52	0.31 - 0.34	0.32 - 0.36	0.51 / 0.50
Eggs	0.064 - 0.084 x 0.040 - 0.048 (Embryonated)	0.067 - 0.073 x 0.043 - 0.046 (Not embryonated)	0.075 - 0.080 x 0.040 - 0.045 (Embryonated)	0.082 x 0.049 —	0.060 - 0.070 x 0.038 - 0.045 (Embryonated)	0.080 - 0.096 x 0.045 - 0.051 (Embryonated)	0.071 - 0.087 x 0.044 - 0.056 (Embryonated)	0.080 - 0.092 x 0.048 - 0.056 (Embryonated)

* Preanal : adanal : postanal. '—' means no data.

Table 3. Morphometric comparison of *Strongyluris calotis* in the present study and previous studies (measurement in millimeter)

Host	*Japalura polygonata donan*	*Japalura polygonata polygonata*	*Japalura swinhonis*	*Japalura polygonata*	*Calotes nigrilabris*	*Japalura splendida*	*Japalura swinhonis*	*Calotes* sp.
Locality	Japan (Okinawa Islands; Yonakuni)	Japan (Okinawa Islands; Kunigami)	Taiwan (Taipei)	Japan (Ryukyu Islands)	Sri Lanka	China (Sichuan)	Taiwan	India (West Bengal)
Reference	The present study[a]	The present study[a]	The present study	Hasegawa & Iwatsuki (1984)	Baylis & Daubney (1923)	Hsü & Hoeppli (1931)	Yamaguti (1935)	Sosta & Chaturvedi (1971)
Male	(n=5)	(n=5)	(n=4)	(n=?)	(n=?)	(n=5)	(n=5)	(n=?)
Worm length	6.7—8.1 (7.4)	9.1—10.8 (10.0)	6.6—7.0 (6.9)	9.0—10.2	8.9—11.1	11.5—13.4	8.6—9.1	12.6—14.0
Worm width	0.29—0.34 (0.32)	0.35—0.41 (0.37)	0.30—0.38 (0.35)	0.32—0.51	0.40—0.50	0.42—0.52	0.21—0.26	0.66—0.75
Pharynx length	0.17—0.20 (0.18)	0.18—0.22 (0.20)	0.16—0.19 (0.18)	0.20—0.22	1.75—2.2	0.21—0.23	0.17—0.18	0.27—0.30
Esophagus length	1.22—1.36 (1.28)	1.27—1.39 (1.33)	1.25—1.33 (1.29)	1.3—1.5		1.47—1.55	1.3—1.5	1.37—1.48
Bulb length	0.17—0.19 (0.18)	0.19—0.21 (0.20)	0.16—0.19 (0.17)	0.24—0.29	—	—	φ0.21—0.28	0.20—0.27
Bulb width	0.18—0.22 (0.20)	0.21—0.22 (0.21)	0.21—0.22 (0.22)	—	—	—		0.22—0.27
Nerve ring from anterior end	0.37—0.42 (0.40)	0.38—0.42 (0.40)	0.36—0.41 (0.38)	0.34—0.49	—	0.40—0.50	—	—
Excretory pore from anterior end	0.81—0.95 (0.89)	0.85—0.94 (0.88)	0.86—1.05 (0.98)	1.05—1.20	—	1.15—1.25	—	—
Spiculae Length	0.56—0.59 (0.57)	0.54—0.68 (0.61)	0.56—0.68 (0.62)	—	0.75—0.80	0.79—0.84	0.64—0.68	0.66—0.84
Caudal papillae number and arrangement[b]	20 (6 : 4 : 4 : 6)[c]	20 (6 : 4 : 4 : 6)[c]	20 (6 : 4 : 4 : 6)[c]	18 (6 : 4 : 4 : 4)	20 (6 : ? : ? : ? [14])[d]	20 (6 : ? : ? : ? [14])[d]	20 (6 : 4 : 4 : 6)	20 (6 : ? : ? : ? [14])[d]
Tail length	—	—	—	0.085—0.103	—	0.080—0.084	0.075—0.112	0.09—0.14
Female	(n=5)	(n=5)	(n=4)	(n=?)	(n=?)	(n=5)	(n=3)	(n=?)
Worm length	7.9—9.2 (8.4)	11.3—12.3 (11.9)	7.1—8.8 (7.8)	9.7—14.9	11.0—13.65	13.1—16.0	9.8—11.9	15.0—20.3
Worm width	0.31—0.38 (0.35)	0.46—0.56 (0.52)	0.35—0.45 (0.39)	0.39—0.60	0.55—0.75	0.45—0.57	0.28—0.30	0.60—0.84
Pharynx length	0.20—0.21 (0.20)	0.21—0.23 (0.22)	0.18—0.22 (0.20)	0.21—0.27	1.75—2.25	0.22—0.28	0.21—0.24	0.27—0.30
Esophagus length	1.37—1.58 (1.45)	1.46—1.62 (1.53)	1.40—1.51 (1.45)	1.4—2.1		1.5—1.9	1.5—1.8	1.5—1.7
Bulb length	0.18—0.22 (0.20)	0.21—0.22 (0.22)	0.20—0.22 (0.21)	0.27—0.36	—	—	φ0.21—0.28	0.30—0.33
Bulb width	0.22—0.25 (0.24)	0.22—0.25 (0.23)	0.24—0.25 (0.25)	—	—	—		0.23—0.27
Nerve ring from anterior end	0.38—0.42 (0.40)	0.42—0.46 (0.43)	0.42—0.47 (0.44)	0.39—0.54	—	0.40—0.47	—	—
Excretory pore from anterior end	0.83—1.02 (0.93)	0.93—1.01 (0.97)	0.79—0.98 (0.92)	0.83—1.39	—	1.00—1.25	—	—
Valva								
Distance from anterior end	4.8—5.7 (5.3)	6.6—7.4 (7.1)	4.3—5.5 (4.8)	5.5—9.1	6.3—8.0	7.7—9.7	—	8.8—12.5
Position[e]	0.60—0.66 (0.62)	0.58—0.60 (0.60)	0.61—0.63 (0.62)	0.57—0.61 (0.59)	0.53—0.62 (0.58)	0.61—0.67 (0.64)	0.58—0.73 (0.66)	0.53—0.66 (0.60)
Tail length	0.13—0.16 (0.14)	0.13—0.18 (0.16)	0.14—0.16 (0.15)	0.16—0.22	0.22—0.25	0.22—0.28	0.23—0.28	0.16—0.38
Egg length	0.072—0.084 (0.080)	0.068—0.080 (0.077)	0.076—0.084 (0.079)	0.079—0.083	0.088—0.098	0.061—0.082	0.081—0.089	—
Egg width	0.040—0.044 (0.041)	0.040	0.040—0.044 (0.041)	0.038—0.045	0.050—0.053	0.037—0.042	0.041—0.047	0.033—0.044

[a] *S. calotis* from *Japalura polygonata* in the Ryukyu Islands, Okinawa, Japan, examined in the present study are a part of specimens examined previously by Hasegawa and Iwatsuki (1984).

[b] Total in number (precloacal : adcloacal : ventrolateral : terminal).

[c] Shown data are based on light microscopic observation, but according to SEM examination two of six terminal papillae were phasmids.

[d] The original records provide only that male worms had 6 precloacal and 14 postcloacal papillae in the caudal region.

[e] Distance between anterior end and vulva / worm length.

Table 4. Morphometric comparison of *Strongyluris* spp. with Oriental distribution (measurements in millimeters)[a]

Species name	*S. chamaeleonis* Baylis et Daubney, 1922	*S. calotis* Baylis et Daubney, 1923	*S. bengalensis* Chakravorty, 1936	*S. karawirensis* Karve, 1938	*S. bufonis* Yamaguti et Mitunaga, 1943	*S. japalurae* Jaing et Lin 1980	(*S. manipurensis* Lakshmipyari et al., 2011)[a]
Host	*Chamaeleo vulgaris; Cophotis ceylanica; Ceratophora stoddarti; Calotes versicolor*	*Calotes nigrilabris*	*Calotes versicolor*	*Calotes versicolor*	*Bufo melanostictus*	*Japalura swinhonis formosensis*	*Calotes versicolor*
Locality	India (Calcutta Zoo)	Sri Lanka	India (Calcutta)	India (Bombay)	Taiwan (Taipei)	Taiwan (Taichung)	India (Manipur)
Reference	Baylis & Daubney (1922)	Baylis & Daubney (1923)	Chakravorty (1936)	Karve (1938)	Yamaguti et Mitunaga (1943)	Jaing & Lin (1980)	Lakshmipyari et al. (2011)
Male	(n=1)	(n=?)	(n=?)	(n=4)	(n=?)	(n=9)	(n=?)
Worm length	6.3	8.9—11.1	9.0—11.5	16.0—18.6	5.8—7.8	7.8—12.7	7.9—11.1
Worm width	0.50	0.40—0.50	0.36—0.47	1.05—1.10	0.3—0.4	0.25—0.50	0.46—0.57
Pharynx length	0.18—0.22	1.75—2.20	0.15—0.22	0.17—0.20	0.17—0.19	0.18—0.23	0.23—0.26
Esophagus length	ca. 1.1		0.98—1.30	1.35—1.41	1.1	1.15—1.69	1.73—2.16
Bulb length	ca. 0.20—0.25	—	0.15—0.22	—	—	0.15—0.25	0.17—0.22
Bulb width	ca. 0.20—0.25	—	0.11—0.18	0.19—0.20	0.19—0.21	0.20—0.24	0.23—0.29
Nerve ring from anterior end	—	—	0.30—0.63	0.42—0.44	0.36—0.41	0.36—0.48	—
Excretory pore from anterior end	—	—	1.1—1.5	1.04—1.11	ca. 1.0	—	0.90—0.91
Spicule length	1.1	0.75—0.80	0.45—0.63	0.57—0.59	0.57—0.63	0.50—0.76	0.55—0.75
Caudal papilla: number and arrangement[b]	18 (6 : 4 : 4 : 4)	20 (6 : ? : ? : ? [14])[c]	18 (6 : 4 : 4 : 4)	22 (6 : 4 : 6 : 6)	20 (6 : 4 : 4 : 6)	20 (6 : 4 : 4 : 6)	18 (6 : 4 : 4 : 4)
Tail length	0.18	—	0.09	0.11	—	0.063—0.068	0.15—0.19
Female	(n=?)	(n=?)	(n=?)	(n=1)	(n=?)	(n=10)	(n=?)
Worm length	8.4—8.8	11.0—13.7	14.0—15.0	23.3	7.9—9.1	8.4—12.1	8.7—11.5
Worm width	0.50—0.70	0.55—0.75	0.57—0.66	1.13	0.3—0.4	0.27—0.51	0.45—0.52
Pharynx length	0.18—0.22	1.75—2.25	0.22—0.27	0.26	0.20—0.21	0.22—0.23	0.21—0.28
Esophagus length	ca. 1.45		1.3—1.5	1.70	1.3—1.5	1.73—2.15	1.44—1.86
Bulb length	ca. 0.20—0.25	—	0.22—0.27	—	—	0.23—0.26	0.16—0.25
Bulb width	ca. 0.20—0.25	—	0.18—0.25	0.25	0.21—0.23	0.25—0.35	0.21—0.30
Nerve ring from anterior end	—	—	0.43—0.45	0.51	0.37—0.44	0.40—0.46	—
Excretory pore from anterior end	—	—	—	1.34	0.9—1.0	—	0.95—1.03
Vulva							
Distance from anterior end	5.4—5.5	6.3—8.0	9.4—10.4	14.2	4.9—5.7	5.46—8.22	5.1—7.5
Position[d]	0.62—0.64 (0.63)	0.53—0.62 (0.58)	0.69	0.61	0.62—0.63	0.65—0.68 (0.67)	0.59—0.65 (0.62)
Tail length	0.30	0.22—0.25	0.22	0.18	0.16—0.18	0.19—0.24	0.15—0.19
Egg length	0.087	0.088—0.098	0.068—0.114	0.070—0.085	0.069—0.081	0.06—0.08	0.05—0.08
Egg width	0.055	0.050—0.053	0.046—0.068	0.050—0.055	0.036—0.046	0.04—0.05	0.03—0.04

[a] In addition to six nominal *Strongyluris* spp. with Oriental distribution (Bursey et al. 2013), '*Strongyluris manipurensis*' described by Lakshmipyari et al. (2011), which we consider to be poorly characterized as an independent species, is shown for reference only.

[b] Total number (precloacal : adcloacal : ventrolateral : terminal).

[c] The original records provide only that male worms had 6 precloacal and 14 postcloacal papillae in the caudal region.

[d] Distance between anterior end and vulva / worm length.

Table 5. Primers used to amplify eight overlapping segments of rDNA of *Strongyluris* worms.

Segment no.	Primer name[a] for amplifying		Sequence	Position of 5'-end[b]
1	F:	F-47	5'-CCCGATTGATTCTGTCGGC-3'	1
	R:	NSR1438/20	5'-GGGCATCACAGACCTGTTAT-3'	1,434
1'	F:	Strongyluris18S_4F	5'-GATTGATTCTGTCGGCGGTT-3'	4
	R:	NSR1438/20	(see above)	1,434
1"	F:	Strongyluris18S_11F	5'-TCTGTCGGCGGTTATATGCT-3'	11
	R:	NSR1438/20	(see above)	1,434
1"'	F:	Strongyluris18S_15F	5'-TCGGCGGTTATATGCTTGTC-3'	15
	R:	NSR1438/20	(see above)	1,434
2	F:	NSF573_Binh/19	5'-CGCGGTAATTCCAGCTCTC-3'	570
	R:	NSR1787/18	5'-CGACGGGCGGTGTGTACA-3'	1,639
3	F:	NSF573_Binh/19	(see above)	570
	R:	S.r.18S-SSU18R	5′-TGATCCTTCYGCAGGTTCAC-3'	1,794
4	F:	NSF1624/20	5'-TTTGTACACACCGCCCGTCG-3'	1,620
	R:	NC13(ITS1)/R[c]	5'-GCTGCGTTCTTCATCGA(T)-3'	2,297
5	F:	NC5(ITS1)/F	5'-GTAGGTGAACCTGCGGAAGGATCATT-3'	1,771
	R:	NC2(ITS2)/R	5'-TTAGTTTCTTTTCCTCCGCT-3'	2,914
6	F:	NC13(ITS2)/F	5'-ATCGATGAAGAACGCAGC-3'	2,280
	R:	28S-408R/20	5'-TTCACGCCCTCTTGAACTCT-3'	3,256
7	F:	B.p.28S/F	5'-AGCGGAGGAAAAGAAACTAA-3'	2,895
	R:	B.p.26S-1270R/22	5'-CAGCTATCCTGAGGGAAACTTC-3'	4,021
8	F:	B.p.28S/F	(see above)	2,895
	R:	NLR1432-1/22	5'-GTTGTTACACACTCCTTAGCGG-3'	4,329

[a]F: forward and R: reverse.

[b]Relative position of the 5'-end of each primer in an rDNA sequence of *Strongyluris calotis* from an Emma Gray's forest lizard (DDBJ/EMBL/GenBank accession no. LC133186). The 5'-end of unfunctional F-47 primer is considered as the beginning of 18S rDNA here.

[c]Primer NC13(ITS1)/R might be functional when it lacked 'T' at the 3'-terminus, i.e. 5'-GCTGCGTTCTTCATCGA-3'.

Table 6. Comparison of morphometrics of different isolates of *Strongyluris calotis* (measurement in millimeter)

Morphotype	Without a pair of united papillae	With a pair of united papillae			
Host	*Pseudocalotes brevipes*	*Calotes emma*	*Japalura swinhonis*	*Japalura polygonata*	*Japalura polygonata*
Locality	Vietnam (Kanton)	Vietnam (Kanton)	Taiwan (Taipei)	Japan (Yonakuni, Okinawa Is.)	Japan (Kunigami, Okinawa Is.)
Reference	The present study	The present study	Chapter 2 in the present study	Chapter 2 in the present study	Chapter 2 in the present study
Male	(n=6)	(n=1)	(n=4)	(n=5)	(n=5)
Worm length	8.1—13.1 (10.4)	11.4	6.6—7.0 (6.9)	6.7—8.1 (7.4)	9.1—10.8 (10.0)
Worm width	0.33—0.56 (0.42)	0.52	0.30—0.38 (0.35)	0.29—0.34 (0.32)	0.35—0.41 (0.37)
Pharynx length	0.19—0.23 (0.21)	0.25	0.16—0.19 (0.18)	0.17—0.20 (0.18)	0.18—0.22 (0.20)
Esophagus length	1.29—1.96 (1.62)	2.13	1.25—1.33 (1.29)	1.22—1.36 (1.28)	1.27—1.39 (1.33)
Bulb length	0.20—0.32 (0.24)	0.23	0.16—0.19 (0.17)	0.17—0.19 (0.18)	0.19—0.21 (0.20)
Bulb width	0.19—0.30 (0.23)	0.27	0.21—0.22 (0.22)	0.18—0.22 (0.20)	0.21—0.22 (0.21)
Nerve ring from anterior end	0.34—0.50 (0.42)	0.62	0.36—0.41 (0.38)	0.37—0.42 (0.40)	0.38—0.42 (0.40)
Excretory pore from anterior end	0.78—1.36 (1.11)	1.38	0.86—1.05 (0.98)	0.81—0.95 (0.89)	0.85—0.94 (0.88)
Spiculae Length	0.72—1.00 (0.85)	ca. 1.15	0.56—0.68 (0.62)	0.56—0.59 (0.57)	0.54—0.68 (0.61)
Caudal papillae number and arrangement[a]	22 (6 : 4 : 4: 8)	20 (6 : 4 : 4 : 6)	20 (6 : 4 : 4 : 6)	20 (6 : 4 : 4 : 6)	20 (6 : 4 : 4 : 6)
Female	(n=4)	(n=1)	(n=4)	(n=5)	(n=5)
Worm length	11.0—16.2 (14.0)	13.1	7.1—8.8 (7.8)	7.9—9.2 (8.4)	11.3—12.3 (11.9)
Worm width	0.68—0.82 (0.74)	0.82	0.35—0.45 (0.39)	0.31—0.38 (0.35)	0.46—0.56 (0.52)
Pharynx length	0.22—0.27 (0.24)	0.30	0.18—0.22 (0.20)	0.20—0.21 (0.20)	0.21—0.23 (0.22)
Esophagus length	1.68—2.05 (1.90)	2.14	1.40—1.51 (1.45)	1.37—1.58 (1.45)	1.46—1.62 (1.53)
Bulb length	0.24—0.27 (0.25)	0.29	0.20—0.22 (0.21)	0.18—0.22 (0.20)	0.21—0.22 (0.22)
Bulb width	0.25—0.27 (0.26)	0.32	0.24—0.25 (0.25)	0.22—0.25 (0.24)	0.22—0.25 (0.23)
Nerve ring from anterior end	0.48—0.60 (0.54)	0.65	0.42—0.47 (0.44)	0.38—0.42 (0.40)	0.42—0.46 (0.43)
Excretory pore from anterior end	1.24—1.56 (1.41)	1.34	0.79—0.98 (0.92)	0.83—1.02 (0.93)	0.93—1.01 (0.97)
Valva					
Distance from anterior end	6.15—9.70 (8.13)	8.15	4.3—5.5 (4.8)	4.8—5.7 (5.3)	6.6—7.4 (7.1)
Position[b]	0.56—0.60 (0.58)	0.62	0.61—0.63 (0.62)	0.60—0.66 (0.62)	0.58—0.60 (0.60)
Tail length	0.23—0.26 (0.27)	0.15	0.14—0.16 (0.15)	0.13—0.16 (0.14)	0.13—0.18 (0.16)
Egg length	0.068—0.080 (0.077)	0.080—0.084 (0.083)	0.076—0.084 (0.079)	0.072—0.084 (0.080)	0.068—0.080 (0.077)
Egg width	0.040—0.044 (0.041)	0.040—0.044 (0.043)	0.040—0.044 (0.041)	0.040—0.044 (0.041)	0.040

[a] Total in number (precloacal : adcloacal : ventrolateral : terminal).Total number and terminal number of papillae contain two phasmids.

[b] Distance between anterior end and vulva / worm length.

Table 7. Nucleotide changes observed in the rDNA of *Strongyluris calotis* of different origins

Host	Locality	DDBJ/EMBL/GenBank accession no.	Nucleotide position where any changes in rDNA are observed[a]																																						
			18S	ITS-1													ITS-2																		28S						
			1474	77	89	152	179	191	213	301	310	383	389	392	408	451	46	49	50	51	58	125	187	188	241	266	284	343	357	378	383	387	412	418	448	530	594	662	765	1068	1187
Calotes emma	Bac Kan Province, Northern Vietnam	LC133186	A	T	A	T	C	C	A	C	T	G	A	A	A	C	G	A	T	G	G	A	A	C	T	G	A	G	T	C	A	G	G	G	C	G	A	G	A	C	A
Calotes emma	Singapore	LC133187	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.							
Japalura polygonata ishigakiensis	Ishigaki Is., Okinawa, Japan	LC133188	.	.	T	.	.	T	G	.	A	A	G	.	.	.	T	G	C	A	T	.	G	T	.	.	G	A	C	.	G	A	.	.	.	A	T	.	A/G	.	C
Pseudocalotes brevipes	Phu Tho Province, Northern Vietnam	LC133189	G	G	.	C	T	T	.	T	A	.	G	C	G	T	.	G	C	A	T	G	G	T	A	A	G	A	C	T	.	.	A	A	T	A	T	C	.	A	
Pseudocalotes brevipes	Bac Can Province, Northern Vietnam	LC133190	.	G	.	C	T	T	.	T	A	.	G	C	G	T	.	G	C	A	T	G	G	T	A	A	G	A	C	T	.	.	A	A	T	A	T	C	.	A	

[a]Nucleotide position is expressed relative to the rDNA sequence of *S. calotis* collected from an Emma Gray's forest lizard in Vietnam (DDBJ/EMBL/GenBank accession no. LC133186). Dots denote an identical base to that of *S. calotis* collected from an Emma Gray's forest lizard in Bac Kan Province, Vietnam, and blanks indicate no data.

Table 8. Recovery of different *Meteterakis* morphotypes from scale-bellied tree lizards in Vietnam

	Locality[a]	Date of collection	Number of lizards examined	Number of parasitized lizards (intensity)[b]			Additional recovery of	Positive host ID
				M. vietnamensis	*M. tamdaoensis*	*M. striaturus*	juvenile worms[c]	
A	Trung Khanh, Cao Bang Province (22°40' N, 106°38' E)	30 April 2012	6	3 (2—18)	0	0	3 (1—12)	CB08, CB18, CB19
B	Tam Dao National Park, Vinh Phuc Province (21°30' N, 105°36' E)	26 November 2012	3	2[d] (4, 15)	2[d] (11, 52)	0	3 (2—36)	TD01, TD02[d], TD04
C	Phu Yen, Son La Province (21°13' N, 104°40' E)	20 February 2013	2	0	0	1 (27)	1 (2)	LS10
D	Xuan Son National Park, Phu Tho Province (21°12' N, 104°50' E)	29 June & 4 July 2012	4	0	0	3 (4, 6, 17)	0	VP03. VP04, VP05
E	Tay Yen Tu, Bac Giang Province (21°11' N, 106°42' E)	9 July 2012	2	2 (8, 10)	0	0	0	BG01, BG02
F	Pu Hu, Thanh Hoa Province (20°28' N, 104°55' E)	11 & 18 June 2012	11	0	8 (4—17)	0	8 (1— 21)	TH01, TH02, TH03, TH04, TH06, TH09, TH10, TH13
G	Kon Ka Kinh National Park, Gia Lai Province (14°22' N, 108°20' E)	29 February 2012	2	2 (8, 23)	0	0	2 (6, 10)	KK02, KK04
H	Vinh Cuu National Park, Dong Nai Province (11°23' N, 107°03' E)	25 January 2013	2	2 (12, 19)	0	0	2 (7, 11)	DN01, DN02
Total			32	10 (geomean 9.1)	10 (geomean 11.9)	4 (geomean 10.2)		

[a] Site plots for each locality on a map are available in Figure 3 of the present thesis.

[b] Number of parasitized lizards is followed by number of recovered adult worms (intensity) in parentheses.

[c] Morphotypes of juvenile worms were not determined, and the data are expressed in the same form as adult worms.

[d] One lizard (TD02) was infected with both A and B morphotypes.

Table 9. Comparison of morphometric characters of different *Meteterakis* morphotypes collected from scale-bellied tree lizards in Vietnam[a]

Morphotype	**A**	**A**	**A**	**B**	**C**
Worm origin (Host ID)	**KK02**	**BG01**	**DN02**	**TD02**	**VP03, VP04, VP05, LS10**
Locality	**G (Kon Ka Kinh)**	**E (Tay Yen Tu)**	**H (Vinh Cuu)**	**B (Tam Dao)**	**C (Phu Yen) and D (Xuan Son)**
Male					
Number of worms observed	n=7	n=4	n=3	n=6	n=7
Body length	3.90—6.14 (4.93)	4.92—6.00 (5.32)	4.39—5.03 (4.72)	3.28—4.22 (3.67)	5.69—7.78 (6.64)
Body width	0.18—0.31 (0.26)	0.24—0.32 (0.28)	0.20—0.25 (0.23)	0.16—0.19 (0.17)	0.24—0.38 (0.32)
Pharynx length	0.064—0.076 (0.069)	0.052—0.060 (0.056)	0.054—0.056 (0.055)	0.052—0.056 (0.054)	0.060—0.064 (0.061)
Esophagus length	0.81—0.97 (0.90)	0.77—0.84 (0.79)	0.67—0.78 (0.72)	0.66—0.80 (0.72)	0.87—1.09 (0.96)
Corpus length	0.66—0.78 (0.71)	0.58—0.64 (0.61)	0.49—0.59 (0.53)	0.51—0.62 (0.57)	0.67—0.86 (0.76)
Length of glandular oesophagus	0.052—0.068 (0.064)	0.056—0.068 (0.060)	0.064—0.068 (0.065)	0.044—0.052 (0.047)	0.060—0.076 (0.067)
Width of glandular oesophagus	0.092—0.116 (0.106)	0.092—0.116 (0.101)	0.096—0.108 (0.101)	0.084—0.104 (0.095)	0.096—0.112 (0.106)
Bulb length	0.100—0.126 (0.117)	0.168—0.200 (0.182)	0.116—0.124 (0.119)	0.092—0.124 (0.107)	0.128—0.148 (0.138)
Bulb width	0.140—0.192 (0.169)	0.144—0.188 (0.159)	0.140—0.160 (0.148)	0.120—0.144 (0.133)	0.160—0.184 (0.171)
Nerve ring from anterior end	0.32—0.34 (0.33)	0.26—0.32 (0.28)	0.26—0.32 (0.29)	0.26—0.29 (0.28)	0.37—0.43 (0.40)
Excretory from anterior end	0.47—0.57 (0.53)	0.44—0.55 (0.48)	0.40—0.50 (0.46)	0.38—0.44 (0.42)	0.51—0.70 (0.60)
Preanal sucker diameter	0.032—0.040 (0.036)	0.040—0.044 (0.041)	0.040—0.044 (0.043)	0.028—0.032 (0.030)	0.044—0.048 (0.047)
Spicule	Equal, alate	Equal, alate	Equal, alate	Equal, non—alate	Equal, non—alate
	0.453—0.561 (0.506)	0.525—0.583 (0.545)	0.528—0.578 (0.554)	0.327—0.494 (0.384)	0.617—0.720 (0.656)
Gubernaculum length	0.072—0.096 (0.085)	0.072—0.100 (0.088)	0.090—0.096 (0.093)	0.072—0.108 (0.082)	0.092—0.108 (0.101)
Gubernaculum width	0.038—0.040 (0.040)	0.042—0.044 (0.044)	0.044—0.046 (0.045)	0.032—0.036 (0.033)	0.048—0.052 (0.050)
Caudal papillae	12 pairs	12 pairs	12 pairs	12 pairs	13 pairs
Tail length	0.20—0.29 (0.25)	0.26—0.29 (0.28)	0.21—0.23 (0.22)	0.18—0.24 (0.21)	0.28—0.33 (0.30)
Female					
Number of worms observed	n=7	n=2	n=3	n=6	n=7
Body length	4.94—7.83 (6.13)	5.22—5.58 (5.40)	4.52—5.17 (4.75)	3.78—4.08 (3.94)	5.25—7.86 (6.42)
Body width	0.24—0.37 (0.30)	0.30—0.30 (0.300)	0.24—0.30 (0.26)	0.18—0.22 (0.20)	0.30—0.48 (0.39)
Pharynx length	0.068—0.084 (0.076)	0.060—0.060 (0.060)	0.054—0.060 (0.057)	0.056—0.060 (0.058)	0.062—0.072 (0.066)
Esophagus length	0.98—1.13 (1.04)	0.86—0.90 (0.88)	0.81—0.82 (0.81)	0.74—0.81 (0.78)	0.92—1.17 (1.02)
Corpus length	0.77—0.90 (0.83)	0.66—0.70 (0.68)	0.62—0.62 (0.62)	0.58—0.64 (0.61)	0.71—0.93 (0.80)
Length of glandular oesophagus	0.064—0.080 (0.073)	0.068—0.068 (0.068)	0.072—0.076 (0.075)	0.044—0.048 (0.047)	0.064—0.072 (0.070)
Width of glandular oesophagus	0.128—0.136 (0.132)	0.124—0.124 (0.124)	0.108—0.112 (0.122)	0.096—0.104 (0.101)	0.108—0.132 (0.122)
Bulb length	0.128—0.152 (0.141)	0.132—0.132 (0.132)	0.116—0.120 (0.119)	0.112—0.128 (0.119)	0.144—0.168 (0.156)
Bulb width	0.184—0.200 (0.191)	0.176—0.176 (0.176)	0.152—0.160 (0.156)	0.144—0.152 (0.147)	0.168—0.216 (0.193)
Nerve ring from anterior end	0.35—0.43 (0.38)	0.33—0.36 (0.34)	0.27—0.30 (0.28)	0.27—0.30 (0.29)	0.33—0.45 (0.39)
Excretory from anterior end	0.54—0.63 (0.58)	0.46—0.50 (0.48)	0.42—0.47 (0.44)	0.41—0.46 (0.42)	0.51—0.68 (0.58)
Vulva from anterior end	2.28—3.06 (2.58)	2.08—2.25 (2.17)	1.94—2.11 (2.01)	1.72—1.90 (1.79)	2.25—3.33 (2.71)
Positon from anterior end (%)[b]	39.1—46.2 (42.4)	39.8—40.3 (40.1)	40.1—43.2 (41.8)	43.8—46.6 (45.3)	40.7—43.8 (42.2)
Tail length	0.30—0.42 (0.36)	0.34—0.34 (0.34)	0.30—0.32 (0.31)	0.26—0.30 (0.29)	0.32—0.46 (0.39)
Eggs length	0.064—0.072 (0.066)	0.064—0.064 (0.064)	0.068—0.072 (0.070)	0.072—0.080 (0.073)	0.066—0.076 (0.071)
Eggs width	0.040	0.040	0.044	0.040—0.042 (0.040)	0.044—0.048 (0.045)

[a] Values not noticeably overlapped with those for morphotype A from locality G (Kon Ka Kinh National Park) are enclosed by squares.

[b] Percentage of distance between vulva and anterior end against whole body length.

Table 10. Endoparasites of lizards in Vietnam recorded during the period of 1966 — 2015.

Lizard species	Number of lizards examined	Recorded parasites*				
		Cestoda	Trematoda	Nematoda	Acanthocephala	Arthropoda
Varanidae						
Clouded monitor *Varanus nebulosus* Gray, 1831	23	*Acanthotaenia shipleyi*; *Acanthotaenia beddardi*; *Acanthotaenia nilotica*; *Acanthotaenia* sp.; *Duthiersia expansa*	*Encyclometra colubrimumorum*; *Singhiatrema vietnamensis*; *Mesocoelium brevicaecum*; *Haplorchis pumilio*; *Artyfechinostomum surfrartyfex*	*Strongyloides mirzai*; *Kalicephalus* sp.; *Oswaldocruzia* sp. 2; '*Herpetostrongylus varani*' sensu Nguyen 2002; *Raillietascaris varani*; *Meteterakis varani*; *Hastospiculum varani*; *Piratuba varanicola*		*Raillietiella orientalis*
Water monitor *Varanus salvator* Laurenti, 1768	20	*Acanthotaenia beddardi*; *Acanthotaenia nilotica*; *Acanthotaenia* sp.; *Kapsulotaenia sandgroundi*; *Duthiersia expansa*; *Scyphocephalus bisulcatus*	*Encyclometra colubrimumorum*; *Singhiatrema vietnamensis*; *Euparadistomum varani*; *Diplodiscus mehrai*; *Mesocoelium brevicaecum*; *Meristocotyle provitellaria*; *Haplorchis pumilio*	*Oswaldocruzia* sp. 2; *Raillietascaris varani*; *Tanque tiara*		
Gekkonidae						
Golden gecko *Gekko badenii* Szezerbak et Nekrasova, 1994	65			*Spauligodon vietnamensis*; *Pharyngodon duci*		
Spiny-tailed house gecko *Hemidactylus frenatus* Schlegel, 1836	149	*Oochoristica chinensis*; *Oochoristica tuberculata*; *Oochoristica* sp. 1; *Oochoristica* sp. 2	*Plagiorchis molini*; *Parabascus lepidotus*; *Postorchigenes ovatus*	*Oswaldocruzia* sp. 1; *Skrjabinodon azerbajdzanicus*	*Pseudoacanthocephalus nguyenthileae*	*Raillietiella frenatus*
Scincidae						
Long-tailed mabuya *Eutropis longicaudata* Hallowell, 1856	38		*Paradistomum orientalis*	*Oswaldocruzia* sp. 1; *Meteterakis mabuyae*		*Raillietiella affinis*
Agamidae						
Scale-bellied tree lizard *Acanthosaura lepidogaster* Cuvier, 1829	32			*Cosmocercoides tonkinensis*		
Emma Gray's forest lizard *Calotes emma* Gray, 1845	6			*Strongyluris calotis*		
Garden fence lizard *Calotes versicolor* (Daudin, 1802)	No data			*Abbreviata deschiensi*		
Eastern butterfly lizard *Leiolepis reevesii* Gray, 1831	20			*Thelandros vietnamensis*		
Vietnamese false bloodsucker *Pseudocalotes brevipes* (Werner, 1904)	7			*Strongyluris calotis*		

* Blank denotes no record.

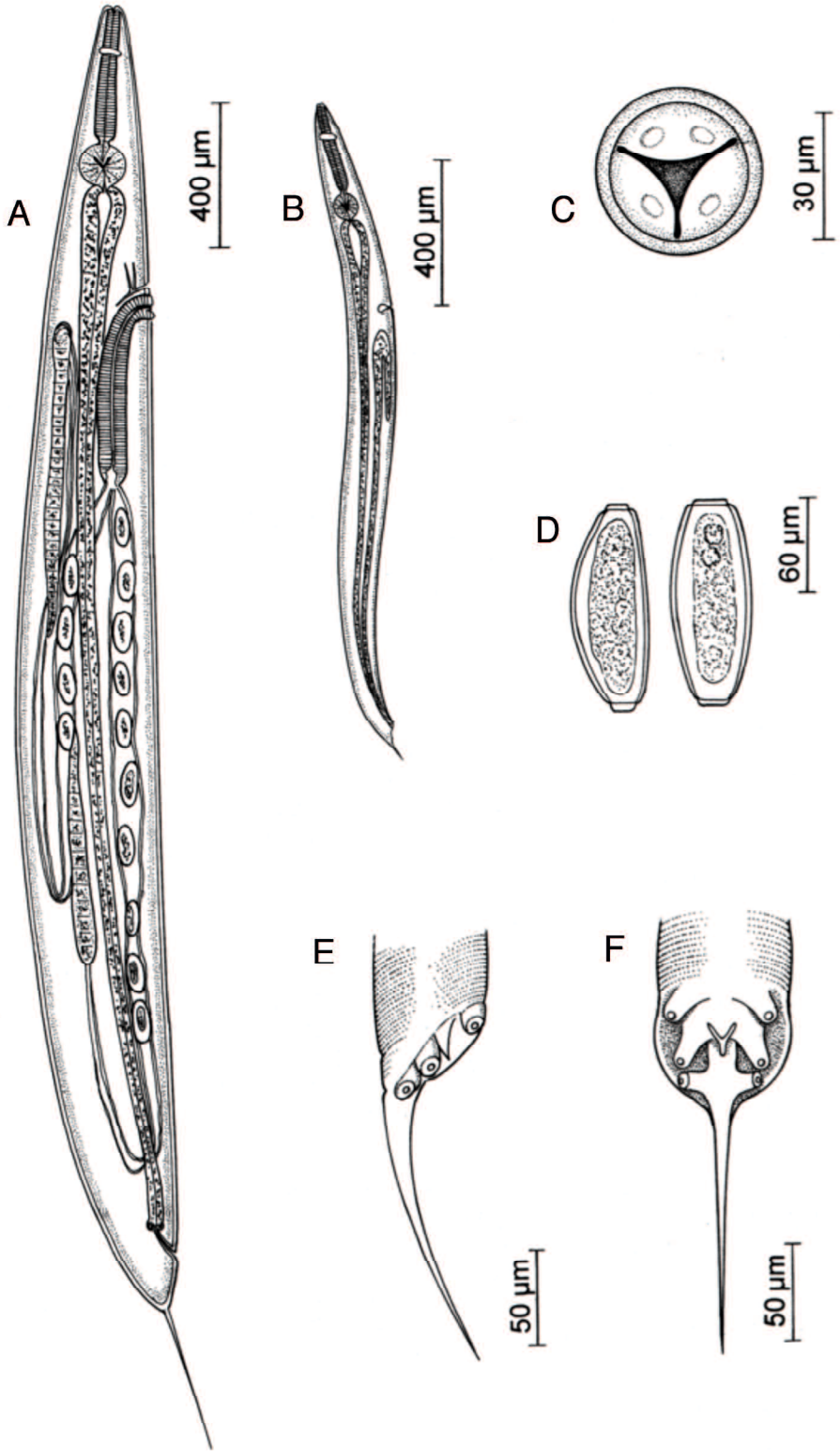

Figure 1. *Pharyngodon duci* n. sp. (**A**) female, entire, lateral view; (**B**) male, entire, lateral view; (**C**) female, en face view; (**D**) eggs; (**E**) male, posterior end, lateral view; and (**F**) male, posterior end, ventral view.

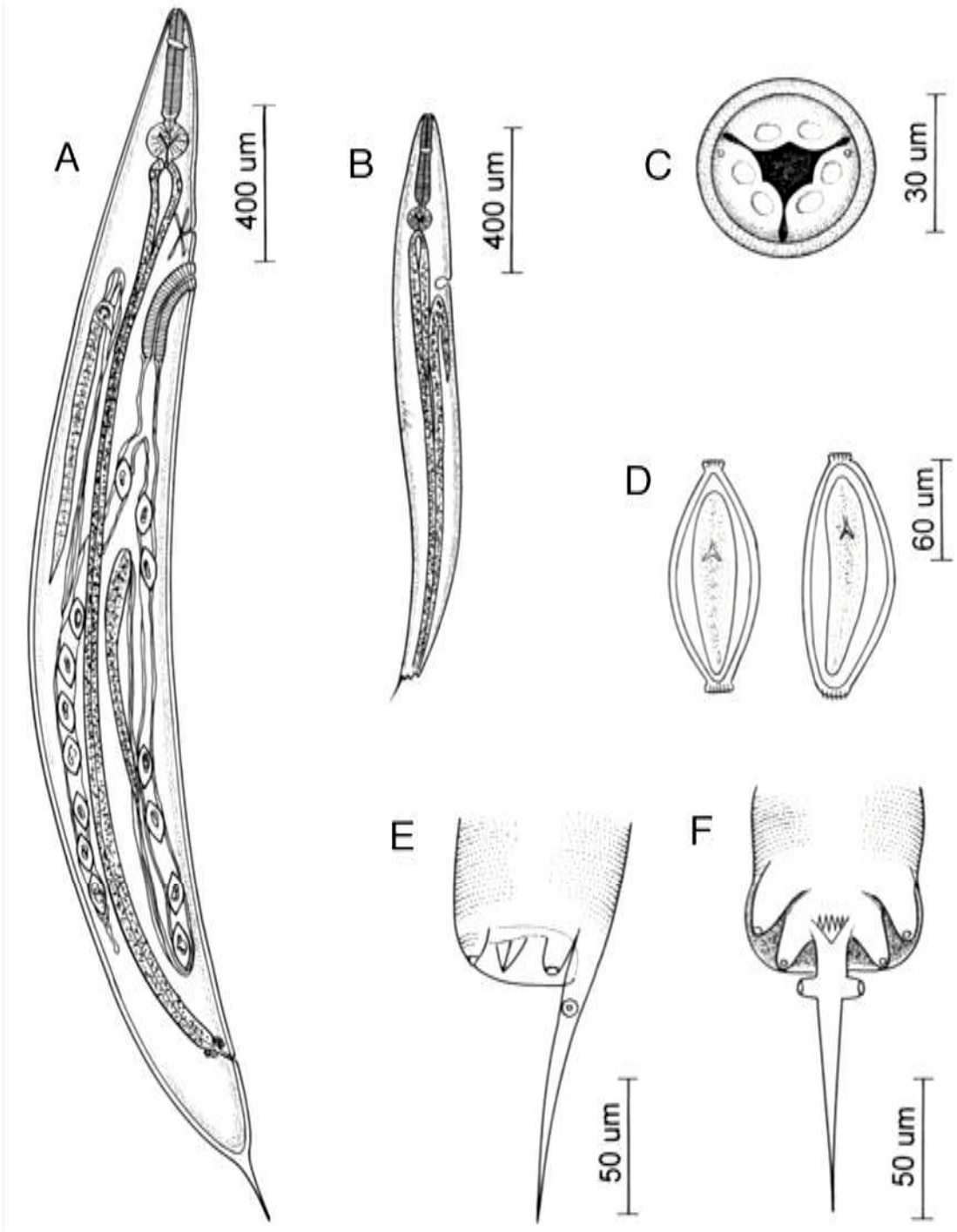

Figure 2. *Spauligodon vietnamensis* n. sp.: (**A**) female, entire, lateral view; (**B**) male, entire, lateral view; (**C**) female, en face view; (**D**) eggs; (**E**) male, posterior end, lateral view; and (**F**) male, posterior end, ventral view.

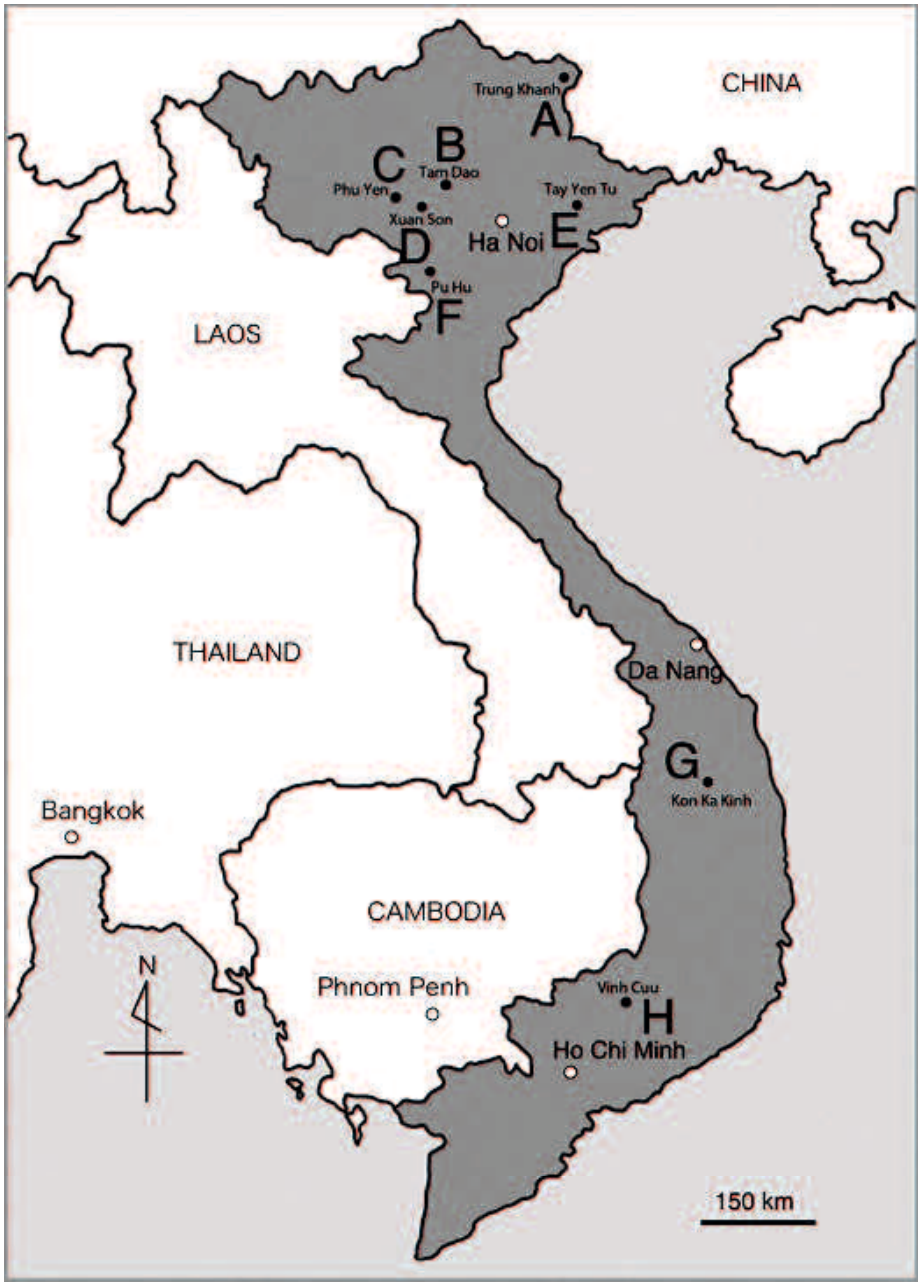

Figure 3. Collection sites of scale-bellied tree lizards.

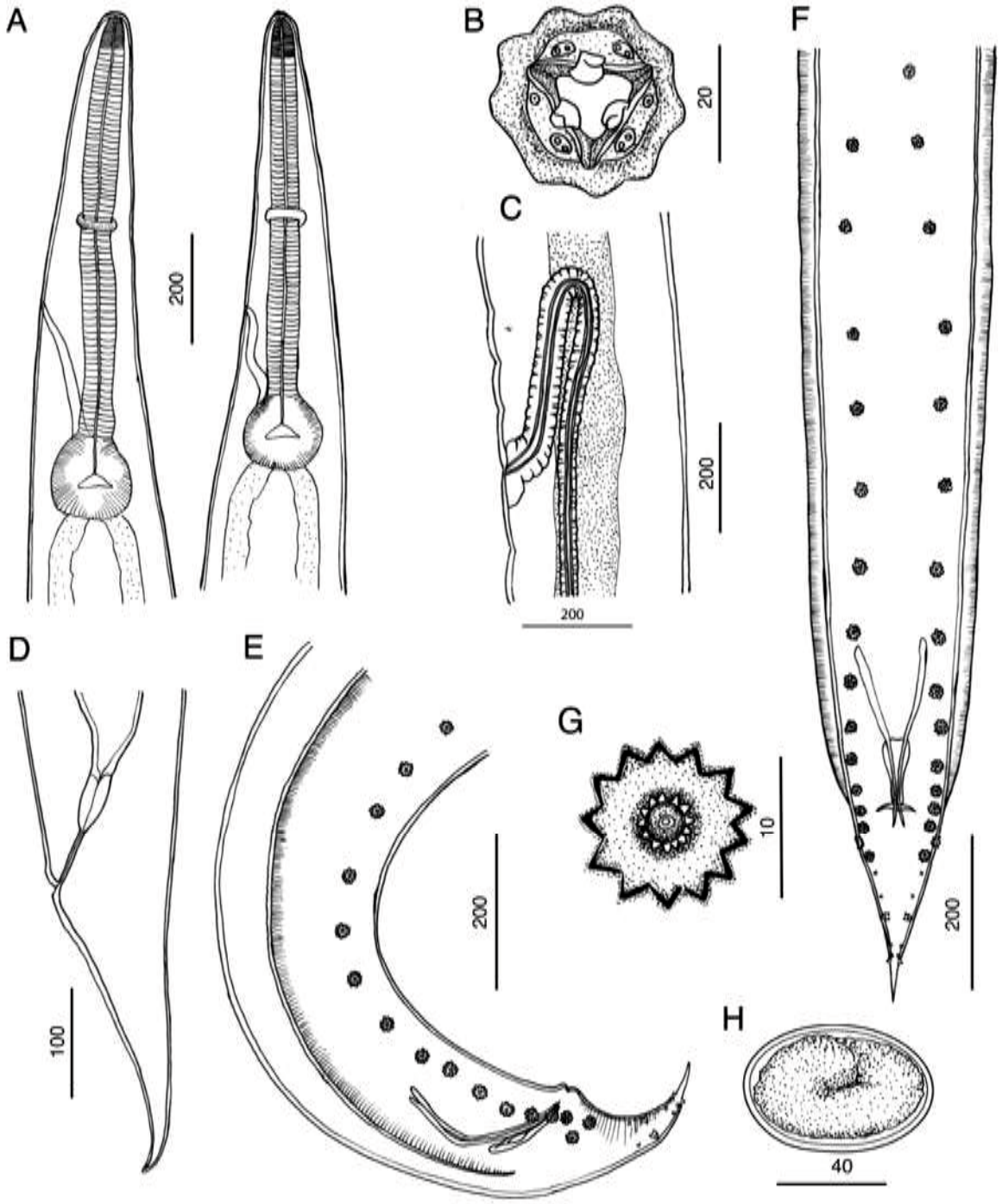

Figure 4. *Cosmocercoides tonkinensis* n. sp. in *Acanthosaura lepidogaster* from Vietnam. (**A**) Lateral view of the anterior end of female and male worms, respectively; (**B**) *En face* view of the anterior end; (**C**) Lateral view of the mid-body of a female around the vulva and vagina; (**D**) Lateral view of the posterior end of a female; (**E**) Lateral view of the posterior end of a male; (**F**) Ventral view of the posterior end of a male; (**G**) Caudal rosette; and (**H**) Embryonated egg in the uterus

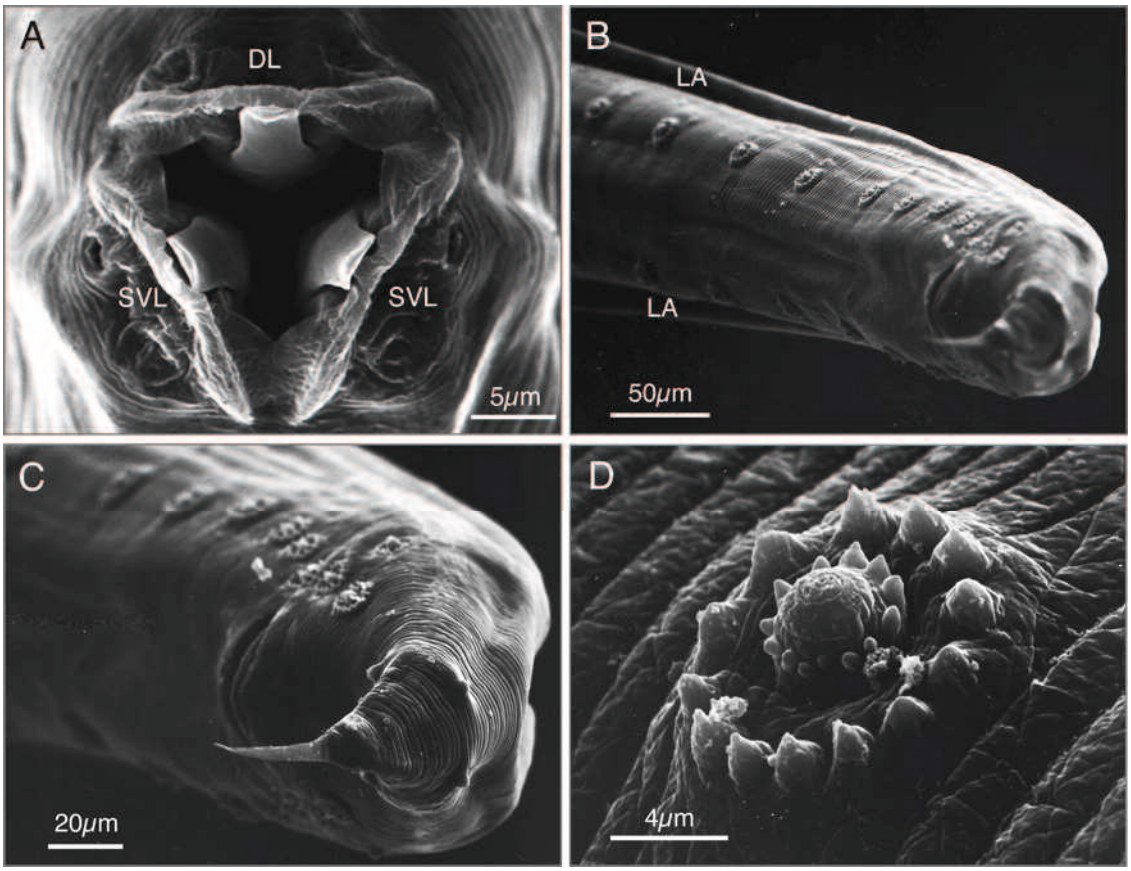

Figure 5. SEM view of a male worm of *Cosmocercoides tonkinensis* n. sp. **(A)** *En face* view of the anterior end (DL: dorsal lip, SVL: subventral lip); (**B**) Oblique view of the posterior end (LA: lateral ala); (**C**) High magnification of the tail; and (**D**) High magnification of a caudal rosette

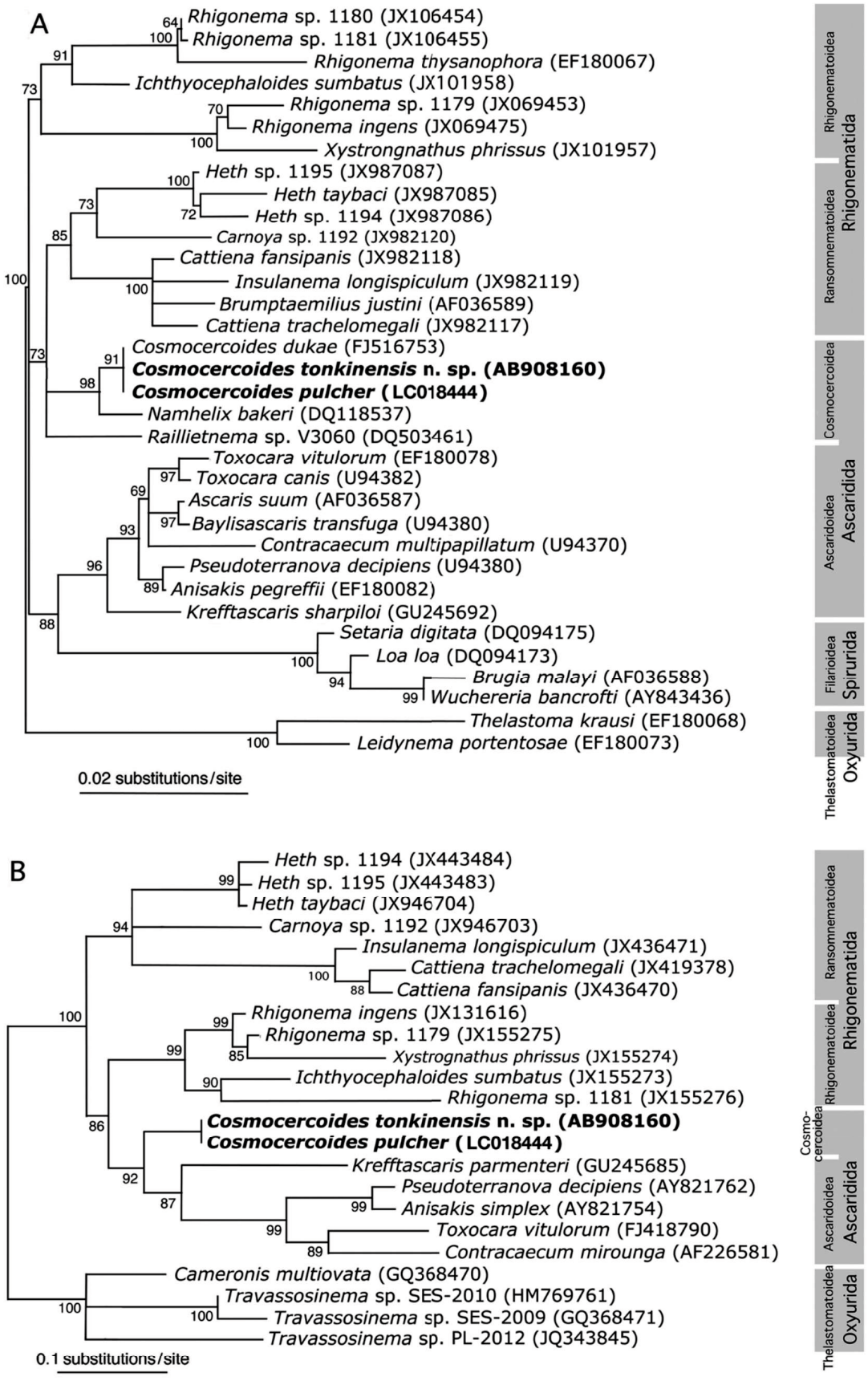

Figure 6. PhyML phylogenetic trees based on the 18S (**A**) and 28S (**B**) rDNA sequences. Species names are shown with their DDBJ/EMBL/GenBank accession nos. in parenthes

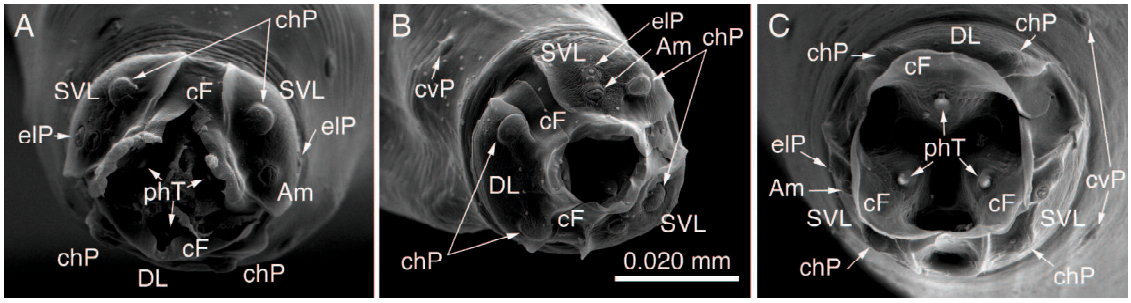

Figure 7. SEM view of the anterior end of *Strongyluris calotis*. (**A**) Male *S. calotis* in *Japalura swinhonis* from Taiwan; (**B**) male *S. calotis* in *Japalura polygonata* on Yonakuni Is., Okinawa, Japan; and (**C**) female *S. calotis* in *J. polygonata* from Kunigami, Okinawa Main Island, Japan. Photographs are at the same magnification and the scale is shown in **B**. Abbreviations: Am, amphid; cF, cuticular flange; chP, cephalic papilla; cvP, cervical papilla; DL, dorsal lip; elP, external labial papilla; phT, pharyngeal teeth; and SVL, subventral lip.

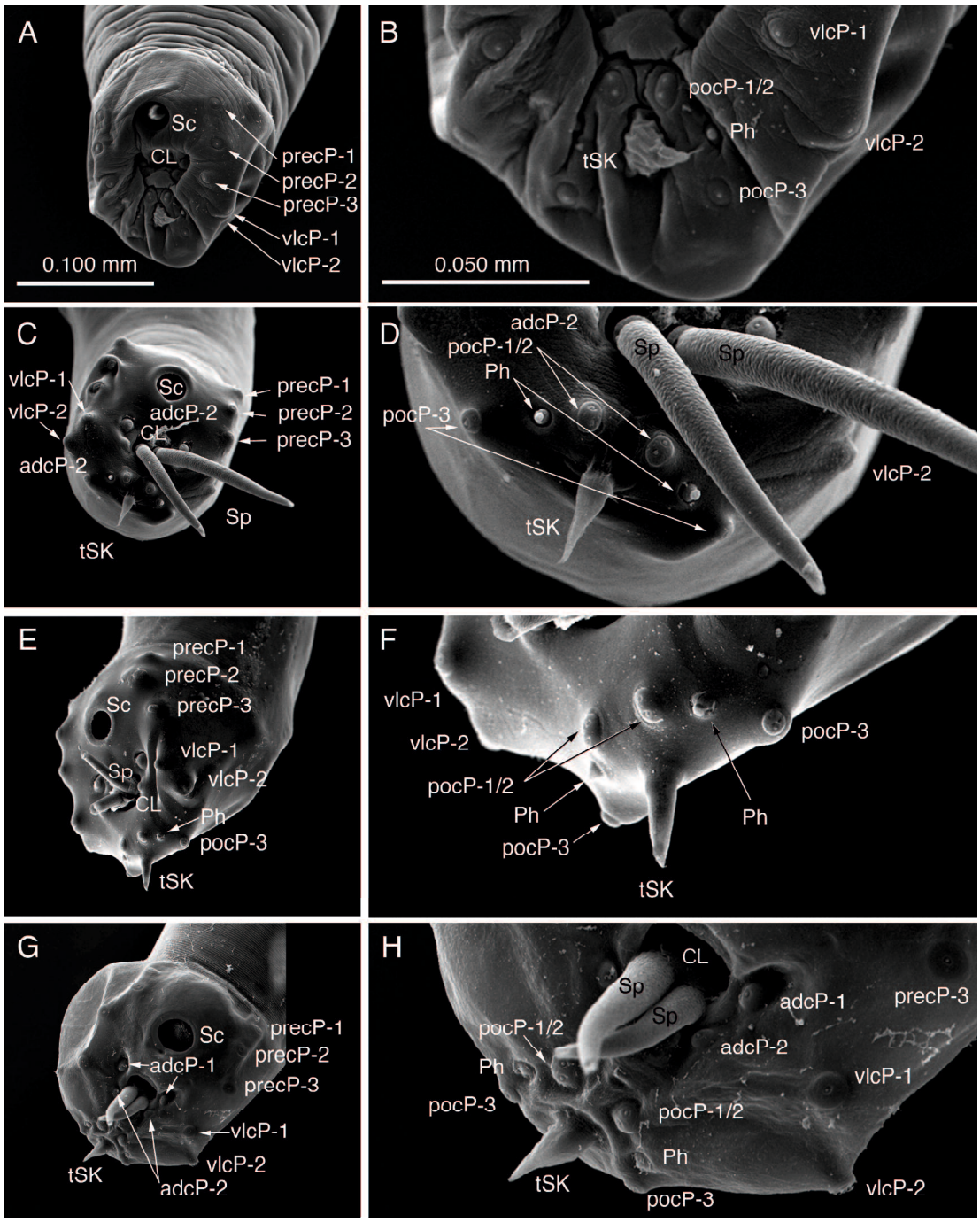

Figure 8. SEM view of the posterior end of male *Strongyluris calotis*. (**A, B**) Worm in *Japalura swinhonis* from Taiwan; (**C, D**) worm in *Japalura polygonata* on Yonakuni Is., Okinawa, Japan; (**E, F**) another worm in *J. polygonata* on Yonakuni Is., Okinawa, Japan; and (**G, H**) worm in *J. polygonata* from Kunigami, Okinawa Main Island, Japan. Photographs on the right side (**B, D, F, H**) are three times higher magnification of a part of each photograph on the left side (**A, C, E, G**), respectively. Photographs placed on the same side are at the same magnification and scales are shown in **A** and **B**. Abbreviations: adcP, adcloacal caudal papilla; CL, cloaca; Ph, phasmid; pocP, postcloacal caudal papilla around the terminal spike; precP, precloacal caudal papilla; Sc, precloacal sucker; Sp, spicule; tSK, terminal spike; and vlcP, ventrolateral caudal papilla around the cloaca. pocP-1/2 denotes united papillae.

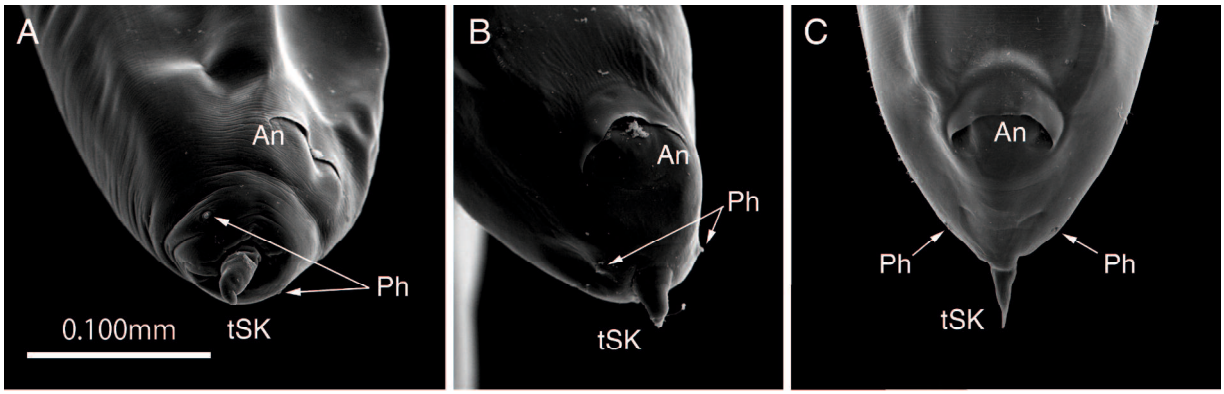

Figure 9. SEM view of the posterior end of female *Strongyluris calotis*. (**A**) Worm in *Japalura swinhonis* from Taiwan; (**B**) worm in *Japalura polygonata* on Yonakuni Is., Okinawa, Japan; and (**C**) worm in *J. polygonata* from Kunigami, Okinawa Main Island, Japan. Photographs are at the same magnification and the scale is shown in **A**. Abbreviations: An, anus; Ph, phasmid; and tSK, terminal spike.

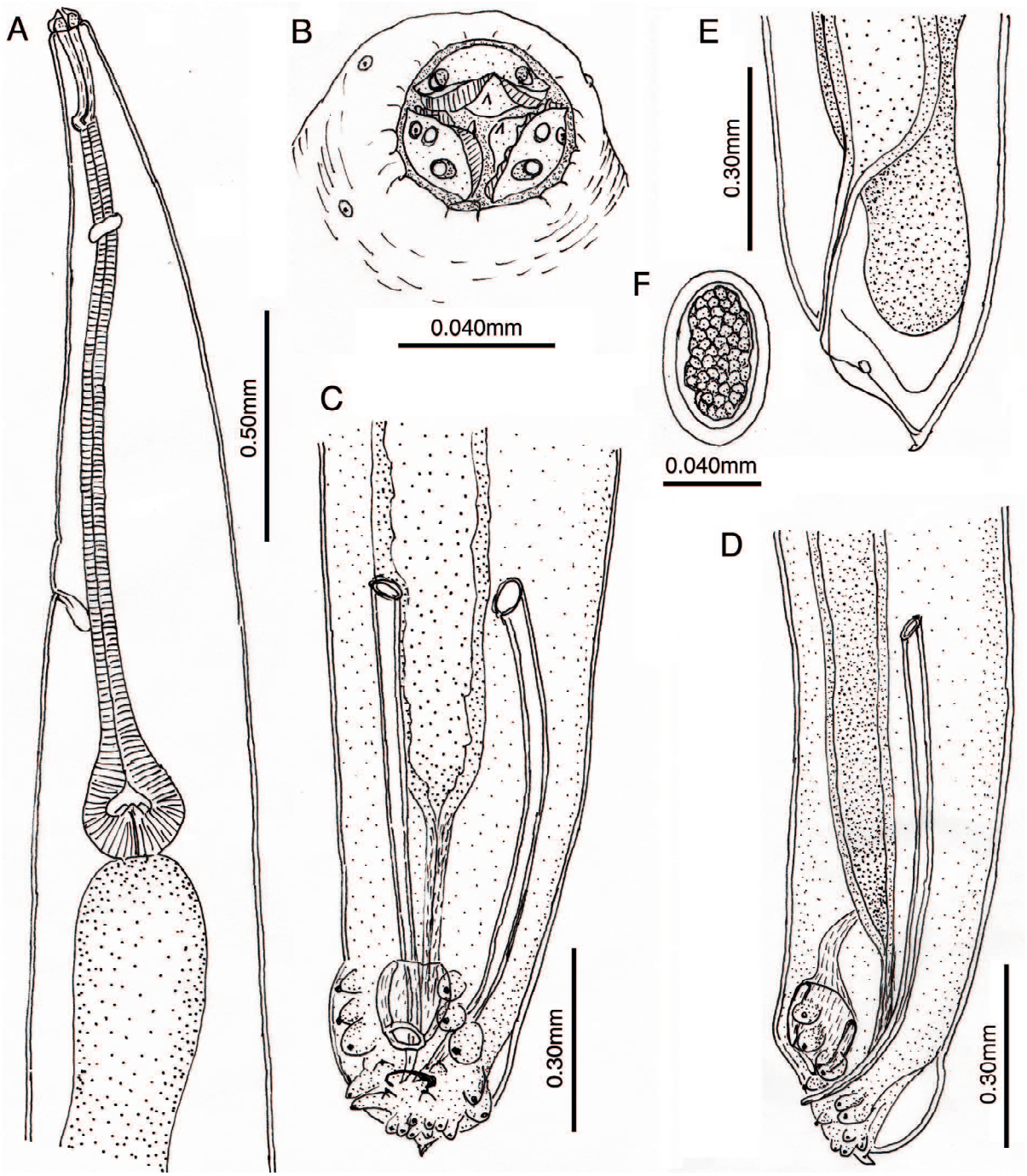

Figure 10. *Strongyluris calotis* in *Pseudocalotes brevipes*. (**A**) Lateral view of the anterior end of a male worm; (**B**) Nearly *en face* view of the anterior end of a male worm; (**C**) Ventral view of the posterior end of a male worm; (**D**) Lateral view of the posterior end of a male worm; (**E**) Lateral view of the posterior end of a female worm; and (**F**) Egg in the uterus.

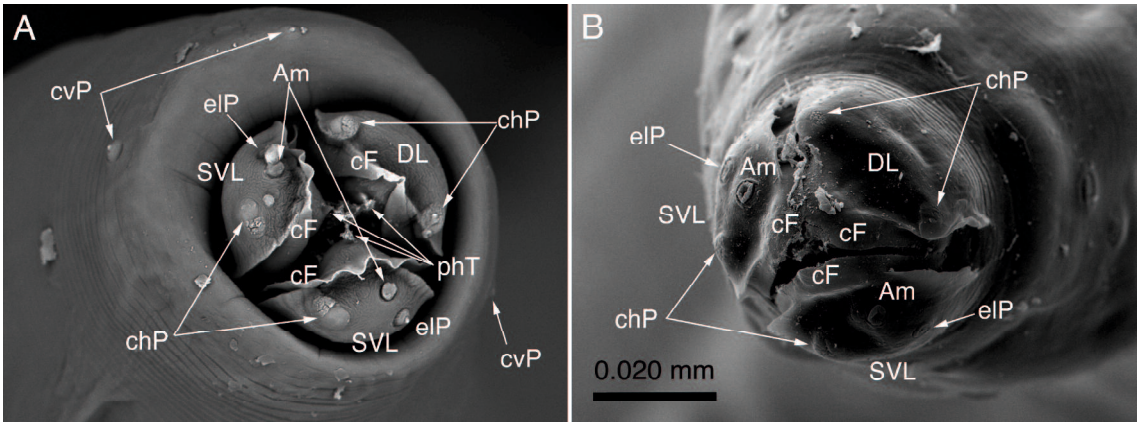

Figure 11. SEM view of the anterior end of a male *Strongyluris calotis* worm. Specimen from *Pseudocalotes brevipes* in Vietnam (**A**), and from *Calotes emma* in Singapore (**B**). Photographs are at the same magnification and the scale is shown in **B**. Abbreviations: Am, amphid; cF, cuticular flange; chP, cephalic papilla; cvP, cervical papilla; DL, dorsal lip; elP, external labial papilla; phT, pharyngeal teeth; and SVL, subventral lip.

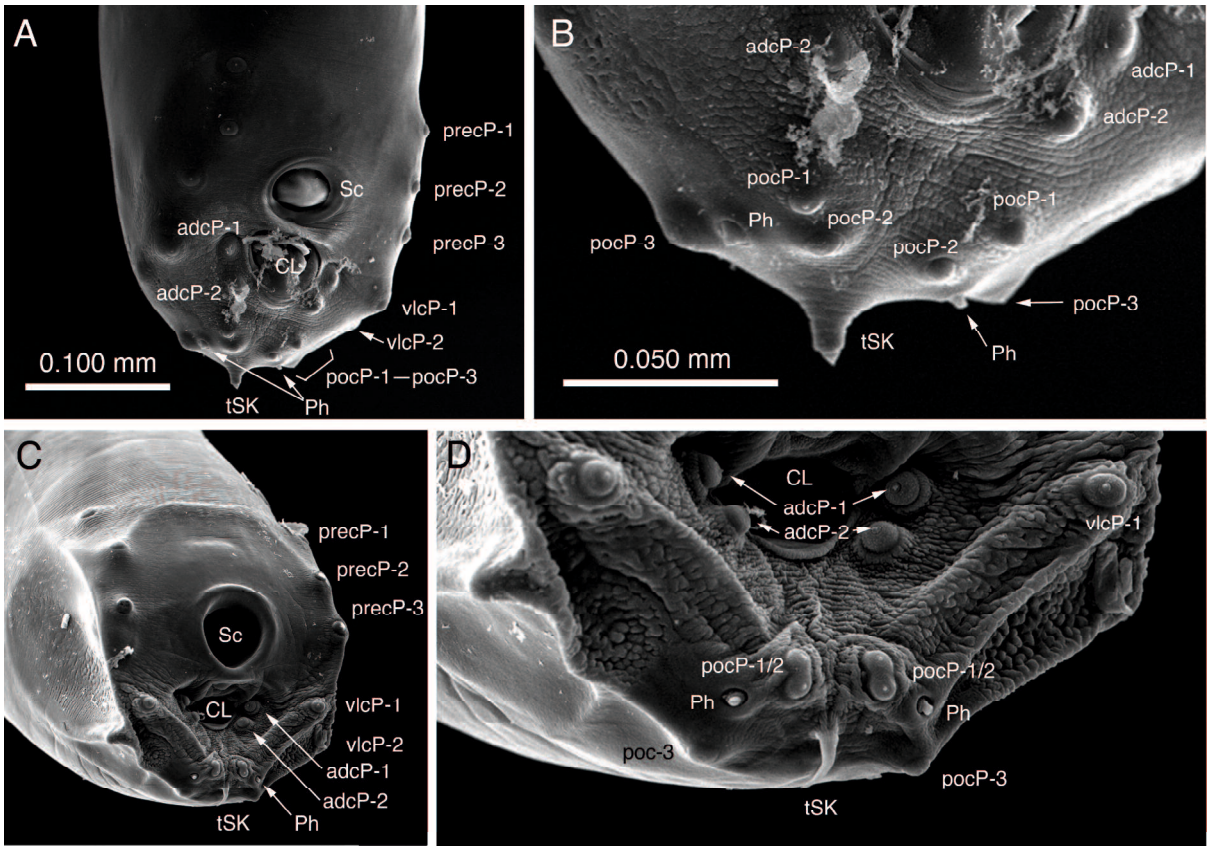

Figure 12. SEM view of the posterior end of a male *Strongyluris calotis* worm. Specimen from *Pseudocalotes brevipes* in Vietnam (**A, B**) and from *Calotes emma* in Singapore (**C, D**). Photographs on the right (**B, D**) are three times higher magnification of a part of each photograph on the left (**A, C**), respectively. Photographs on the same side are at the same magnification and scales are shown in **A** and **B**. Abbreviations: adcP, adcloacal caudal papilla; CL, cloaca; Ph, phasmid; pocP, postcloacal caudal papilla around the terminal spike; precP, precloacal caudal papilla; Sc, precloacal sucker; tSK, terminal spike; and vlcP, ventrolateral caudal papilla around the cloaca. pocP-1/2 denotes united papillae.

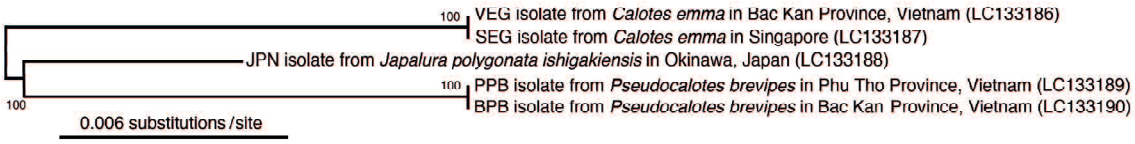

Figure 13. Unrooted ML phylogenetic tree based on concatenated ITS-1 and ITS-2 nucleotide sequence of 892-bp length.

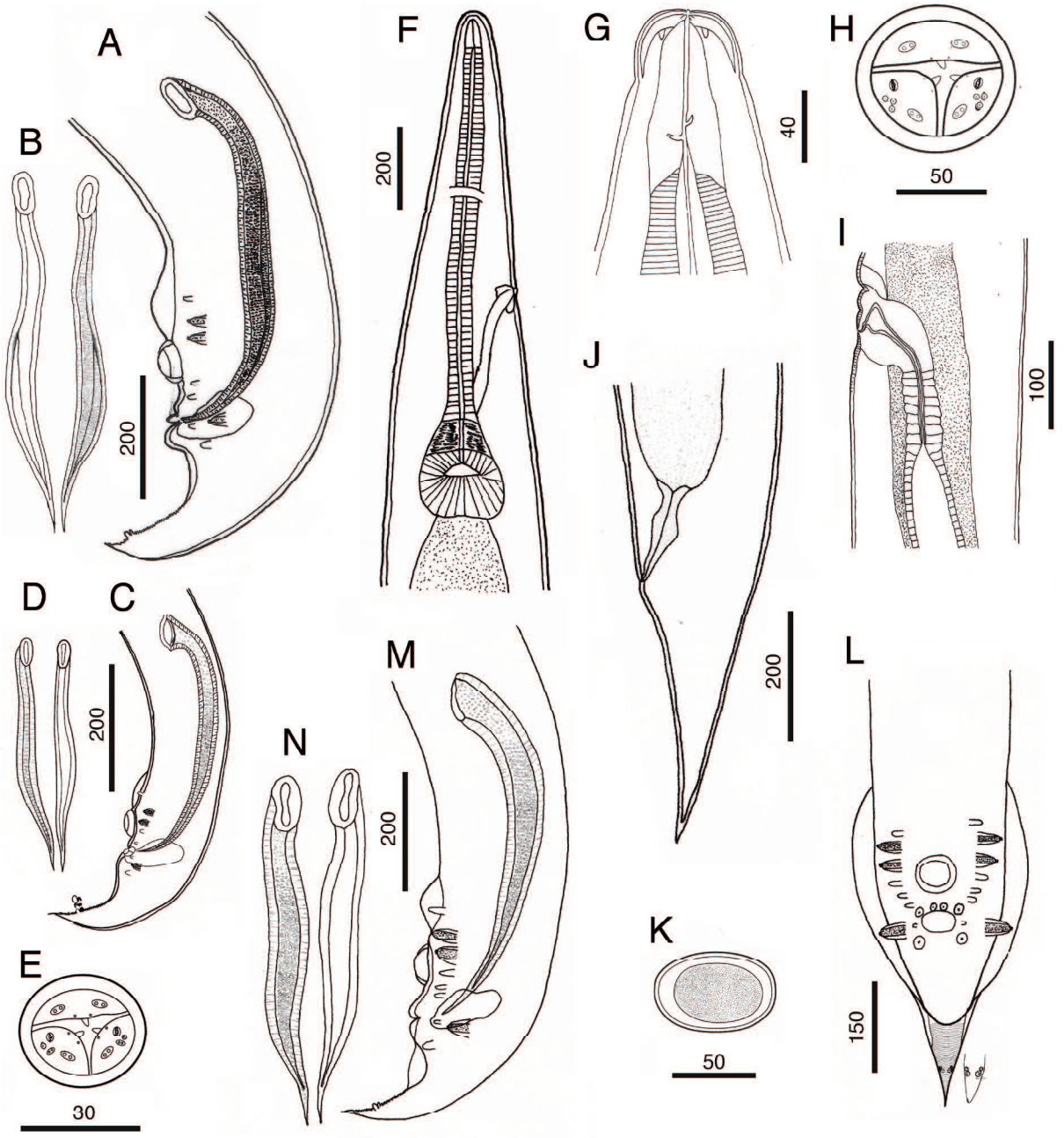

Figure 14. Different *Meteterakis* morphotypes collected from scale-bellied tree lizards in Vietnam: (**A**) and (**B**) morphotype A; (**C**)–(**D**) morphotype B; and (**E**)–(**N**) morphotype C. (**A**) Posterior end of male, lateral view; (**B**) alate spicules, ventral view; (**C**) posterior end of male, lateral view; (**D**) non-alate spicules, ventral view; (**E**) anterior end of female, *en face* view; (**F**) anterior end of female, ventral view; (**G**) anterior end of female, ventral view; (**H**) anterior end of female, *en face* view; (**I**) vulva of female, lateral view; (**J**) posterior end of female, lateral view; (**K**) egg; (**L**) posterior end of male, ventral view; (**M**) posterior end of male, lateral view; and (**N**) non-alate spicules, ventral view. Scale bars are in μm.

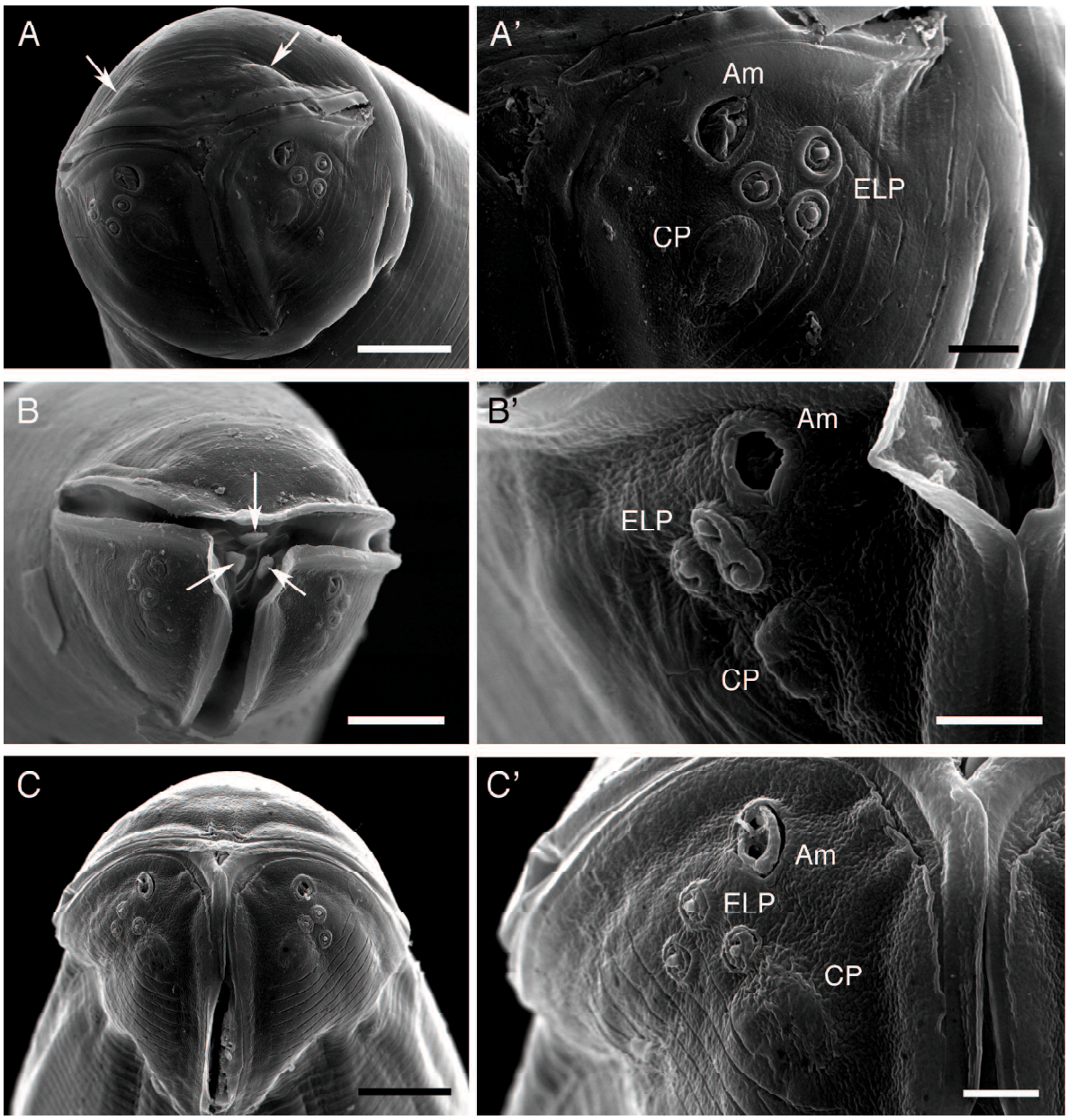

Figure 15. SEM view of the anterior end of male *Meteterakis* worms of different morphotypes: (**A**) and (**A'**) morphotype A; (**B**) and (**B'**) morphotype B; and (**C**) and (**C'**) morphotype C. (**A**)–(**C**) *En face* to oblique views of anterior end, scale bar = 10 μm; and (**A'**)–(**C'**) external surface of subventral labia at a higher magnification, scale bar = 3 μm. Arrows shown in (**A**) indicate cephalic papillae on the dorsal lip, and arrows in (**B**) indicate pharyngeal teeth. Abbreviations: Am, amphid; CP, cephalic papilla; ELP, external labial papilla.

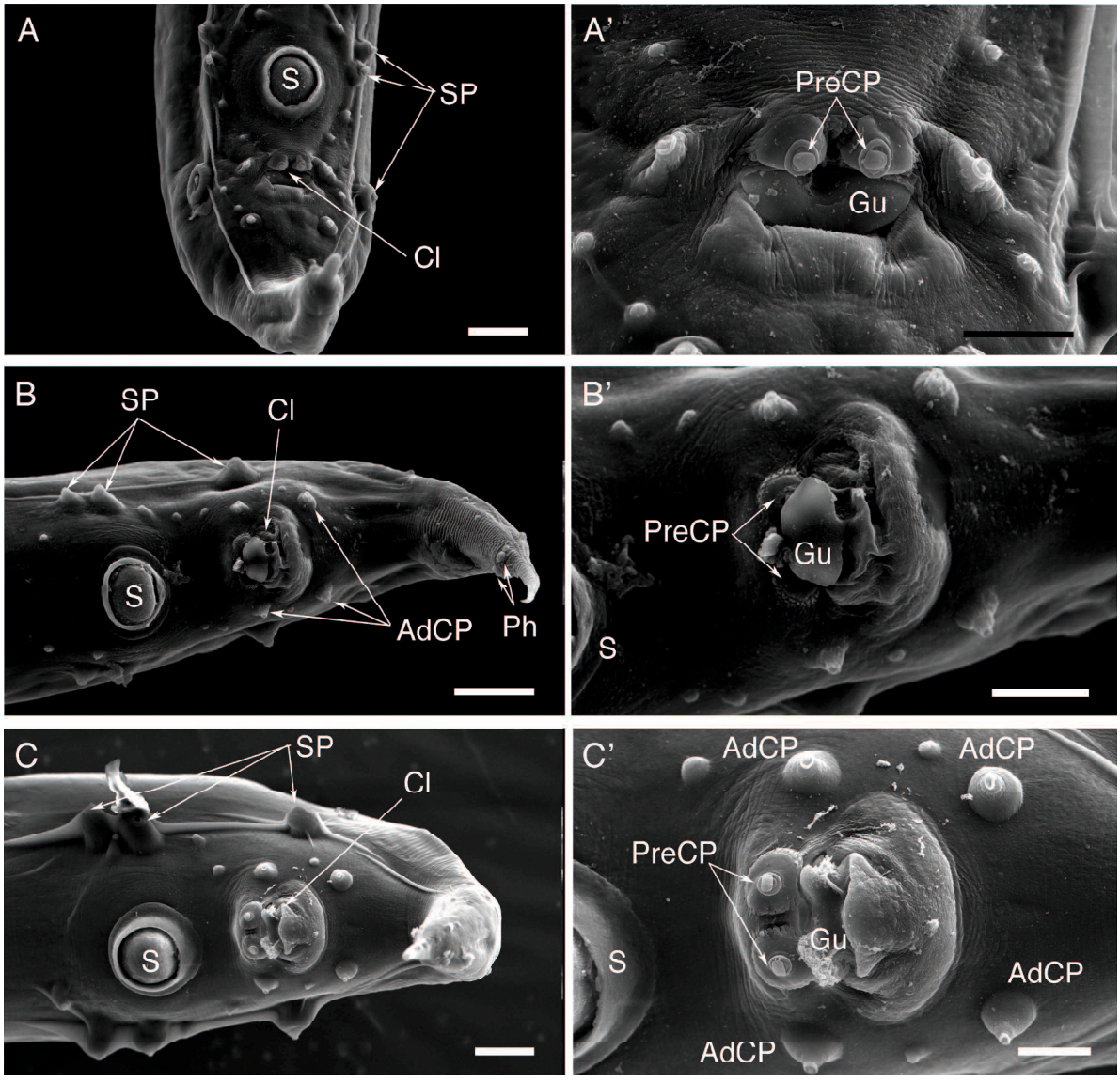

Figure 16. SEM view of the posterior end of male *Meteterakis* worms of different morphotypes: (**A**) and (**A'**) morphotype A; (**B**) and (**B'**) morphotype B; and (**C**) and (**C'**) morphotype C. (**A**)–(**C**) Ventral view of posterior end, scale bar = 20 μm; and (**A'**)–(**C'**) ventral view around the cloaca at a higher magnification, scale bar = 10 μm. Abbreviation: adCP, adcloacal caudal papilla; Cl, cloaca; Gu, gubernaculum; Ph, phasmid; preCP, precloacal papilla; S, precloacal sucker; SP, stout caudal papilla.

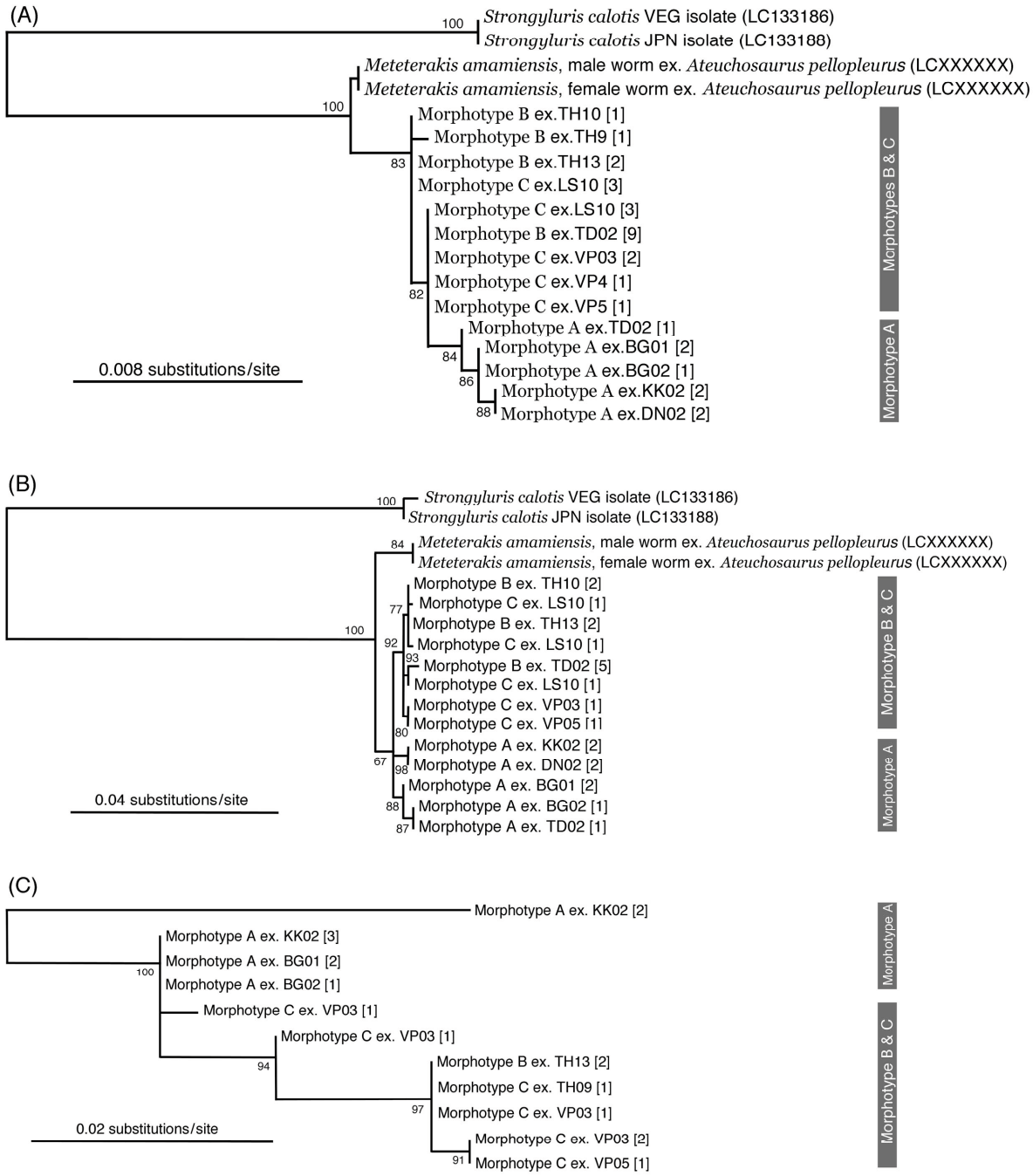

Figure 17. PhyML phylogenetic trees based on the 18S (**A**), 28S (**B**), and ITS-2 region of rDNA nucleotide sequences. Species names are shown with their DDBJ/EMBL/ GenBank accession nos. in parentheses, whereas mophotypes are shown with the host ID and number of different DNA samples from the same host in square parentheses.

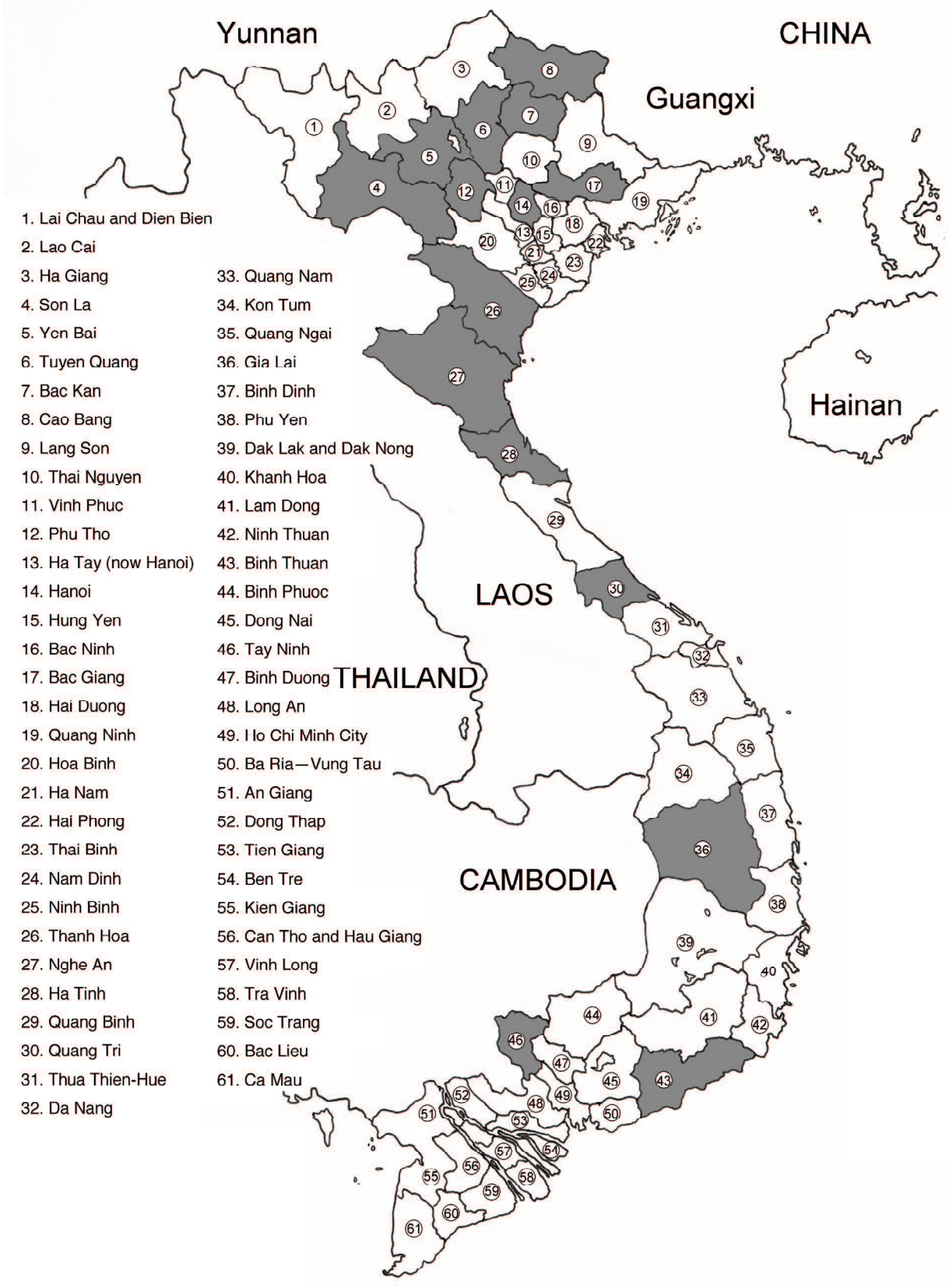

Figure 18. Map of Vietnam illustrating 61 provinces. The provinces coloured grey denote localities where parasites have been recorded in lizards.

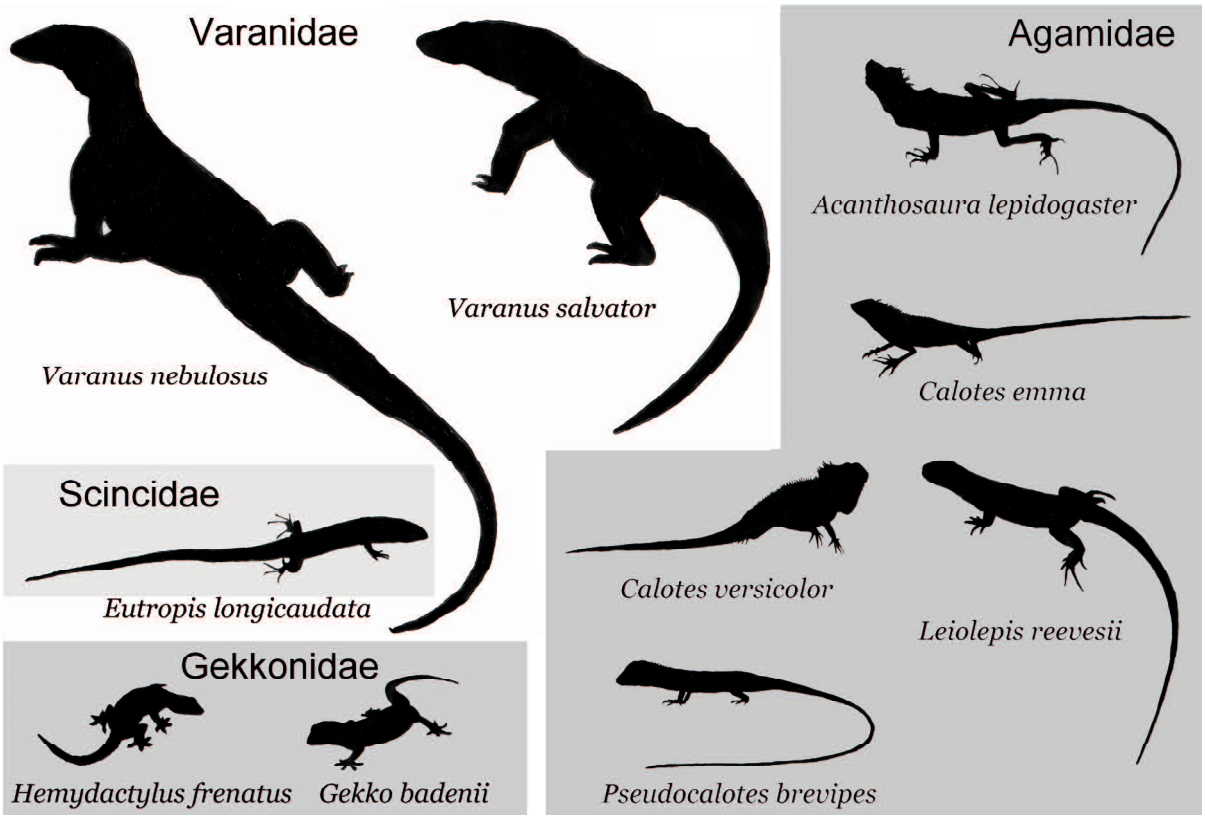

Figure 19. Shadow pictures of lizards that have been recorded as hosts for endoparasites in Vietnam. Lizards of different snout-vent length (SVL) are shown without reflecting their sizes. Approximate SVLs are as follows: *V. nebulosus*, 120 cm; *V. salvator*, 80 cm; *A. lepidogaster*, *L. reevesii* and *E. longicaudata*, 14 cm; and the remaining five species, 8 cm.