

Study of speciation and species taxonomy
of *Meteterakis* (Nematoda: Heterakidae)
from the East Asian islands

Naoya SATA

Graduate School of Science
Kyoto University

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東アジア島嶼域産寄生性線虫 *Meteterakis* 属の種分化と種分類に関する研究

佐田 直也

和文要旨

Meteterakis 属は、爬虫両生類の消化管に寄生し、中間宿主を必要としない寄生性線虫の分類群である。東アジア島嶼域からは3種が記載され、これらは、本州から琉球列島中部において、異所的に分布していることが知られていた。これら3種は、複数のトカゲ類とカエル類を宿主としており、特に東アジア島嶼域において洋上分散を経験したトカゲ属は、本線虫類の代表的な宿主と見なされている。本論文では、宿主城が広く、分散能の高い宿主を利用する、東アジア島嶼域産 *Meteterakis* 属線虫の種多様性と種分化様式の解明に取り組んだ。

東アジア島嶼域産 *Meteterakis* 属線虫の分布域解明のために、当該地域から、主要宿主であるトカゲ属を採集し、解剖調査を行った。結果、*M. japonica* の東日本と九州南部の下甕島からなる隔離分布、西日本における未同定種の分布を明らかにした。さらに、琉球列島南部の石垣島と西表島、台湾北部から *Meteterakis* 属線虫を初めて記録し、いずれも未同定種であった。これらの分布は、側所的または異所的であった。

次に、東アジア島嶼域産 *Meteterakis* 属線虫の進化史の推定のために、DNA 塩基配列を用いた分子系統解析を行った。結果、東アジア島嶼域産 *Meteterakis* 属線虫は、大きく2つの系統群（J・A-グループ）に分かれた。これら2系統群の分布は排他的、かつ、モザイク状であった。J-グループは、日本本土に産する *M. japonica* と沖縄諸島に産する *M. ishikawanae* から、A-グループは、奄美・小宝島に産する *M. amamiensis* と、西日本産・石垣島産・西表島産・台湾北部産の4未同定種により構成された。2系統群の分布境界と宿主の動物地理学的境界は一致せず、このことは、2系統群の分化は、本地域における宿主相の分断に起因しないことを示唆した。各系統群内の分岐パターンと宿主の動物地理学的境界を比較した結果、J-グループ内の種分化は宿主相の分断に起因すると推測された。一方、A-グループでは系統群内の遺伝的分化のパターンが、爬虫両生類相形成史から期待されるパターンと不一致であった。このことからA-グループの各種は、宿主相の形成とは独立に、周辺地域から分散し、分化したと考えられた。また、*M. japonica* の東日本と下甕島からなる隔離分布と、本土産種の集団遺伝学的解析の結果は、日本本土では、*M. japonica* の祖先が西日本を含む広域に分布していたが、後に下甕島を除く西日本において、侵入した西日本産未同定種の祖先に排除され、現在見られる2系統群のモザイク状分布が形成されたことを示唆し

た。以上より、東アジア島嶼域産 *Meteterakis* 属線虫の種多様性は、宿主相の分断との共分化、宿主相の分断から独立した周縁的種分化、種間の排他的相互作用によって形成されたと推定された。これらは、宿主体が広く、宿主の分散能が高い寄生虫の種分化要因として重要であると考えられた。

そして、西日本産と台湾北部産の未同定種について、分類学的地位を検討した。結果、両未同定種は、交接刺や側翼などで固有の形質状態を有していたため、それぞれが未記載種であると判断し、西日本産を *M. occidentalis*、台湾北部産を *M. formosensis* として新種記載した。さらに、側翼が本属の種判別に有効という結論に達し、*Meteterakis* 属の種分類における新たな判別形質を見いだした。

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INTRODUCTION

Elucidating the evolutionary history of living things leads us to a better understanding of the diversification process of organisms. Host-parasite relationships are considered as an important factor that has affected the parasite species diversity.

Meteterakis is a parasitic-nematode genus that parasitizes reptiles and amphibians, without intermediate hosts in the life cycle. In the East Asian islands, *Meteterakis* infests several hosts including *Plestiodon* lizards, which are known to have experienced oversea dispersals beyond well-established herpetofaunal boundaries in this area.

Several studies comparing the intra-specific genetic diversity of parasites with different host usages and ranges suggested that parasites that are specific to mobile hosts or with wide host ranges show reduced intra-specific genetic divergences because of their highly dispersible ability. Nonetheless, *Meteterakis* from the East Asians islands still exhibit the certain species diversity, so their diversification factors remain to be unclarified.

To clarify the species-diversification factors of *Meteterakis* from the East Asian islands, the distribution pattern, species diversity and inter-specific diversification process were investigated by morphological, molecular phylogenetic and population genetic analyses.

MATERIALS AND METHODS

To collect *Meteterakis* specimens from the East Asian islands, host lizards and frogs were dissected. The obtained specimens were morphologically identified with optical microscope.

The phylogenetic analyses were conducted based on mitochondrial and nuclear DNA sequences. Phylogenies were reconstructed using maximum likelihood method. Additionally, population genetic parameters, i.e. nucleotide and gene diversities and net genetic distances, were also calculated.

RESULTS

Distribution pattern of Meteterakis in the East Asian islands

An unidentified *Meteterakis* species was recorded from the western Japanese Archipelago. Shimokoshikijima Island is exceptional in harboring *M. japonica* despite that the island is located off Kyushu, western Japanese Archipelago. *Meteterakis japonica* was recorded from the various localities in the eastern Japanese Archipelago. Unidentified *Meteterakis* specimens were collected from Southern Ryukyus and Taiwan, respectively.

Phylogenetic and population genetic analyses of Meteterakis

The both mitochondrial and nuclear phylogenetic trees showed that the East Asian *Meteterakis* was divided into the two major clades (defined herein as J-, A-groups). J-group consisted of *M. japonica* indigenous to the Japanese Archipelago and *M. ishikawanae* distributed in the Okinawa Islands; and A-group comprised the unidentified *Meteterakis* species from the western Japanese Archipelago, Southern Ryukyus and Taiwan, and *M. amamiensis* inhabiting Amami Islands. The distributions of the respective groups were not overlapped each other. The A-group species were substantially diverged each other, and the precise relationships within A-group could not be resolved.

The nucleotide diversity of *M. japonica* was higher than that of unidentified *Meteterakis* from the western Japanese Archipelago. The gene diversities of these two units were higher and showed almost same values. The net-genetic distance between the unidentified *Meteterakis* from the western Japanese Archipelago and *M. amamiensis* was clearly smaller than that between the two J-group species.

Taxonomic study

Each unidentified *Meteterakis* species from western Japanese Archipelago and Taiwan possessed unique morphological characteristics, so they were described as *M. occidentalis*, and *M. formosensis*, respectively. Although *M. occidentalis* and *M. amamiensis* exhibit similar morphological features, lateral alae can be used as a valuable diagnostic character for discriminating these species.

DISCUSSION

The geographic ranges of J- and A-groups were discordant with the well-established biogeographic province of reptiles and amphibians including *Meteterakis* hosts (Japanese Archipelago; Central Ryukyus; Southern Ryukyus; Taiwan) negating

co-divergence of the major group of *Meteterakis* nematodes with the host fauna in the East Asian islands. Thus, species-level diversification within each of the major clades were compared with the host biogeography in followings.

The divergence within J-group was concordant with the host biogeography, and simply interpreted as a result of the vicariance events of their hosts. In contrast, the phylogenetic relationships within A-group was discordant with the biogeographic patterns of the hosts, i.e. although the host faunal vicariance between Southern Ryukyus and Taiwan occurred in relatively recent age, the substantial diversification among the *Meteterakis* lineages of these area was detected. This fact implies that these endemic species were formed by multiple peripatric speciation rather than *in situ* diversification in accordance with host vicariance.

The disjunct distribution of *M. japonica*, the comparison of the net genetic distances and the nucleotide diversity suggested that the ancestor of *M. japonica* had been initially indigenous to the Japanese Archipelago, and then the ancestor of *M. occidentalis* invaded to the region and replaced with the former.

The present study revealed that the species diversity of *Meteterakis* from the East Asian islands has been formed by co-divergences with their host faunal vicariations, peripatric speciation and exclusive interactions between species. These factors may also play an important role in diversification of parasites having mobile hosts or wide host ranges.

The morphological analysis revealed that lateral alae could discriminate two morphologically closely related species. Thus, lateral alae can be regarded as one of the key diagnostic characters of this nematode group.

Distribution of Parasitic Nematodes in Japan with Host–Parasite Relationship of Lizards of *Plestiodon* (Reptilia: Squamata: Scincidae)

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Distribution of Parasitic Nematodes in Japan with Host–Parasite Relationship of Lizards of *Plestiodon* (Reptilia: Squamata: Scincidae)

NAOYA SATA

Department of Zoology, Graduate School of Science, Kyoto University, Sakyo, Kyoto, 606-8502, Japan
(e-mail: sata@zoo.zool.kyoto-u.ac.jp)

ABSTRACT: Four Japanese species of *Plestiodon* (Reptilia: Scincidae, formerly *Eumeces*): *Plestiodon japonicus*, *Plestiodon finitimus*, *Plestiodon latiscutatus*, and *Plestiodon oshimensis oshimensis* collected from Honshu, Shikoku, and Kyushu of the Japanese main islands and Amamioshima Island of the Ryukyus, Japan, were examined to make a more exact list of the parasitic nematodes and to reveal their distribution patterns. Six nematode species were found, including 2 species of Heterakidae (*Meteterakis japonica* and *Meteterakis amamiensis*), 1 species of Rhabdiasidae (*Kurilonema markovi*), 1 species of Trichostrongylidae (*Oswaldocruzia socialis*), 1 species of Cosmocercidae (Cosmocercidae gen. sp.), and 1 species of Pharyngodonidae (Pharyngodonidae gen. sp.). Six new hosts were recorded and 19 new localities were reported. *Meteterakis amamiensis* and *K. markovi* were distributed on both sides of Watase's line, which is known as a geographical distribution boundary between the Oriental and Palaearctic regions in Japan. A parapatric distribution of *M. japonica* and *M. amamiensis* in the Japanese main islands and coexistence of *K. markovi* and *Neoentomelas asatoi* in Amamioshima Island were revealed.

KEY WORDS: Japan, lizard, *Plestiodon*, nematode, *Meteterakis japonica*, *Meteterakis amamiensis*, *Kurilonema markovi*, *Oswaldocruzia socialis*, Cosmocercidae, Pharyngodonidae.

The parasitic nematode fauna of Japanese lizards have been studied by Hasegawa (1985, 1990, 1992a), Goldberg et al. (1993), Telford (1997), and Bursey et al. (2005) based on specimens collected from limited areas in Japan. Their studies showed the presence of 5 species of parasitic nematodes and a few other helminths in Japanese *Plestiodon* lizards (formerly *Eumeces*).

Recent genetic and taxonomic studies on Japanese *Plestiodon* revealed that 3 species are distributed parapatrically in the Japanese main islands: *Plestiodon japonicus* in Western Japan, *Plestiodon finitimus* in Eastern Japan, and *Plestiodon latiscutatus* on the Izu Peninsula and Izu Islands (Okamoto et al., 2006; Okamoto and Hikida, 2009, 2012). In addition, a recent phylogeographical study of *Plestiodon marginatus* in the Ryukyus suggested the specific status of 2 subspecies of *P. marginatus*, rather than subspecific status (Kurita and Hikida, 2014). Table 1 is a list of the parasitic nematodes of Japanese *Plestiodon* species ordered according to recent host taxonomy. This table indicates that the parasitic nematode fauna of Japanese *Plestiodon* has been only partially identified. In this study, to make a more exact list of the parasitic nematodes of Japanese *Plestiodon* and to reveal their distribution patterns, 137 specimens of 4 species of *Plestiodon* were examined.

MATERIALS AND METHODS

One hundred thirty-seven specimens from 4 species of Japanese *Plestiodon* lizards collected from Honshu, Shikoku, and Kyushu of the Japanese main islands and

Amamioshima Island of the Ryukyus were examined: *P. japonicus* ($n = 83$) was collected from 26 localities; *P. finitimus* ($n = 44$) from 19 localities; *P. latiscutatus* ($n = 6$) from 3 localities, and *Plestiodon oshimensis* ($n = 4$) from 2 localities. Both specimens from the zoological collection of Kyoto University Museum (KUZ) and specimens newly captured in 2011–2012 were examined. The latter specimens were also deposited in the KUZ collection.

Captured specimens were euthanized by an injection of sodium pentobarbital. Body cavities of all of specimens were dissected by making a longitudinal incision beginning at the throat and ending at the vent. Body cavities were searched for parasitic nematodes with a dissecting microscope and the digestive tract and lungs were removed. Excised organs were dissected longitudinally and the lumens searched. Nematodes obtained from individual hosts were fixed with 5% hot glycerin–ethyl alcohol (100% glycerin: 70% ethyl alcohol, 1:19 dilution) and placed in a drop of 100% glycerin under a cover glass to allow them to clear. Cleared preparations were used for identification with a light microscope. Host lizards were fixed with 10% formalin and preserved in 75% ethyl alcohol.

Identification of nematodes was generally carried out to the species level, but some were done to genus or family level. Nematode specimens that could not be identified morphologically (i.e., degraded, injured, or larval individuals and cysts) were excluded from the study. Some nematode specimens were deposited in the KUZ collection as vouchers (KUZ Z630–Z674). New localities were recorded for nematodes that were identified to species level and were found for the first time in a particular prefecture or on a particular island.

RESULTS

Table 1 lists the parasitic nematodes of Japanese *Plestiodon* based on past and present studies and on any recent host taxonomic changes.

Table 1. Parasitic nematodes found in Japanese *Plestiodon* lizards.

Host	Nematode	Infection site	Reference
<i>Plestiodon japonicus</i>	<i>Aplectana macintoshii</i>	Large intestine	Goldberg et al. (1993)
	Cosmocercidae gen. sp.	Rectum	This study
	<i>Meteterakis japonica</i> *	Rectum	This study
	<i>Meteterakis amamiensis</i> *	Rectum, small intestine	This study
	<i>Kurilonema markovi</i> *	Lung	This study
	<i>Oswaldocruzia socialis</i> *	Rectum, small intestine	This study
<i>Plestiodon finitimus</i>	<i>M. japonica</i>	Rectum	This study, Telford (1997), Hasegawa and Asakawa (2004)
	<i>K. markovi</i>	Lung	This study, Telford (1997), Szczerbak and Sharpilo (1969)
<i>Plestiodon latiscutatus</i>	<i>M. japonica</i>	Large intestine	Bursey et al. (2005)
	<i>K. markovi</i>	Lung	This study, Bursey et al. (2005)
<i>Plestiodon marginatus</i>	<i>Parapharyngodon</i> sp.	Rectum	Hasegawa (1985), Hasegawa and Asakawa (2004)
<i>Plestiodon oshimensis</i>	<i>M. amamiensis</i>	Rectum, small intestine	This study, Hasegawa (1990)
	<i>K. markovi</i> *	Lung	This study
	Pharyngodonidae gen. sp*	Rectum	This study
? <i>Plestiodon kishinouyei</i>	<i>Meteterakis</i> sp.	? Rectum	Hasegawa (1992b), Hasegawa and Asakawa (2004)

*New host record.

Thirty-eight and six-tenths percent (32/83) of *P. japonicas*; 36.4% (16/44) of *P. finitimus*; 66.7% (4/6) of *P. latiscutatus*; and 100% (4/4) of *P. oshimensis* harbored parasitic nematodes in this study. No digestive-tract parasitic nematodes were obtained from *P. latiscutatus*.

***Plestiodon japonicus* (Peters 1864)**

Eighty-three specimens collected from Kyushu, Shikoku, and western Honshu of the Japanese main islands and some adjacent islets were used for this study. Body cavities and digestive tracts of all 83 hosts, and lungs of 66 of the 83 specimens, were examined for parasitic nematodes. Information about the locality where the host lizards were captured is shown in Table 2. Mean intensities are reported as the mean $\pm$ standard error and range.

***Meteterakis japonica* (Wilkie, 1930)**

(Syn. *Spinicauda japonica* Wilkie, 1930; *Africana howardi* Li, 1933).

Prevalence and mean intensity: One of 83 hosts was infected (1.2%, 7).

Site of infection: Rectum.

Locality record in this study: Tokushima, Tokushima Prefecture, Shikoku.

Type host: Wilkie (1930) described the host of *M. japonica* as the “Bull-frog” and did not provide the definite scientific name of this frog in the original

description of the nematode. He guessed this frog to be *Rana japonica*.

Type locality: Tokyo, Japan.

Other reported hosts in Japan: *Bufo japonicus japonicus* (see Yamaguti, 1935); *Bufo japonicus formosus* (see Yamaguti, 1941; Goldberg and Bursey, 2002); *Bufo gargarizans miyakonis* (see Hasegawa, 1984; Hasegawa and Iwatsuki, 1993); *Rana ornativentris*, *Takydromus tachydromoides*, and *Plestiodon finitimus* (see Telford, 1997); *P. latiscutatus* (see Bursey et al. 2005).

Additional locality records in Japan: Shirahama, Wakayama Prefecture, Honshu (Yamaguti, 1935); Shiga Prefecture, Honshu (Yamaguti, 1941); Miyakojima Island of the Ryukyus (Hasegawa, 1984; Hasegawa and Iwatsuki, 1993); Hanno, Saitama Prefecture, Honshu (Telford, 1997); Kanagawa Prefecture, Honshu (Goldberg and Bursey, 2002; Bursey et al., 2005).

Specimens deposited: KUZ Z641 (1 vial).

Geographical range: Shikoku (this study) and Eastern Japan.

Remarks

Although the life cycle of *Meteterakis* has not been investigated, the members of the Heterakoidea, including *Meteterakis*, are considered to be monoxenous. Infection occurs when eggs containing infective-stage larvae are ingested by a suitable host

Table 2. Locality where host lizards of *Plestiodon* were captured.

<i>Plestiodon</i>	Locality	Geographical coordinates	N
<i>Plestiodon japonicus</i>	Matsuyama, Ehime Prefecture	33°50'38.0"N; 132°46'02.5"E, 33°51'N; 132°47'E, 33°51'N; 132°46'E	8
	Yamada, Nakatsu, Oita Prefecture	33°27'04.6"N; 131°02'12.3"E	1
	Kagoshima, Kagoshima Prefecture	31°35'39.76"N; 130°27'31.55"E, 31°35'40.11"N; 130°33'1.60"E	5
	Kanoya, Kagoshima Prefecture	31°28'23.67"N; 130°49'11.56"E	1
	Shimokoshikijima Island, Kagashima Prefecture	31°41'N; 139°44'E	1
	Iojima Island, Kagoshima Prefecture	30°46'50.16"N; 130°16'49.58"E	2
	Nagata, Yakushima Island, Kagoshima Prefecture	30°23'53.41"N; 130°25'41.51"E	1
	Isso, Yakushima Island, Kagoshima Prefecture	30°27'N; 130°29'E	3
	Kizu, Kyoto Prefecture	34°44'31.53"N; 135°51'17.33"E	2
	Kyoto, Kyoto Prefecture	35°01'N; 135°47'E, 35°01'N; 135°45'E, 35°02'N; 135°47'E	23
	Miyama, Nantan, Kyoto Prefecture	35°16'50.06"N; 135°41'44.06"E	1
	Mt. Oe, Fukuchiyama, Kyoto Prefecture	35°26'52.85"N; 135°6'43.36"E	3
	Kikuchi, Kumamoto Prefecture	32°59'13"N; 130°48'54"E	2
	Kumamoto, Kumamoto Prefecture	32°47'N; 130°41'E	3
	Kochi, Kochi Prefecture	33°34'54.73"N; 133°32'48.54"E	1
	Motoyama, Kochi Prefecture	33°45'35.86"N; 133°34'54.02"E	1
	Takashima, Shiga Prefecture	35°22'N; 135°48'E, 35°26'N; 135°58'E, 35°22'N; 135°58'E	5
	Yoshida, Unnan, Shimane Prefecture	35°07'31"N; 132°53'53"E	1
	Tokushima, Tokushima Prefecture	34°04'30.9"N; 134°33'19.8"E	1
	Minami, Tokushima Prefecture	33°43'57.4"N; 134°31'39.9"E	1
	Fukuejima Island, Nagasaki Prefecture	32°41'33.11"N; 128°50'48.51"E	1
	Awaji Island, Awaji, Hyogo Prefecture	34°30'37.46"N; 134°55'30.04"E	2
	Akiota, Hiroshima Prefecture	34°24'48"N; 132°40'30"E	1
	Kaita, Hiroshima Prefecture	34°22'19.1"N; 132°34'11.4"E	1
	Hatsukaichi, Hiroshima Prefecture	34°29'28"N; 132°5'8"E	5
	Yu, Iwakuni, Yamaguchi Prefecture	34°02'N; 132°12'E	7
<i>Plestiodon finitimus</i>	Toyohashi, Aichi Prefecture	34°46'14"N; 137°23'36"E	2
	Mt. Rokusyo, Aichi Prefecture	35°03'35"N; 137°17'02"E	1
	Mt. Hakkoda, Aomori, Aomori Prefecture	40°38.779"N; 140°51.131'E, 40°39.033"N; 140°51.009'E	5
	Yamana, Takasaki, Gunma Prefecture	36°16'N; 139°01'E	2
	Mt. Ryokami, Saitama Prefecture	36°02'34.5"N; 138°51'43.8"E	1
	Yogo, Nagahama, Shiga Prefecture	35°39'41.5"N; 136°11'06.7"E	1
	Shizuoka, Shizuoka Prefecture	34°55'26.74"N; 138°21'58.71"E, 34°57'31.87"N; 138°21'10.09"E	4
	Shimada, Shizuoka Prefecture	34°49'27.30"N; 138°7'38.93"E	1
	Arai, Kosai, Shizuoka Prefecture	34°41'24.01"N; 137°33'2.00"E	1
	Hamamatsu, Shizuoka Prefecture	34°42'41.89"N; 137°43'28.72"E	1
	Fukuroi, Shizuoka Prefecture	34°44'N; 137°58'E, 34°44'N; 137°59'E, 34°43'N; 137°58'E	7
	Fuji, Shizuoka Prefecture	35°8'26.87"N; 138°41'54.20"E	1
	Hachioji, Tokyo Metropolis	35°37.772"N; 139°21.539'E	1
	Mt. Chausu, Ina, Nagano Prefecture	35°49'N; 137°51'E	4
	Omachi, Nagano Prefecture	36°36'15.1"N; 137°47'59.3"E, 36°34'57"N; 137°49'40"E	2
	Nagano, Nagano Prefecture	36°38'52.70"N; 138°10'34.53"E	3
	Shimokitayama, Nara Prefecture	34°0'39.17"N; 135°56'57.63"E	3
	Miyagawa, Mie Prefecture	34°11'35"N; 136°10'08"E	1
	Kofu, Yamanashi Prefecture	35°40'N; 138°34'E	3
<i>Plestiodon latiscutatus</i>	Susono, Shizuoka Prefecture	35°12'N; 138°51'E	3
	Izu, Shizuoka Prefecture	34°50'52.3"N; 138°55'00.5"E 34°50'48.7"N; 138°54'52.5"E	2
	Shimoda, Shizuoka Prefecture	34°40'54.41"N; 138°56'56.37"E	1
<i>Plestiodon oshimensis</i>	Nazeasani, Amamioshima Island, Kagoshima Prefecture	28°24.021'N; 129°28.560'E	3
	Sumiyo, Amamioshima Island, Kagoshima Prefecture	28°15.592'N; 129°24.525'E	1

(Anderson, 2000). *Meteterakis* is a parasite of amphibians and reptiles of the Oriental and Palaearctic regions. There are 3 described species of *Meteterakis* in Japan: *Meteterakis ishikawanae* Hasegawa, 1987; *Meteterakis amamiensis* Hasegawa, 1990, and *M. japonica* (Wilkie, 1930).

Plestiodon japonicus is a new host for *M. japonica*. Tokushima Prefecture is a new locality record of this nematode.

One *Meteterakis* specimen collected from Tokushima Prefecture could not be identified to species level because of the poor condition of the specimen. Because *M. japonica* were collected from the same host, this specimen has been identified tentatively as *Meteterakis* cf. *japonica* and was deposited as KUZ Z642 and excluded from this record.

***Meteterakis amamiensis* Hasegawa, 1990**

Prevalence and mean intensity: Thirteen of 83 hosts infected (15.7%, 16.85 ± 5.82 , 1–71).

Site of infection: Rectum, small intestine.

Locality record in this study: Kyoto, Kyoto Prefecture, Honshu; Kumamoto, Kumamoto Prefecture, Kyushu; Kagoshima, Kagoshima Prefecture, Kyushu; Shimokoshikijima Island, Kagoshima Prefecture; Issu, Yakushima Island, Kagoshima Prefecture.

Type host: *Ateuchosaurus pellopleurus*, *Plestiodon oshimensis* (referred to as *Eumeces marginatus oshimensis*), and *Odorrana splendida* (referred to as *Rana ishikawae*).

Type locality: Setouchi and Mt. Yuwan, Amamioshima Island, Japan.

Other reported hosts in Japan: Unknown.

Additional locality records in Japan: Unknown.

Specimens deposited: KUZ Z645, Z656, Z658, Z661, Z662, Z664, Z666 (7 vials).

Geographical range: Western Japan (this study); Amamioshima Island, the Ryukyus (Hasegawa, 1990).

Remarks

Plestiodon japonicus is a new host for *M. amamiensis*. Kyoto, Kumamoto, and Kagoshima Prefectures and Shimokoshikijima and Yakushima Islands are new locality records of this nematode. These were the first locality records of *M. amamiensis* from the Japanese main islands. This species is distributed on both sides of Watase's line, which is known as a geographical distribution boundary

between the Oriental and Palaearctic regions in Japan (Hikida, 2002).

Some specimens of *Meteterakis* and 1 specimen of the Heterakidae collected from Kyoto Prefecture could not be identified to species level because they were in the larval form. Because *M. amamiensis* was collected from the same hosts, these specimens were identified as *Meteterakis* cf. *amamiensis* or Heterakidae gen. sp. and were deposited as KUZ Z665, Z667, and Z657 and excluded from this record.

***Oswaldocruzia socialis* Morishita, 1926**

Prevalence and mean intensity: Two of 83 hosts were infected (2.4%, 3, 3).

Site of infection: Rectum, small intestine.

Locality record in this study: Hatsukaichi, Hiroshima Prefecture, Honshu.

Type host: Not designated.

Type locality: Not designated.

Other reported hosts in Japan: *Rana japonica*, *R. ornativentris*, *Rana tagoi tagoi*, *Rana nigromaculata*, *Rhacophorus arboreus*, *Rhacophorus schlegelii*, *B. japonicus*, and *T. tachydromoides* (Goldberg et al., 2004). Although this nematode was also recorded from *Elaphe quadrivirgata* and *Rhabdophis tigrinus*, Hasegawa and Asakawa (2004) regarded these records as pseudoparasitism.

Additional locality records in Japan: Tokyo, Chiba, Saitama, Kanagawa, Shizuoka, Gifu, Shiga and Kyoto Prefectures, Honshu (Goldberg et al., 2004).

Specimens deposited: KUZ Z647, Z651 (2 vials).

Geographical range: Honshu Island, Japan.

Remarks

Although some researchers considered *O. socialis* a synonym of *Oswaldocruzia filiformis* (Burse et al., 2005), I follow the classification of Hasegawa and Asakawa (2004) and regard *O. socialis* as a valid species.

Plestiodon japonicus is a new host for *O. socialis*. Hiroshima Prefecture is a new locality record of this nematode.

***Kurilonema markovi* Szczerbak and Sharpilo, 1969**

(Syn. *Entomelas markovi* Baker, 1980).

Prevalence and mean intensity: Seventeen of 66 hosts were infected (25.8%, 3.06 ± 0.5 , 1–8).

Site of infection: Lung.

Locality record in this study: Takashima, Shiga Prefecture, Honshu; Kyoto, Fukuchiyama and Kizu, Kyoto Prefecture, Honshu; Hatsukaichi, Hiroshima Prefecture, Honshu; Nakatsu, Oita Prefecture, Kyushu; Shimokoshikijima Island, Kagoshima Prefecture.

Type host: *Plestiodon finitimus*.

Type locality: Kunashiri Islands, Hokkaido Prefecture, Japan.

Other reported hosts in Japan: *Plestiodon finitimus* (Szczerbak and Sharpilo, 1969; Telford, 1997; Kuzmin and Sharpilo, 2002); *Plestiodon latiscutatus* (Bursey et al., 2005); *T. tachydromoides* (Telford, 1997; Bursey et al., 2005).

Additional locality records in Japan: Tokyo Metropolis, Honshu; Hanno, Saitama and Niigata Prefectures, Honshu (Telford, 1997); Hakone, Kanagawa Prefecture, Honshu (Bursey et al., 2005).

Specimens deposited: KUZ Z644, Z646, Z648–Z650, Z652–Z655, Z659, Z660, Z663, Z668 (13 vials).

Geographical range: Honshu, Kyushu, Shimokoshikijima Island (this study), and Kunashiri Island.

Remarks

Baker (1980) designated *Kurilonema* as a synonym of *Entomelas*. However, Kuzmin and Sharpilo (2002) redescribed *Kurilonema markovi* and regarded *Kurilonema* as a valid genus. Members of the Rabdiasidae, including *Kurilonema*, have both parasitic and free-living generations and no intermediate host. Some species of this family are thought to have a paratenic host (Anderson, 2000).

Plestiodon japonicus is a new host for *K. markovi*. Shiga, Kyoto, Hiroshima, and Oita Prefectures, and Shimokoshikijima Island are new locality records of this nematode.

Cosmocercidae gen. sp.

Prevalence and mean intensity: One of 83 hosts was infected (1.2%, 3).

Site of infection: Rectum.

Locality record in this study: Iojima Island, Kagoshima Prefecture.

Specimens deposited: KUZ Z643 (1 vial).

Remarks

Because male specimens of this species were not obtained, these nematodes could not be identified to the genus level.

Plestiodon finitimus Okamoto and Hikida, 2012

Forty-four specimens collected from eastern Honshu of the Japanese main islands were used for this study. Body cavities and digestive tracts of all 44 specimens and lungs of 39 specimens were examined for parasitic nematodes. Locality information of the host lizards is shown in Table 2.

Meteterakis japonica (Wilkie, 1930)

Prevalence and mean intensity: Six of 44 hosts were infected (13.6%, 2.5 ± 0.62 , 1–5).

Site of infection: Rectum.

Locality record in this study: Takasaki, Gunma Prefecture, Honshu; Hachioji, Tokyo Metropolis, Honshu; Kofu, Yamanashi Prefecture, Honshu; Hamamatsu and Fukuroi, Shizuoka Prefecture, Honshu.

Specimens deposited: KUZ Z632, Z634, Z636, Z637, Z639 (5 vials).

Remarks

Gunma, Yamanashi, and Shizuoka Prefectures are new locality records of this nematode. Some specimens of *Meteterakis* and 1 specimen of Heterakidae collected from Gunma, Shizuoka, and Aichi Prefectures could not be identified to the species level because they were larval forms or because the condition of the specimens was bad. Because *M. japonica* were collected from the same host or locality, these specimens were identified tentatively as *Meteterakis* cf. *japonica* or Heterakidae gen. sp. and deposited as KUZ Z630, Z631, Z638, and Z633 and were excluded from this record.

Kurilonema markovi Szczerbak and Sharpilo, 1969

Prevalence and mean intensity: Five of 39 hosts were infected (12.8%, 2.8 ± 0.8 , 1–5).

Site of infection: Lung.

Locality record in this study: Mt. Hakkoda, Aomori Prefecture, Honshu; Shimokitayama, Nara

Prefecture, Honshu; Mt. Ryokami, Saitama Prefecture, Honshu.

Specimens deposited: KUZ Z635, Z640 (2 vials).

Remarks

Aomori and Nara Prefectures are new locality records of this nematode.

Plestiodon latiscutatus Hallowell, 1861

Six specimens collected from Izu Peninsula of Japan were used for this study. Body cavities, lung and digestive tracts of all 6 specimens were examined for parasitic nematodes. Locality information of host lizards is shown in Table 2.

Kurilonema markovi Szczerbak and Sharpilo, 1969

Prevalence and mean intensity: Four of 6 hosts were infected (66.7%, 4.25 ± 1.25 , 1–7).

Site of infection: Lung.

Locality record in this study: Izu and Susono, Shizuoka Prefecture, Honshu.

Specimens deposited: KUZ Z669, Z670 (2 vials).

Remarks

Shizuoka Prefecture is a new distribution record of this nematode.

Plestiodon oshimensis (Thompson, 1912)

Four specimens collected from Amamioshima Island of Japan were used for this study. Body cavities, lungs, and digestive tracts of all 4 specimens were examined for parasitic nematodes. Locality information of the host lizards is shown in Table 2.

Meteterakis amamiensis Hasegawa, 1990

Prevalence and mean intensity: Two of 4 hosts were infected (50%, 3 ± 1 , 2–4).

Site of infection: Rectum and small intestine.

Locality record in this study: Naze, Amamioshima Island, Kagoshima Prefecture.

Specimens deposited: KUZ Z673, Z674 (2 vials).

Remarks

General information is reported under *P. japonicus*.

Kurilonema markovi Szczerbak and Sharpilo, 1969

Prevalence and mean intensity: Two of 4 hosts were infected (50%, 2 ± 1 , 1–3).

Site of infection: Lung.

Locality record in this study: Naze, Amamioshima Island, Kagoshima Prefecture.

Specimen deposited: KUZ Z672 (1 vial).

Remarks

Plestiodon oshimensis is a new host for *K. markovi*. Amamioshima Island is a new locality record of *K. markovi*. This nematode was recorded only from northeastern parts of Japan in previous studies (Bursey et al., 2005; Szczerbak and Sharpilo, 1969; Telford, 1997). This was the first record of *K. markovi* from south of Watase's line.

Pharyngodonidae gen. sp.

Prevalence and mean intensity: One of 4 hosts was infected (25%, 1).

Site of infection: Rectum.

Locality record in this study: Sumiyo, Amamioshima Island (Kagoshima Prefecture).

Specimen deposited: KUZ Z671 (1 vial).

Remarks

Because male specimens of this species were not obtained, this nematode could not be identified at the genus level. This is the first record of Pharyngodonidae from *P. oshimensis*.

DISCUSSION

This study revealed that *M. japonica* was distributed in Eastern Honshu and Shikoku, and *M. amamiensis* in Western Honshu and Kyusyu. The distribution boundary in Honshu is considered to be located in the Kinki Region, in a region of the border located between Kyoto Prefecture and Shiga Prefecture. To the author's knowledge, *Meteterakis* has been reported from *P. japonicus*, *P. finitimus*, *P. latiscutatus*, *T. tachydromoides*, *B. j. japonicus*, *B. j. formosus*, *R. japonica*, and *R. ornativentris* on the Japanese main islands. The above 3 species of *Plestiodon* lizards are known to have a parapatric distribution pattern in the Japanese main islands: *P. japonicus* is distributed in Western Japan, *P. finitimus*

in Eastern Japan (except the Izu Peninsula), with *P. latiscutatus* on the Izu Peninsula and the Izu Islands (Okamoto et al., 2006; Okamoto and Hikida, 2012). The distribution boundary of the 2 *Meteterakis* does not correspond with any of the distribution boundaries of the 3 *Plestiodon* species, because the present and previous studies showed that *P. japonicus* collected from Kyushu and Western Honshu harbored *M. amamiensis*; *P. japonicus* collected from Shikoku harbored *M. japonica*; and *P. finitimus* and *P. latiscutatus* also harbored *M. japonica* (Burse et al., 2005; Telford, 1997). The 2 subspecies of *B. japonicus* are known to have parapatric distribution on the Japanese main island: *B. j. japonicus* is distributed in Western Japan and *B. j. formosus* in Eastern Japan (Matsui, 1984). The distribution boundary of the 2 *Meteterakis* does not correspond with that of the 2 subspecies of *B. japonicus*, because *M. japonica* was collected from Tokushima, Tokushima Prefecture and Shirahama, Wakayama Prefecture, where *B. j. japonicus* is distributed. The parapatric distribution of western and eastern lineages of *R. japonica* and *R. ornativentris* has been revealed by allozyme studies (Sumida and Nishioka, 1994, 1996) and the distribution boundary of the 2 *Meteterakis* does not correspond with that of the 2 lineages of *R. japonica*, because the boundary is located east of that between the 2 *Meteterakis*. The distribution boundary of 2 lineages of *R. ornativentris* was considered to be located in the Kinki Region by Sumida and Nishioka (1996), but this speculation is uncertain because the previous study used only a small number of specimens from limited localities (Sumida and Nishioka, 1996). Thus, the distribution boundaries of the 2 species of *Meteterakis* and 2 lineages of *R. ornativentris* could not be compared. The infection of *Meteterakis* in *T. tachydromoides* is considered to be accidental, because the prevalence of *M. japonica* in *T. tachydromoides* was extremely low relative to that of *P. finitimus* collected from the same area (Telford, 1997). Consequently, the distribution patterns of the host species and lineages may not have any influence on the distribution patterns of *M. japonica* and *M. amamiensis*.

This study also revealed that *K. markovi* infected the lungs of all 4 species of *Plestiodon* investigated in this study, and its distribution ranged from Kunashiri Island, Hokkaido, to Amamioshima Island of the Ryukyus, Japan. On the other hand, *Neotentmelas asatoi* has been reported from the lung of the scincid lizard, *A. pellopleurus*, in Amamioshima Island (Hasegawa, 1990). Although the parasitic generation

of both of these nematodes infects the lungs of scincid lizards, and although both belong to the family Rhabdiasidae, *K. markovi* and *N. asatoi* apparently coexist on Amamioshima Island without evident competitive exclusion. This suggests high host specificity for the 2 scincid hosts, *K. markovi* and *N. asatoi*, for *Plestiodon* and *A. pellopleurus*, respectively.

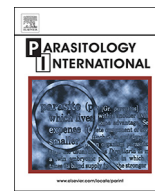
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Allopatric speciation of *Meteterakis* (Heterakoidea: Heterakidae), a highly dispersible parasitic nematode, in the East Asian islands

Naoya Sata

Department of Zoology, Graduate School of Science, Kyoto University, Sakyo, Kyoto 606-8502, Japan

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ABSTRACT

To clarify how the species diversity of highly dispersible parasites has developed, molecular phylogenetic analyses of *Meteterakis* spp., multi-host endoparasitic nematodes of reptiles and amphibians from the East Asian islands, were conducted. The results demonstrated the existence of two major clades, the J- and A-groups, with exclusive geographic ranges that are discordant with the host faunal province. However, diversification within the J-group was concordant with the host biogeography and suggested co-divergence of this group with vicariance of the host fauna. In contrast, the phylogenetic pattern within the A-group was discordant with host biogeography and implied diversification by repeated colonization. In addition, the mosaic distribution pattern of a J-group and an A-group species in the Japanese Archipelago, along with comparison of population genetic parameters and the genetic distance from their closest relatives, suggested the initial occurrence of a J-group lineage followed by exclusion in the western part of this region caused by invasion of an A-group lineage. Thus, the present study suggested that the species diversity of highly dispersible parasites including *Meteterakis* is formed not only by co-divergence with host faunal vicariance but also by peripatric speciation and exclusive interactions between species.

1. Introduction

Parasites exhibit high species diversity, and elucidation of their diversification factors is a fundamental question in evolutionary biology. Some researchers expected that several ecological factors, such as host specificity, mobility of hosts and parasites, and life cycle, influenced the patterns of parasite species diversity [1,2], because these may affect the dispersal ability of parasites; this can influence the intra-specific genetic diversity and the rate of allopatric speciation.

Dispersal ability is one of the most important factors influencing intra- and inter-specific diversity [3]. Low dispersal ability leads to a low rate in gene flow and promotes inter-population genetic divergence, and high dispersal leads to a high rate in gene flow and suppresses inter-population genetic divergence. Several studies have compared the degree of intra-specific genetic divergences between parasite species which exhibit different host use and range [4–8]. These studies revealed that parasites having mobile hosts or wide host ranges tended to have reduced intra-specific genetic divergence, and concluded that the mobility of hosts and host richness (the number of possible host taxa) are major determinants of the dispersal ability of parasites [4–8]. Nonetheless, highly dispersible parasite taxa have a certain species diversity [e.g., [9]], and their diversification process remains to be studied.

Given that parasite dispersal ability is mostly regulated by host movements [e.g., [6]], and high dispersal ability should increase the chance of geographic range expansion [10], two modes of allopatric speciation of highly dispersible parasites are possible: (1) isolation of entire host fauna leads to vicariant allopatric speciation of parasites; and (2) extreme geographic range expansion ability increases the chances of peripatric speciation, caused by dispersal to an unoccupied area followed by speciation. To clarify the extent of contributions of these modes of allopatric speciation, one of the best strategies is an inference of historical biogeography of a group of parasites which encompasses allopatric closely related species and uses multiple hosts and/or a mobile host with a well-established biogeographic background.

Meteterakis Karve, 1930 (Heterakoidea; Heterakidae) is a multi-host parasitic nematode which mainly parasitizes the rectum of several species of lizards and frogs [11]; it has neither intermediate hosts nor a free-living phase [12]. Four named species of *Meteterakis* with exclusive distributions are currently known from the East Asian islands: *Meteterakis japonica* (Wilkie, 1930) in the eastern Japanese Archipelago (Fig. 1B), *Meteterakis amamiensis* Hasegawa, 1990 in the western Japanese Archipelago and Amamioshima Island (Amami Group of the Central Ryukyus, Fig. 1B, C), *Meteterakis ishikawanae* Hasegawa, 1987 in the Okinawa Group of the Central Ryukyus (Fig. 1C), and *Meteterakis*

E-mail address: sata@zoo.zool.kyoto-u.ac.jp.

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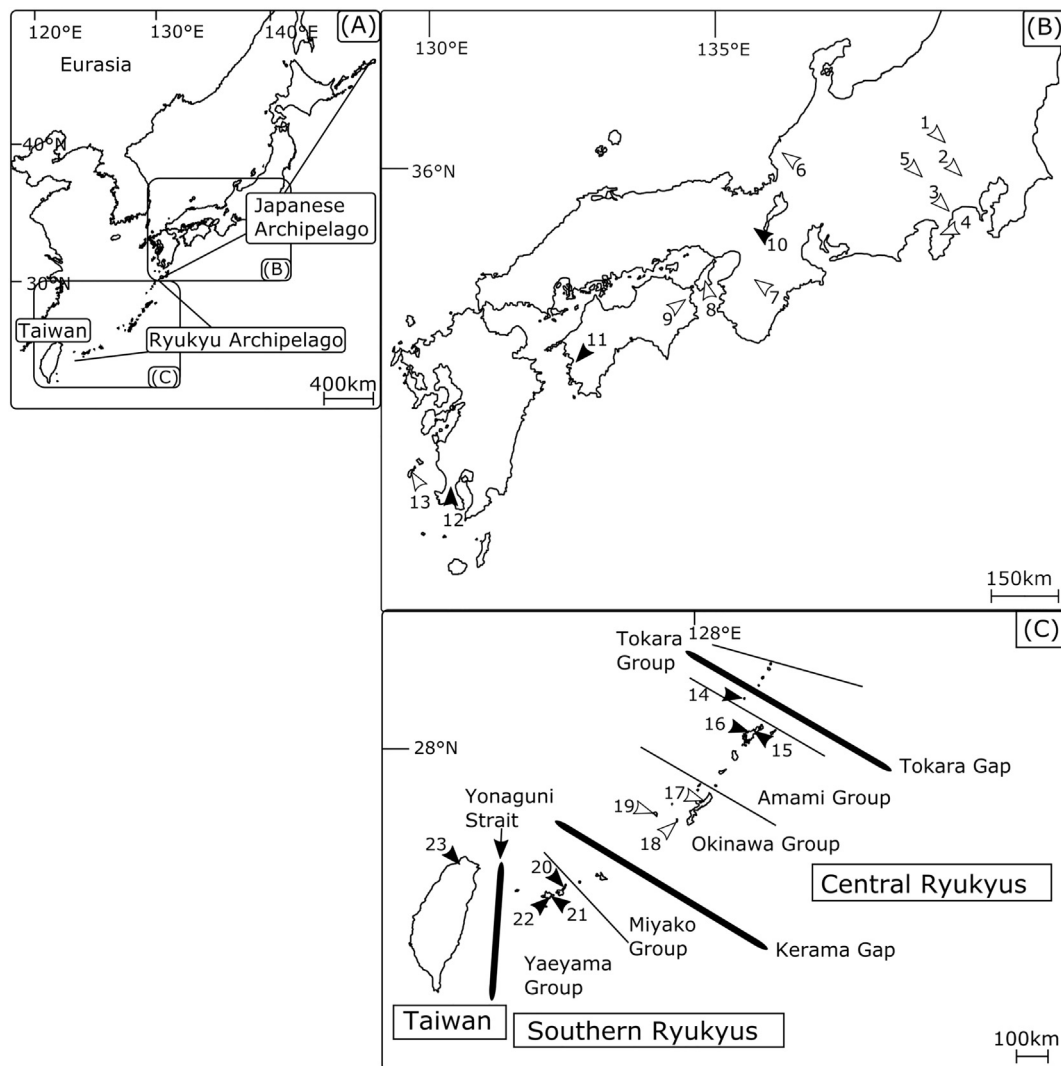


Fig. 1. Map of the East Asian islands showing: A, the entire study area in the East Asian islands; B, sampling localities in the Japanese Archipelago; C, sampling localities in the Ryukyus and Taiwan, and the boundaries of known biogeographic provinces (thick lines; see text for details). Open and solid symbols indicate J-group and A-group, respectively. See Table 1 for further details of sampling localities.

govindi Karve, 1930 in southern Taiwan (Fig. 1C). *M. govindi* is also found in India, Bangladesh, Myanmar, and China [11]. In addition, another undescribed species may occur in Miyakojima Island, the Miyako Group of the Southern Ryukyus: Hasegawa [13] reported individuals of *M. japonica* from this island, which differed from the true *M. japonica* in lacking a gubernaculum [14,15].

The known host species of *Meteterakis* in the East Asian islands consist of several frogs in the genera *Rana* Linnaeus, *Odorrana* Fei et al., *Bufo* Laurenti, and *Duttaphrynus* Frost et al.; lizards in the genera *Plestiodon* Duméril & Bibron, *Eutropis* Fitzinger, *Ateuchosaurus* Gray, *Takydromus* Daudin, and *Japalura* Gray; and a turtle in the genus *Geoemyda* Gray [13,14,16–28]. The extremely rare occurrence of *Meteterakis* in *Takydromus* and *Japalura* [20,23] strongly suggested that their infections were accidental.

The East Asian islands are composed of several island groups: the Japanese Archipelago, the Ryukyu Archipelago (northern and southern Tokara Groups, Amami Group, Okinawa Group, Miyako Group, and Yaeyama Group), and Taiwan (Fig. 1A, C). The species and subspecies distribution patterns of the native amphibians and terrestrial reptiles, including *Meteterakis* hosts, divide the East Asian islands into four biogeographical regions [29]. There are: (1) the Japanese Archipelago (including northern Tokara Group); (2) the Central Ryukyus (southern Tokara Group, Amami Group, and Okinawa Group); (3) the Southern

Ryukyus (Miyako Group and Yaeyama Group); and (4) Taiwan (Fig. 1A, C). Three sea straits, the Tokara Gap, Kerama Gap, and Yonaguni Strait (Fig. 1C), correspond to the biogeographical boundaries between the Japanese Archipelago and the Central Ryukyus, the Central and Southern Ryukyus, and the Southern Ryukyus and Taiwan, respectively. These straits have not been closed by land bridge since their establishment [29]. A recent review of biogeographic patterns of terrestrial reptiles in this region [30] suggested that: the major part of the Southern Ryukyu fauna was formed by isolation from Taiwanese fauna during the Pliocene (5.33–2.58 Ma) [31]; the major part of the Central Ryukyu fauna was formed by isolation from the other land areas during the late Miocene (23.03–5.33 Ma) [31] and; and that the Japanese Archipelago fauna was formed by isolation from the surrounding areas during the middle Miocene and by secondary interchange with the continent occurring during the Pliocene and Pleistocene (2.58–0.01 Ma) [31].

Several studies have also suggested overseas dispersal of some lizards between these major biogeographic regions [30,32–35]. Lizards, whose range expansion was also due to overseas dispersal, include *Plestiodon* and *Ateuchosaurus*, which are known hosts of *Meteterakis* spp. [30,33–35]. Thus, some of the hosts of *Meteterakis* from this area have high mobility.

Because *Meteterakis* species use highly dispersible lizard lineages,

they are expected to be highly dispersible themselves. Furthermore, the mutually exclusive distribution of *Meteterakis* species in the East Asian islands (Supplementary Fig. 1) implies diversification by allopatric speciation events. Thus, *Meteterakis* spp. from the East Asian islands are an excellent model to confirm the two modes of allopatric speciation of highly dispersible parasites. If the phylogenetic relationships among *Meteterakis* species are concordant with the established historical biogeography of the hosts [e.g., [29,30]], it would suggest the occurrence of allopatric speciation by host faunal isolation. If any colonization between major host biological provinces was detected, it would suggest the occurrence of peripatric speciation. To elucidate the modes of allopatric speciation in *Meteterakis*, *Meteterakis* specimens were collected from the entire East Asian islands, and their inter-specific phylogenetic relationships were inferred based on molecular data. Population genetic characteristics and relative ages of the two species from the Japanese Archipelago were also compared, to consider how the present geographic arrangements within the Archipelago have been formed.

2. Materials and methods

2.1. Sample collection

During 2011–2015, a total of 593 individuals of six genera of three families of lizards (Scincidae: *Plestiodon*, *Ateuchosaurus*, *Scincella* Mittleman, *Eutropis*; Lacertidae: *Takydromus*; Agamidae: *Japalura*) and 11 individuals of two families and two genera of frogs (Ranidae: *Rana*; Bufonidae: *Bufo*), which represent the majority of the known hosts of *Meteterakis* in the East Asian islands (except for *Scincella*) [13,14,16–28], were collected from 161 localities. Scincid lizards were mainly collected as the representative lizard host, because lizards of Lacertidae and Agamidae were considered to be accidental hosts [20,23]. Hosts were collected and handled in accordance with the Regulations of Animal Experimentations at Kyoto University (approval numbers: H24014 and H2711). To further elucidate the distribution patterns of *Meteterakis* spp., ten specimens of *Plestiodon marginatus* Hallowell and ten specimens of *Plestiodon barbouri* (Van Denburgh), which have been deposited in the Zoological Collection of Kyoto University (KUZ), were also dissected: *P. marginatus*, KUZ R27929, R27940–R27943, R27945, R27947, R36436, R36438, R36467; and *P. barbouri*, KUZ R29284–R29289, R30386, R35051–R35056.

Hosts were euthanized by oral injection with sodium pentobarbital (35–70 mg/kg) and then dissected. Their intestines were examined under a stereoscopic microscope; helminths were collected when they were present in the intestines. The nematodes obtained were fixed in hot 70% glycerin ethanol, cleared in 100% glycerin, and morphologically identified to species as far as possible. The identified specimens were preserved in 70% or 99% ethanol at 4 °C or –20 °C until DNA extraction. A total of 71 *Meteterakis* individuals, identified to species or genus level, were used for subsequent DNA analyses (Table 1). The locality names of the samples used in this study are listed in Table 1, and their sampling sites are shown in Fig. 1. *Strongyluris* sp. (Heterakoidea; Heterakidae) collected from *Japalura polygonata polygonata* (Hallowell) captured from Okinawajima Island was used as an outgroup for phylogenetic inference. Several *Meteterakis* specimens have been deposited in KUZ as vouchers (*M. japonica*: KUZ Z1762–Z1765 and Z1767; *M. amamiensis* from the Japanese Archipelago: KUZ Z1766; *M. amamiensis* from Amamioshima Island and southern Tokara Group: KUZ Z1769–Z1770; *M. ishikawanae*: KUZ Z1771–Z1773; *Meteterakis* sp. 1: KUZ Z1774; *Meteterakis* sp. 2: KUZ Z1775–Z1776; and *Meteterakis* sp. 3: KUZ Z1777).

2.2. DNA sequencing

Genomic DNA was extracted from the nematodes using standard phenol chloroform extraction or the following method. The whole body of each individual was incubated at 50 °C for 120 min followed by 95 °C

for 20 min in a solution of 5 µl proteinase K (final concentration of proteinase K was 2 mg/ml) and 20 µl lysis buffer, which contained 10 mM Tris-HCl (pH 8.0), 150 mM NaCl, 10 mM EDTA (pH 8.0), and 0.1% SDS. Then 20 µl of TE buffer (10 mM pH 8.0 Tris-HCl, 1 mM pH 8.0 EDTA) was added, and each DNA extract was preserved at 4 °C. The four fragments of mitochondrial DNA, cytochrome c oxidase subunits I (COI) and II (COII), cytochrome b (cytb), and 12S ribosomal DNA (12S); and nuclear DNA fragments including the 3' end of 18S rDNA, internal transcribed spacer (ITS) 1, 5.8S rDNA, ITS 2, and the 5' end of 28S rDNA (including variable domains D1–D3) (18S–28S) were amplified by polymerase chain reaction (PCR) using a TaKaRa Ex Taq kit (Takara Bio, Japan) and a GeneAmp PCR Systems 2700 or 9700 (Applied Biosystems, USA), or MyCycler (Bio-Rad Laboratories, USA). The PCR mixture for the COII fragment contained 2.5 mM MgCl₂ in addition to the normal reaction mix provided by the manufacturer (Takara Bio, Japan). PCR conditions were as follows: five cycles of 40 s at 94 °C, 40 s at 45 °C, and 1 min at 72 °C; 35 cycles of 40 s at 94 °C, 40 s at 51 °C, and 1 min at 72 °C for COI; 35 cycles of 45 s at 94 °C, 45 s at 46–51 °C, and 1 min at 72 °C for COII; 35 cycles of 45 s–1 min at 94 °C, 1 min at 46–48 °C, and 1 min at 72 °C for cytb; 35 cycles of 45 s at 94 °C, 45 s at 48 °C, and 1 min at 72 °C for 12S; and 35 cycles of 30 s at 94 °C, 30 s at 53–56 °C, and 3.5 min at 72 °C for 18S–28S. These cycles were initiated with an initial denaturation step at 94 °C for 3 min, and followed by a final extension at 72 °C for 5 min. All the primers used in this study are listed in Table 2. The PCR products were purified by ExoSAP-IT reagent (Affymetrix, USA). Sequencing reactions were performed using a BigDye Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems, USA). The products were cleaned by ethanol precipitation and sequenced with an Applied Biosystems 3130xl Genetic Analyzer (Applied Biosystems, USA).

2.3. Phylogenetic and population genetic analyses

The sequences were edited and aligned with MEGA 5 [36]. The sequences for each gene or region were aligned separately using Clustal W [37]. The regions difficult to align because of alignment gaps in 12S and 18S–28S fragments were removed manually. Phylogenetic relationships were inferred with maximum likelihood (ML) method using TREEFINDER v March 2011 [38]. Both the mitochondrial (sequences of all loci were concatenated) and nuclear DNA data sets were collapsed to only those with unique haplotypes (individuals possessing identical sequences in the sequenced loci were treated as those with the same haplotype, even if some of them had missing loci). Although the nuclear DNA of *Meteterakis* individuals from Ishigakijima Island and Iriomotejima Island were identical, the samples were treated separately in phylogenetic analysis. The collapsed mitochondrial and nuclear datasets of *Meteterakis* individuals and the outgroup consisted of 33 and 13 haplotypes, respectively. Appropriate substitution models for the partitioned data set were selected based on AICc by TREEFINDER v March 2011. The selected models were: 1st, 2nd, and 3rd codon positions of COI, HKY + G, TN + G; 1st, 2nd, and 3rd codon positions of COII, HKY + G, HKY, TN; 1st, 2nd, and 3rd codon positions of cytb, HKY + G, HKY + G, TN + G; 12S, HKY + G; and all positions of 18S–28S, J2 + G. Support for each clade of the ML trees was assessed using bootstrap analysis with 1000 pseudoreplicates.

To compare demographic histories and relative original time of the two lineages from the Japanese Archipelago, *M. japonica* and a lineage of *M. amamiensis* found on the Japanese Archipelago (see Section 4.1), nucleotide and haplotype diversities and net genetic distances from their closest relatives were calculated. The nucleotide and haplotype diversities of *M. japonica* and *M. amamiensis* from the Japanese Archipelago were calculated with Arlequin v 3.5.2.2 [39]. These were examined using 611 bp of COI sequences of ten *M. japonica* individuals from ten localities, and 36 *M. amamiensis* individuals from three localities in the Japanese Archipelago. The net genetic distances were calculated with MEGA 5 using the concatenated mitochondrial DNA

Table 1

Meteterakis spp. used in this study and its localities. “N” indicates the number of specimens included in the analyses. The locality numbers are consistent with the numbers shown in Fig. 1.

<i>Meteterakis</i> spp.	N	Host species	Locality No.	Locality name	Geographical coordinates
<i>Meteterakis japonica</i>	1	<i>Plestiodon finitimus</i>	1	Japanese Archipelago	
<i>M. japonica</i>	1	<i>P. finitimus</i>	2	Yamana, Takasaki, Gunma Pref.	36°16.650'N; 139°01.998'E
<i>M. japonica</i>	1	<i>P. finitimus</i>	3	Nakayama, Hachioji, Tokyo Metro.	35°37.772'N; 139°21.539'E
<i>M. japonica</i>	1	<i>P. finitimus</i>	4	Odawara, Kanagawa Pref.	35°17'7.7"N; 139°12'36.4"E
<i>M. japonica</i>	1	<i>P. latiscutatus</i>	5	Mt. Kasturagi, Izunokuni, Shizuoka Pref.	35°00'43.1"N; 138°55'13.1"E
<i>M. japonica</i>	1	<i>P. finitimus</i>	6	Kofu, Yamanashi Pref.	35°40'N; 138°34'E
<i>M. japonica</i>	1	<i>P. finitimus</i>	7	Kosui, Fukui, Fukui Pref.	36°03.284'N; 136°16.718'E
<i>M. japonica</i>	1	<i>Plestiodon japonicus</i>	8	Shimoichi, Nara Pref.	34°17'35.6"N 135°47'22.4"E
<i>M. japonica</i>	1	<i>P. japonicus</i>	9	Minamiawaji, Hyogo Pref. (Awajishima Is.)	34°14'56.9"N; 134°47'48.4"E
<i>M. japonica</i>	1	<i>P. japonicus</i>	10	Tokushima, Tokushima Pref.	34°04.494'N; 134°33.352'E
<i>Meteterakis amamiensis</i>	22	<i>P. japonicus</i>	11	Kyoto, Kyoto Pref.	35°01.723'N; 135°47.169'E
<i>M. amamiensis</i>	5	<i>P. japonicus</i>	12	Oura, Uwajima, Ehime Pref.	33°13.946'N; 132°33.228'E
<i>M. amamiensis</i>	13	<i>P. japonicus</i>	13	Haruyama, Kagoshima, Kagoshima Pref.	31°35'39.76"N; 130°27'31.55"E
<i>M. japonica</i>	1	<i>P. japonicus</i>	14	Shimokoshiki, Satsumasendai, Kagoshima Pref. (Shimokoshikijima Is.)	31°42.517'N; 129°43.970'E
<i>M. amamiensis</i>	2	<i>Plestiodon oshimensis</i> , <i>Ateuchosaurus pellopleurus</i>	15	Southern Tokara Group Kodakurajima, Toshima, Kagoshima Pref. (Kodakurajima Is.)	29°13'22.6"N; 129°19'39.2"E
<i>M. amamiensis</i>	6	<i>P. oshimensis</i>	16	Central Ryukyus Nazehiramatsum, Amami, Kagoshima Pref. (Amamioshima Is.)	28°24.021'N; 129°28.560'E
<i>M. amamiensis</i>	2	<i>A. pellopleurus</i>	17	Oganeku, Yamato, Kagoshima Pref. (Amamioshima Is.)	28°21'30.8"N; 129°20'11.7"E
<i>Meteterakis ishikawanae</i>	1	<i>A. pellopleurus</i>	18	Ogimi, Ogimi, Okinawa Pref. (Okinawajima Is.)	26°41.448'N; 128°07.527'E
<i>M. ishikawanae</i>	2	<i>A. pellopleurus</i>	19	Tokashiki, Tokashiki, Okinawa Pref. (Tokashikijima Is.)	26°12'48.80"N; 127°21'26.98"E
<i>M. ishikawanae</i>	4	<i>A. pellopleurus</i>	20	Nishime, Kumejima, Okinawa Pref. (Kumejima Is.)	26°21'44.28"N; 126°45'53.35"E
<i>Meteterakis</i> sp. 1	1	<i>Plestiodon stimpsonii</i>	21	Southern Ryukyus Nosoko, Ishigaki, Okinawa Pref. (Ishigakijima Is.)	24°29.362'N; 124°15.157'E
<i>Meteterakis</i> sp. 2	1	<i>P. stimpsonii</i>	22	Mihara, Taketomi, Okinawa Pref. (Iriomotejima Is.)	24°20.395'N; 123°54.830'E
<i>Meteterakis</i> sp. 2	1	<i>P. stimpsonii</i>	23	Otomi, Taketomi, Okinawa Pref. (Iriomotejima Is.)	24°17.912'N; 123°51.230'E
<i>Meteterakis</i> sp. 3	1	<i>Plestiodon chinensis</i>		Taiwan Bali, New Taipei city, Taiwan	25°08'53.1"N; 121°25'47.3"E

sequences based on Kimura's two-parameter model to infer the relative original time of the two lineages from the Japanese Archipelago: *M. japonica* ($N = 10$) vs *M. ishikawanae* ($N = 7$); *M. amamiensis* in the Japanese Archipelago ($N = 38$) vs *M. amamiensis* in Amami and the southern Tokara Groups ($N = 10$) (see Results), *Meteterakis* sp. 1 ($N = 2$), *Meteterakis* sp. 2 ($N = 2$), and *Meteterakis* sp. 3 ($N = 1$) (see Results and Discussion).

3. Results

3.1. Sample collection and identification

Meteterakis specimens were obtained from 26 localities and analysed specimens from 23 localities (Fig. 1B–C). *Meteterakis* infection was observed in species of *Plestiodon* (except for *P. marginatus* Hallowell (0/43) in the Okinawa Group of the Central Ryukyus) and *Ateuchosaurus pellopleurus* (Hallowell). The *Meteterakis* obtained were identified as three known species and three undescribed species. The known species were: *M. japonica* from the eastern Japanese Archipelago (sites 1–9, Fig. 1B) and Shimokoshikijima Island off the western coast of Kyushu (site 13; the island population was formerly identified as *M. amamiensis* by Sata [28]); *M. amamiensis* from Amamioshima Island of the Amami Group (sites 15–16, Fig. 1C), Kodakurajima Island of the southern Tokara Group (site 14, Fig. 1C), and the western Japanese Archipelago (sites 10–12, Fig. 1B); and *M. ishikawanae* from Okinawajima Island (site 17, Fig. 1C), Tokashikijima Island (site 18, Fig. 1C) and Kumejima Island, (site 19, Fig. 1C) of the Okinawa Group. The three putative undescribed *Meteterakis* were obtained from Ishigakijima Island, Iriomotejima Island of the Yaeyama Group (sites 20–22, Fig. 1C), and the northern Taiwan (site 23 of Fig. 1C).

The morphological identification for *Meteterakis* species from Ishigakijima Island, Iriomotejima Island, and Taiwan were based on nine (one adult male, and eight adult females), four (two adult males, and two adult females), and ten (six adult males, and four adult females) individuals, respectively, and morphological differences from each other as well as from other described species were revealed. Further details of them will be described in future taxonomic papers.

3.2. Mitochondrial phylogenetic tree

COI, COII, cytb, and 12S were successfully sequenced for 71, 66, 53, and 70 individuals, respectively. The length of the concatenated mitochondrial DNA sequences was ca. 2100 bp. Sequence data were deposited in DNA Data Bank of Japan (DDBJ), and accession numbers are provided in Supplementary Table 1. The mitochondrial phylogenetic tree is shown in Fig. 2. East Asian islands *Meteterakis* diverged into two major strongly supported clades. One clade consisted of *M. japonica* and *M. ishikawanae* (J-group) and the other encompassed *M. amamiensis* and three *Meteterakis* spp. from Ishigakijima Islands, Iriomotejima Islands, and Taiwan (A-group). Each of the *Meteterakis* species from Ishigakijima Island (*Meteterakis* sp. 1), Iriomotejima Island (*Meteterakis* sp. 2), and Taiwan (*Meteterakis* sp. 3) formed a distinct clade. The interspecific relationships of A-group could not be resolved by the present dataset. The tree also showed genetic distinction between the two populations of *M. amamiensis* from the Amami and southern Tokara Groups (sites 14–16, Fig. 1C), and the western Japanese Archipelago (sites 10–12, Fig. 1B).

Table 2
PCR and cycle sequencing (CS) primers used in this study.

Primer name	Sequence (5'–3')	Reference
COI (PCR)		
NemF1_t1	TGTAAAACGACGGCCAGT	[42]
	CRACWGTWAATCAYAARAATATTGG	
NemF2_t1	TGTAAAACGACGGCCAGT	[42]
	ARAGATCTAATCATAAGATATYGG	
NemF3_t1	TGTAAAACGACGGCCAGT	[42]
	ARAGTTCTAATCATAARGATATTGG	
NemR1_t1	CAGGAAACAGCTATGAC	[42]
	TAAACTTCWGGRTGACCAAAAAATCA	
NemR2_t1	CAGGAAACAGCTATGAC	[42]
	TAWACYTCWGGRTGMCCAAAAAYCA	
NemR3_t1	CAGGAAACAGCTATGAC	[42]
	TAAACCTCWGGATGACCAAAAAATCA	
COI (CS)		
M13F	TGTAAAACGACGGCCAGT	[43]
M13R	CAGGAAACAGCTATGAC	[43]
COII (PCR and CS)		
211F	TTTCTAGTTATATAGATTGRTTYAT	[44]
210R	CACCAACTCTTAAATATTATC	[44]
CIIFmete	ATCAAAACKGGRGAATTTTTRGTAG	This study
CIIRmete	GTTACYTCYAAAGCAATAGGCA	This study
cytb (PCR and/or CS)		
1F	GRAATTTTGGTAGTATRTTRG	[45]
1R	AGMACGYAAAATWGYAWAAGC	[45]
cybF1mete	GKGGRTACAYTTTAATAATGCT	This study
cybR1mete	TCCTTRAMTCAATAATAAGG	This study
12S (PCR and CS)		
12SF	GTTCCAGAATAATCGGCTA	[46]
12SR	ATTGACGGATGRTTTGTACC	[46]
18S–28S (PCR and CS)		
ritf	GCGGCTTAATTTGACTCAACACGG	[47]
FmeteIT	ATGGCCGTTCTAGTTGGTG	This study
1500R	GCTATCTGAGGGAACCTTCG	[47]
18S–28S (CS)		
ITS5	GGAAGTAAAGTCGTAAACAAGG	[47]
meteF1	ATTCTTTGGCTTGCAATGG	This study
meteF3	TGAGCACTAAGATTTCGAAC	This study
meteF4	ATTGTTTCGTTTACCACAAAG	This study
meteF5	GTGTAATTTGCCGTCGATATG	This study
meteR1	AACRGGGATTAGACGCCAAC	This study
meteR2	TGTGCGTTTCGAAATCTTAGTG	This study
RI12ne	ATGCTTAARTTCRGGGGTAATC	This study
300R	CAACTTTCCCTCACGGTACTTG	[47]
ECD2	CTTGGTCCGTGTTCAAGACGGG	[47]

3.3. Nuclear phylogenetic tree

18S–28S was successfully sequenced for 37 individuals. The length of the 18S–28S sequences was ca. 2500 bp. Sequence data were deposited in DDBJ, and accession numbers are provided in Supplementary Table 1. Any of the resultant nuclear sequences were homozygous, thus that of each sample was treated as a single haplotype. The nuclear phylogenetic tree is shown in Fig. 3. The tree shows two strongly supported clades, A- and J-groups, which were consistent with the mitochondrial tree. The inter-specific relationships of the A-group were not resolved in this tree, but genetic divergence was also found between *M. amamiensis* from the Amami and southern Tokara Groups (sites 14–16, Fig. 1C) and *M. amamiensis* from the western Japanese Archipelago (sites 10–12, Fig. 1B). *Meteterakis* sp. 1 from Ishigakijima Island of the Yaeyama Group (site 20, Fig. 1C) and *Meteterakis* sp. 2 from Iriomotejima Island of the Yaeyama Group (sites 21–22, Fig. 1C) possessed identical 18S–28S sequences. Each of the species from Taiwan, and the Yaeyama Group formed distinct clades.

3.4. Population genetic analysis

The nucleotide diversity values of *M. amamiensis* from the western Japanese Archipelago (sites 10–12, Fig. 1B) and *M. japonica* from the eastern Japanese Archipelago and Shimokoshikijima Island (sites 1–9, 13, Fig. 1B) were $0.91 \pm 0.50\%$ and $1.63 \pm 0.92\%$, respectively, and haplotype diversities of the two lineages were both 1.00 (Table 3). The net genetic distance between *M. amamiensis* from the western Japanese Archipelago and its closest relative (*M. amamiensis* from the Amami and southern Tokara Groups) (0.069) was clearly smaller than that between the two J-group species (0.097) (Table 4).

4. Discussion

4.1. *Meteterakis* lineages and their distribution

The present study obtained six species of *Meteterakis* from the East Asian islands; *M. japonica*, *M. amamiensis*, *M. ishikawanae*, and three undescribed species. Analyses also clarified a deep genetic divergence between *M. amamiensis* populations of the Japanese Archipelago (sites 10–12, Fig. 1) and the Amami and southern Tokara Groups (sites 14–16), comparable with inter-specific differentiation among other species. Thus, seven lineages of *Meteterakis* with mutually exclusive geographic ranges were detected. In the remaining part of this paper I have abbreviated as follows: *M. amamiensis* from the western Japanese Archipelago (WJ), *M. amamiensis* from the Amami and southern Tokara Groups (MA), *Meteterakis* sp. 1 from Ishigakijima Island (SP1), *Meteterakis* sp. 2 from Iriomotejima Islands (SP2), *Meteterakis* sp. 3 from Taiwan (SP3), *M. japonica* from the eastern Japanese Archipelago and Shimokoshikijima Island (MJ), and *M. ishikawanae* from the Okinawa Group (MI).

4.2. Major divergence of *Meteterakis* and comparison with host faunal province

The congruence of major diversification patterns between mitochondrial and nuclear DNA phylogenetic trees suggest that these should reflect the true phylogenetic relationships of the lineages. Both trees exhibited two major distinct clades (J- and A-groups) with mutually exclusive geographic ranges. Both clades had disjunct ranges (J-group: eastern Japanese Archipelago, Shimokoshikijima Island, and the Okinawa Group, sites 1–9, 13 and 17–19, Fig. 1B–C; A-group: western Japanese Archipelago except Shimokoshikijima Island, the southern Tokara Group, the Amami Group, the Yaeyama Group of the Southern Ryukyus, and the northern Taiwan, sites 10–12, 14–16, and 20–23, Fig. 1B–C). Thus, none of the geographic ranges of the major clades was concordant with any of the well-established biogeographic provinces of the host taxa – the Japanese Archipelago, the Central Ryukyus, the Southern Ryukyus, or Taiwan (see [29,30]). The discordance negates the hypothesis that the diversification between J- and A-groups was formed by co-divergence with vicariance events of the host fauna. The next part of this paper discusses species-level diversification within each of the major clades to infer the allopatric speciation modes of *Meteterakis*.

4.3. Allopatric speciation factors of *Meteterakis*

The J-group encompassed two deeply diverged species with allopatric distribution, *M. japonica* and *M. ishikawanae*; their geographic ranges corresponded to major biogeographic provinces of the hosts, the Japanese Archipelago and the Central Ryukyus, respectively. Thus, the divergence within the J-group was concordant with the host biogeography, and can be simply interpreted as a result of the vicariance event of host fauna. According to the biogeographic studies of the host taxa [e.g., [29,30]], a major part of the host fauna has persisted since the initial isolation of the Japanese Archipelago and the Central Ryukyus from the continent. Therefore, the J-group species should also be

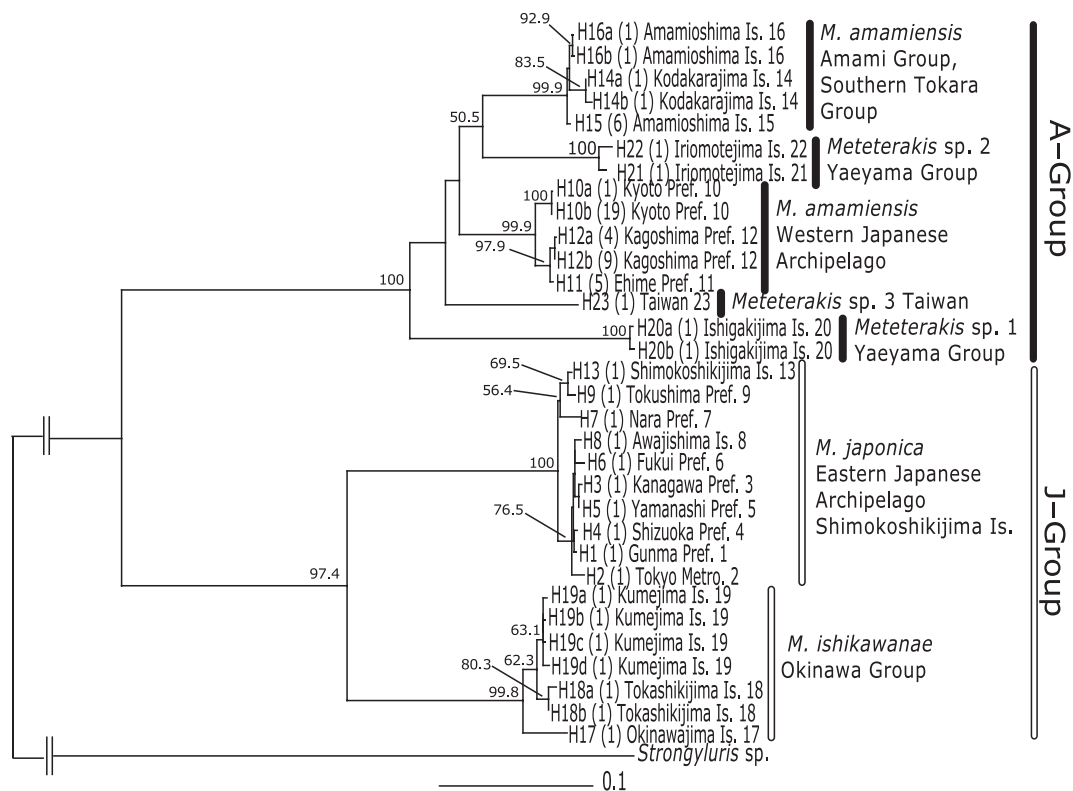


Fig. 2. A maximum likelihood tree of mtDNA haplotypes of *Meteterakis* spp. from the East Asian islands. Bootstrap values of 50% or higher are shown above the branches. Numbers preceded by “H” indicate haplotype names, and these numbers correspond with the locality numbers in Fig. 1. The numbers in parentheses following haplotype names indicate the number of specimens for each haplotype. Each haplotype names are accompanied by its locality name and number. Details of the locality names and numbers are listed in Table 1 and Fig. 1. Details of the haplotypes are listed in Supplementary Table 1.

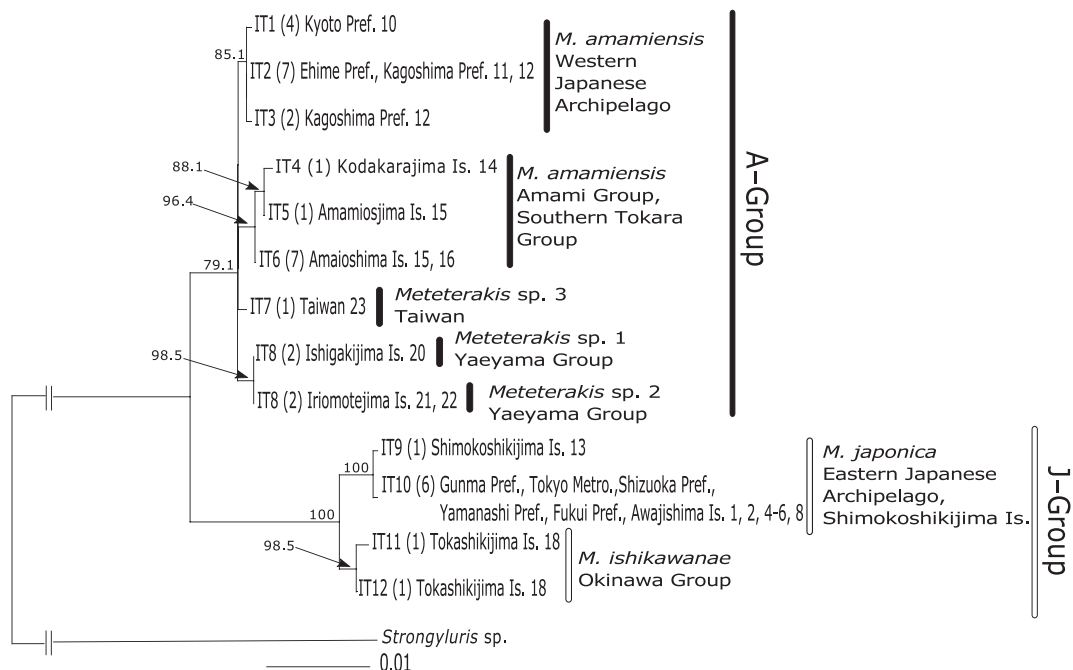


Fig. 3. A maximum likelihood tree of nuclear DNA haplotypes of *Meteterakis* spp. from the East Asian islands. Bootstrap values of 50% or higher are shown above the branches. Numbers preceded by “IT” indicates haplotype name. The numbers in parentheses after haplotype name indicate the number of specimens, included. Each haplotype names are accompanied by its locality name and number. Details of the locality names and numbers are listed in Table 1 and Fig. 1. Details of the haplotypes are listed in Supplementary Table 1.

Table 3

Nucleotide diversity (π) and haplotype diversity (h) values of *Meteterakis amamiensis* in the western Japanese Archipelago (WJ) and *M. japonica* (MJ) in the eastern Japanese Archipelago and Shimokoshikijima Island with standard deviations.

Population	Number of locality	N	π (%)	h
WJ	3	36	0.91 ± 0.50	1.00
MJ	10	10	1.63 ± 0.92	1.00

Table 4

Net genetic distances between *Meteterakis* species. WJ: *Meteterakis amamiensis* in the western Japanese Archipelago; MA: *M. amamiensis* in Amamioshima Island and Kodakarajima Island; SP1: *Meteterakis* sp. 1 in Ishigakijima Island; SP2: *Meteterakis* sp. 2 in Iriomotejima Island; SP3: *Meteterakis* sp. 3 in Taiwan; MJ: *Meteterakis japonica* in the eastern Japanese Archipelago and Shimokoshikijima Island; MI: *Meteterakis ishikawanae* in the Okinawa Group.

		A-group					J-group	
		WJ	MA	SP1	SP2	SP3	MJ	MI
A-group	WJ							
	MA	0.069						
	SP1	0.096	0.105					
	SP2	0.078	0.076	0.111				
	SP3	0.075	0.085	0.114	0.092			
J-group	MJ	0.133	0.147	0.151	0.147	0.147		
	MI	0.132	0.138	0.153	0.148	0.149	0.097	

regarded as parts of the oldest members of the fauna of these islands.

In the A-group, the present results detected deep differentiation between WJ and MA occurring in the Japanese Archipelago and Central Ryukyus respectively, which is concordant with the host biogeography at a glance. However, the diversification pattern in the entire A-group was discordant with the biogeographic patterns of the hosts. Given the identical host composition between Ishigakijima Island and Iriomotejima Island, and the close relationships of host taxa on these islands and Taiwan [e.g., [29,30]], shared species of *Meteterakis* between Ishigakijima Island and Iriomotejima Island and a close relationship between species of the Yaeyama Group and Taiwan were expected. However, any of these areas accommodate specific lineages with deep divergence from any other species (Figs. 2, 3). The substantial diversification among the lineages of the Southern Ryukyus and Taiwan should negate *in situ* diversification in accordance with host vicariance, and rather implies that these endemic species were formed by independent invasions from the surrounding area and the speciation that followed. Although their geographic origin could not be detected, the present phylogenetic patterns suggested that multiple peripatric speciation formed the species diversity of the A-group.

The hypothesis of diversification of A-group taxa by multiple colonization can explain the mosaic geographic arrangement of the A- and J-groups. The present study clarified disjunct distribution of MJ separated by the range of WJ (MJ: sites 1–9, 13, Fig. 1B; WJ: sites 10–12, Fig. 1B). These patterns suggest the following scenario: (1) ancestral MJ was distributed across the entire Japanese Archipelago including its western part, and (2) invasion of the ancestral WJ to the western Japanese Archipelago except for Shimokoshikijima Island occurred, and then the ancestral MJ was excluded by some exclusive interaction with WJ there. The comparison of the net genetic distances of WJ and MJ from their closest lineages (Table 4) suggested a younger origin of WJ.

The differential biogeographic histories of WJ and MJ were also supported by demographic parameters. Although both lineages had equally large haplotype diversities ($h = 1.0$), MJ exhibited greater nucleotide diversity ($\pi = 1.63 \pm 0.92\%$) than WJ ($\pi = 0.9 \pm 0.5\%$) (Table 3). The higher π value of MJ than of WJ implies that the population of MJ has been maintained for a certain length of time,

whereas WJ has experienced a recent increase in population size by geographic range expansion [40]. Thus, the present results collectively support the above scenario, in which WJ was formed by peripheral isolation from some other region followed by range expansion in the western Japanese Archipelago. The closest genetic affinity between MJ populations of the eastern Japanese Archipelago (sites 1–9) and Shimokoshikijima Island (site 13) implies recent expansion of WJ in western Japanese Archipelago. Similarly, MA may have a younger origin than MI in the Central Ryukyus, according to the shallower genetic distance between MA and WJ than between MJ and MI (Table 4). Occurrence of the A-group but absence of the J-group in the Amami Group can thus be regarded as a result of the exclusion of an old resident (the J-group) by a younger colonizer (the A-group) in the northern part of the Central Ryukyus.

The co-divergence of the J-group with host faunal divergence and the peripatric speciation of the A-group suggested that both modes of allopatric speciation may have formed the present species diversity of highly dispersible parasites. The relative importance of these modes may differ from lineage to lineage, given the contrasting phylogeographic patterns between the two groups. All *Meteterakis* species exhibited mutually exclusive geographic distribution, despite multiple colonization, at least in the A-group. This implies the inhibition of co-existence by some exclusive interaction between species. Such interaction, as well as the two modes of allopatric speciation, may play an important role in range expansion and diversification of highly dispersible parasites.

4.4. Implications from peripatric speciation of *Meteterakis*

Criscione & Bloin [41] compared phylogeographical patterns of a digenae trematode parasite [*Plagioporus shawi* (McIntosh, 1939)] and its salmonid hosts (*Oncorhynchus* spp.). They found concordant divergent patterns between the parasites and the hosts, and concluded that host biogeographical history can explain the broad-scale phylogeography of parasites. In contrast, the present study clarified the biogeographic pattern of *Meteterakis* spp. in the East Asian islands, which was formed by simple allopatric speciation of the J-group through vicariance of host fauna, followed by invasion of the A-group and peripatric speciation, and exclusive interaction between the divergent lineages. Thus, the present study suggests that peripatric speciation can lead to the inconsistent phylogeographical pattern between highly dispersible parasites and their hosts. However, the present study could not reveal the origin of each of the A-group species, although the ordinary peripatric speciation model requires “parental” species in the geographic origin. Detection of the parental species or geographic origin requires additional sampling of *Meteterakis* spp. in the continent, and inference of well-supported phylogeny among the species. According to the present conclusion, continental lineage close to the J-group is expected to be relictual or absent, whereas the known A-group species in the island region, which may have been formed by repeated isolation after colonization from somewhere else, are expected to be polyphyletic with respect to the continental species. These predictions will be tested by future phylogenetic studies focusing on continental *Meteterakis* spp.

4.5. Taxonomic implications

The phylogenetic trees revealed the genetic distinctiveness of SP3 collected from northern Taiwan (Figs. 2, 3). SP3 also had several divergent morphological characters from any other known species. Although the present nuclear DNA sequences of SP1 from Ishigakijima Island (site 20, Fig. 1C) and SP2 from Iriomotejima Island (sites 21–22, Fig. 1C) were identical, the mitochondrial DNA sequences and morphological characters suggested their distinct differentiation from any other species. Given the distinctiveness in the morphology and DNA sequences, each of the three *Meteterakis* lineages—SP1 from

Ishigakijima Island, SP2 from Iriomotejima Island, and SP3 from Taiwan—should be regarded as different lineages. The mitochondrial and nuclear phylogenetic trees clarified the existence of two genetically distinct lineages in *M. amamiensis*, WJ and MA. Further morphological comparisons to describe the WJ, and future taxonomic descriptions of these four lineages, are needed.

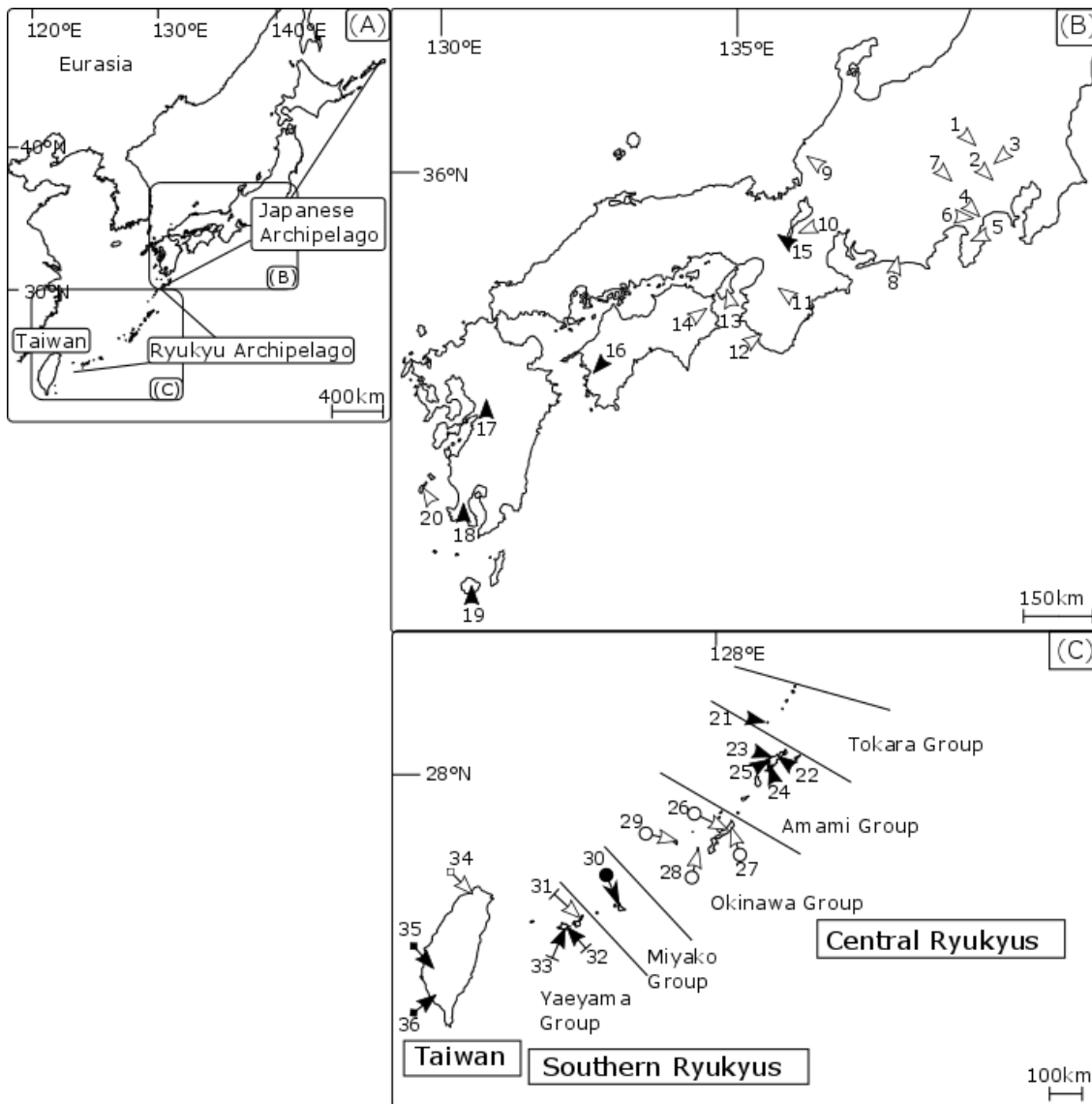
Supplementary data to this article can be found online at <https://doi.org/10.1016/j.parint.2018.04.008>.

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Supplementary Figure 1

Maps of the East Asian islands showing the distribution of *Meteterakis* species, which based on the present and previous studies [1–12]. Arrows with numbers indicate recorded localities of *Meteterakis* species. 1–14, 20: *Meteterakis japonica*; 15–19, 21–25: *M. amamiensis*; 26–29: *M. ishikawanae*; 30: *M. japonica*-like undescribed species (see main text for details); 31: *Meteterakis* sp. 1; 32 and 33 *Meteterakis* sp. 2; 34: *Meteterakis* sp. 3; 35 and 36: *M. govindi*.

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Supplementary Table 1. DDBJ accession numbers of *Meteterakis* spp. and *Strongyluris* sp. used in this study. The locality numbers are consistent with Fig 1 and Table 1. The haplotype names are consistent with Fig 2 and 3.

Locality No.	Sample name	Haplotype name		DDBJ accession numbers					Analyses		
		mt	n	COI	12S	cytb	COII	18S-28S	Phylogenetic tree	Net genetic distance	Genetic diversity
1	<i>Meteterakis japonica</i>	H1	IT10	LC185713	LC185907	LC185785	LC185839	LC185978	1	1	1
2	<i>M. japonica</i>	H2	IT10	LC185714	LC185908	LC185786	LC185840	LC185979	1	1	1
3	<i>M. japonica</i>	H3	-	LC185715	LC185909	LC185787	LC185841	-	1	1	1
4	<i>M. japonica</i>	H4	IT10	LC185716	LC185910	LC185788	LC185842	LC185980	1	1	1
5	<i>M. japonica</i>	H5	IT10	LC185717	LC185911	LC185789	LC185843	LC185981	1	1	1
6	<i>M. japonica</i>	H6	IT10	LC185718	LC185912	LC185790	LC185844	LC185982	1	1	1
7	<i>M. japonica</i>	H7	-	LC185719	LC185913	LC185791	LC185845	-	1	1	1
8	<i>M. japonica</i>	H8	IT10	LC185720	LC185914	LC185792	LC185846	LC185983	1	1	1
9	<i>M. japonica</i>	H9	-	LC185721	LC185915	LC185793	LC185847	-	1	1	1
10	<i>Meteterakis amamiensis</i>	H10b	-	LC185722	LC185916	-	LC185848	-	1	1	1
		H10b	IT1	LC185723	LC185917	LC185794	-	LC185984	1	1	1
		H10b	-	LC185724	LC185918	LC185795	-	-	1	1	1
		H10b	IT1	LC185725	LC185919	LC185796	LC185849	LC185985	1	1	0
		H10b	IT1	LC185726	LC185920	LC185797	LC185850	LC185986	1	1	1
		H10b	IT1	LC185727	LC185921	LC185798	LC185851	LC185987	1	1	1
		-	-	LC185728	-	-	LC185852	LC185988	0	0	1
		H10b	-	LC185729	LC185922	-	LC185853	-	1	1	0

		H10b	-	LC185730	LC185923	-	LC185854	-	1	1	1
		H10a	-	LC185731	LC185924	LC185799	LC185855	-	1	1	1
		H10b	-	LC185732	LC185925	LC185800	LC185856	-	1	1	1
		H10b	-	LC185733	LC185926	LC185801	LC185857	-	1	1	1
		H10b	-	LC185734	LC185927	LC185802	LC185858	-	1	1	1
		H10b	-	LC185735	LC185928	LC185803	LC185859	-	1	1	1
		H10b	-	LC185736	LC185929	LC185804	LC185860	-	1	1	1
		H10b	-	LC185737	LC185930	LC185805	LC185861	-	1	1	1
		H10b	-	LC185738	LC185931	LC185806	LC185862	-	1	1	1
		H10b	-	LC185739	LC185932	-	LC185863	-	1	1	0
		H10b	-	LC185740	LC185933	-	LC185864	-	1	1	1
		H10b	-	LC185741	LC185934	LC185807	LC185865	-	1	1	1
		H10b	-	LC185742	LC185935	-	LC185866	-	1	1	1
11	<i>M. amamiensis</i>	H11	IT2	LC185743	LC185936	LC185808	LC185867	LC185989	1	1	1
		H11	-	LC185744	LC185937	LC185809	LC185868	-	1	1	1
		H11	IT2	LC185745	LC185938	-	LC185869	LC185990	1	1	1
		H11	IT2	LC185746	LC185939	-	LC185870	LC185991	1	1	1
		H11	IT2	LC185747	LC185940	-	LC185871	LC185992	1	1	1
12	<i>M. amamiensis</i>	H12b	-	LC185748	LC185941	LC185810	-	-	1	1	1
		H12b	-	LC185749	LC185942	-	LC185872	-	1	1	1
		H12a	-	LC185750	LC185943	LC185811	LC185873	-	1	1	1
		H12b	IT2	LC185751	LC185944	LC185812	LC185874	LC185993	1	1	1
		H12a	IT2	LC185752	LC185945	-	LC185875	LC185994	1	1	1

		H12b	IT3	LC185753	LC185946	LC185813	LC185876	LC185995	1	1	1
		H12b	IT3	LC185754	LC185947	LC185814	LC185877	LC185996	1	1	1
		H12b	IT2	LC185755	LC185948	LC185815	LC185878	LC185997	1	1	1
		H12a	-	LC185756	LC185949	LC185816	LC185879	-	1	1	1
		H12b	-	LC185757	LC185950	LC185817	-	-	1	1	1
		H12b	-	LC185758	LC185951	-	LC185880	-	1	1	1
		H12b	-	LC185759	LC185952	LC185818	LC185881	-	1	1	1
		H12a	-	LC185760	LC185953	-	LC185882	-	1	1	1
13	<i>M. japonica</i>	H13	IT9	LC185761	LC185954	LC185819	LC185883	LC185998	1	1	1
14	<i>M. amamiensis</i>	H14b	-	LC185762	LC185955	LC185820	LC185884	-	1	1	1
		H14a	IT4	LC185763	LC185956	LC185821	LC185885	LC185999	1	1	1
15	<i>M. amamiensis</i>	H15	IT6	LC185764	LC185957	LC185822	LC185886	LC186000	1	1	1
		H15	IT6	LC185765	LC185958	LC185823	LC185887	LC186001	1	1	1
		H15	IT5	LC185766	LC185959	-	LC185888	LC186002	1	1	1
		H15	IT6	LC185767	LC185960	LC185824	LC185889	LC186003	1	1	1
		H15	IT6	LC185768	LC185961	-	LC185890	LC186004	1	1	1
		H15	IT6	LC185769	LC185962	LC185825	LC185891	LC186005	1	1	1
16	<i>M. amamiensis</i>	H16b	IT6	LC185770	LC185963	LC185826	LC185892	LC186006	1	1	1
		H16a	IT6	LC185771	LC185964	LC185827	LC185893	LC186007	1	1	1
17	<i>Meteterakis ishikawanae</i>	H17	IT12	LC185772	LC185965	LC185828	LC185894	LC186008	1	1	1
18	<i>M. ishikawanae</i>	H18a	IT11	LC185773	LC185966	LC185829	LC185895	LC186009	1	1	1
		H18b	IT12	LC185774	LC185967	-	LC185896	LC186010	1	1	1
19	<i>M. ishikawanae</i>	H19a	-	LC185775	LC185968	-	LC185897	-	1	1	1

		H19b	-	LC185776	LC185969	LC185830	LC185898	-	1	1	1
		H19d	-	LC185777	LC185970	LC185831	LC185899	-	1	1	1
		H19c	-	LC185778	LC185971	LC185832	LC185900	-	1	1	1
20	<i>Meteterakis</i> sp. 1	H20b	IT8	LC185779	LC185972	LC185833	LC185901	LC186011	1	1	1
		H20a	IT8	LC185780	LC185973	LC185834	LC185902	LC186012	1	1	1
21	<i>Meteterakis</i> sp. 2	H21	IT8	LC185781	LC185974	LC185835	LC185903	LC186013	1	1	1
22	<i>Meteterakis</i> sp. 2	H22	IT8	LC185782	LC185975	LC185836	LC185904	LC186014	1	1	1
23	<i>Meteterakis</i> sp. 3	H23	IT7	LC185783	LC185976	LC185837	LC185905	LC186015	1	1	1
outgroup	<i>Strongyluris</i> sp.	-	-	LC185784	LC185977	LC185838	LC185906	LC186016	1	0	0

1, sequences used in analysis; 0, sequences not used in analysis.

Two new skink-endoparasitic species of *Meteterakis* (Nematoda, Heterakidae, Meteterakinae) from East Asian islands

Naoya Sata¹

¹ Department of Zoology, Graduate School of Science, Kyoto University, Sakyo, Kyoto 606-8502, Japan

<http://zoobank.org/2922776D-5C7B-4444-AEA3-6BAC0FDC6F57>

Corresponding author: Naoya Sata (sata@zoo.zool.kyoto-u.ac.jp)

Abstract

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Here, two new nematodes of *Meteterakis* Karve, 1930 from Taiwan and the western Japanese Archipelago that are endoparasitic to scincid lizards are described. The Taiwanese *Meteterakis formosensis* **sp. n.** and the Japanese *Meteterakis occidentalis* **sp. n.** can be distinguished from other congeners by the following characteristics: spicules 437–537 μm in length in *M. formosensis* **sp. n.** and 359–538 μm in *M. occidentalis* **sp. n.**; spicules with narrow alae, funnel-shaped, proximal ends ventrally bent; prevulval flap well-developed; gubernaculum mass absent; precloacal sucker with diameter of 35–47 μm in *M. formosensis* **sp. n.** and of 32–36 μm in *M. occidentalis* **sp. n.**; 9–15 caudal papillae on both lateral sides in *M. formosensis* **sp. n.** and 10–14 in *M. occidentalis* **sp. n.**; and relatively narrow lateral alae, ending at region near proximal end of spicule in male or at region anterior to anus in female. *Meteterakis formosensis* **sp. n.** is distinguished from *M. occidentalis* **sp. n.** by possessing spicules with hyaline pointed distal ends and well-developed cuticular backing structures. The present study suggests that lateral alae can be used as diagnostic character among the *Meteterakis* species, and it revealed that meteterakine nematodes mature in the host's small intestine and then migrate to the rectum to oviposit.

Introduction

Meteterakis Karve, 1930 is a parasitic nematode genus, which is specific to amphibians (frogs and caecilians) and reptiles (lizards and land turtles) (Baker 1984, 1987, Hasegawa and Asakawa 2004, Zhang and Zhang 2011, Junker et al. 2015), and currently consists of 27 species. They are distributed in South, Southeast and East Asian regions, as well as in Oceania and Sao Tome Island, which is in the Gulf of Guinea (Baker 1984, 1987, Junker et al. 2015). Four species of *Meteterakis* have been recorded from the East Asian islands, which consist of the islands from the Japanese Archipelago to Taiwan: *M. japonica* (Wilkie, 1930), inhabiting the eastern Japanese Archipelago and Shimokoshikijima Island, an islet west off Kyushu, western Japan; *M. amamiensis* Hasegawa, 1990, indigenous to the western Japanese Archipelago and northern the Ryukyu Archipelago (Kodakarajima and Amamioshima

Islands); *M. ishikawanae* Hasegawa, 1987, described from the Okinawan Islands; and the Burmese *M. govindi* Karve, 1930, which is the type species of the genus, from southern Taiwan (Karve 1930, Wilkie 1930, Yamaguti 1935, 1941, Hasegawa 1987, 1990, 1992, Telford Jr 1997, Goldberg and Bursey 2002, Bursey et al. 2005, Norval et al. 2014, Sata 2015, 2018). Although *M. japonica* was once reported from Miyakojima Island in the southern Ryukyu Archipelago (Hasegawa 1984), the individuals from the islet can be distinguished from the “true” *M. japonica* by the absence of a gubernaculum (*M. japonica* possesses a gubernaculum; Wilkie 1930, Inglis 1958).

A molecular phylogenetic study revealed that the East-Asian insular *Meteterakis* nematodes are divided into two major clades (Sata 2018). One phylogroup contains *M. japonica* and *M. ishikawanae*, while the other major clade contains *M. amamiensis* and three distinct unidentified nematodes from Ishigakijima and Iriomotejima islands in

the southern Ryukyu Archipelago and Taiwan. The study also highlights the deep-genetic divergence between the *M. amamiensis* populations inhabiting Kodakarajima and Amamioshima (type locality) Islands and those distributed in the western Japanese Archipelago (Sata 2018).

Clarifying the systematic accounts of the aforementioned unidentified species will lead to a better understanding of the species diversity and evolutionary history of *Meteterakis* parasites inhabiting the East Asian islands. In the present study, therefore, the taxonomic states of the unidentified Taiwanese species and the *M. amamiensis* populations in the western Japanese Archipelago are investigated, and each is described as a new species.

Methods

The *Meteterakis* specimens examined in this study were obtained from the scincid lizard hosts, *Plestiodon chinensis* (Gray, 1838) and *Plestiodon japonicus* (Peters, 1864), which were collected from Taiwan and the western Japanese Archipelago (Fig. 1), respectively. The host lizard specimens were identified based on Okamoto and Hikida (2012) and Kurita et al. (2017). Hosts were collected and handled in accordance with the Regulations of Animal Experimentations at Kyoto University (approval numbers: H24014 and H2711). All captured lizards were euthanized by an injection of sodium pentobarbital. The body cavity of each specimen was dissected by a longitudinal incision, and then the digestive tract was removed. The excised organs were dissected longitudinally, and the lumens were investigated. The obtained nematode individuals were fixed with a hot 5% solution of glycerin in 70% ethyl alcohol. To clear the nematode specimens, they were placed in 50% solution of glycerin in 70% ethyl alcohol, then incubated 2–3 days at 60 °C to gradually evaporate the ethyl alcohol. The cleared specimens were observed with a light microscope (OLYMPUS BX53). The measurements in males were given for a holotype, followed by the range of paratypes in parentheses; for females, averages were provided, followed by the range of the paratypes in parentheses. All measurements were described in micrometers (µm) unless otherwise stated. Both the nematode and reptile specimens examined in this study have been deposited in the Zoological Collection of Kyoto University (KUZ).

For comparison, the following *Meteterakis* specimens deposited in the KUZ collection were examined: *M. amamiensis* (sensu Sata 2015): KUZ Z2015 from Yakushima Town, Kagoshima Prefecture, Japan (Yakushima Island), Japan (site 5); KUZ Z1769 from Kodakarajima, Toshima Village, Kagoshima Prefecture (Kodakarajima Island), Japan (site 6); KUZ Z673, Z674 from Amami City, Kagoshima Prefecture, Japan (Amamioshima Island) (site 7); KUZ Z1770 from Yamato Village, Kagoshima Prefecture, Japan (Amamioshima Island) (site 8); *M. japonica*: KUZ Z1762 from Odawara City, Kanagawa Prefecture, Japan; KUZ Z1763 from Izunokuni City, Shizuoka Prefecture, Japan; KUZ Z630–Z632 from Fukuroi City, Shizuoka

Prefecture, Japan; KUZ Z636 from Kofu City, Yamanashi Prefecture, Japan; KUZ Z637, Z638 from Takasaki City, Gunma Prefecture, Japan; KUZ Z639 from Hachioji City, Tokyo, Japan; KUZ Z641, Z642, Z1765 from Tokushima City, Tokushima Prefecture, Japan; and KUZ Z645, Z1767 from Nagahama, Satsumasendai City, Kagoshima Prefecture, Japan (Shimokoshikijima Island) (Sata 2018).

Systematics

Meteterakis formosensis sp. n.

<http://zoobank.org/51F7462B-95D2-43AD-A4DA-0ECBD7E019FA>
Fig. 2

Meteterakis sp. 3; Sata 2018: figs 2, 3 (in part), table 1 (in part).

Type materials. Holotype: KUZ Z1779, whole specimen, adult male, obtained from the rectum of a *Plestiodon chinensis* specimen (KUZ R69425), collected from Mt. Guanyinshan, Bali District, New Taipei City, Taiwan (25°08'53.1"N, 121°25'47.3"E; elevation 199 m) (site 10 in Fig. 1) on 20 March 2013. Paratypes: KUZ Z1777, Z1778, Z1783, Z1992 and Z1993, whole specimens, five adult males; KUZ Z1780–Z1782, three adult females, obtained from the same host specimen of the holotype; KUZ Z1994, one prepared slide of male spicules; and KUZ Z1995, a section of the anterior end and a remaining body (KUZ Z1994 is also derived from this individual), obtained from the same host specimen of the holotype.

Additional material. The following scincid lizard specimens, which were collected from Taiwan, were also dissected to reveal the geographic range of the new Taiwanese taxon: *P. chinensis* from Taipei City (KUZ R51443, R51444 and R51449–R51453), from New Taipei City (KUZ R46132, R46134 and R46136–R46139), and from Miaoli County (KUZ R70946, R70948–R70951, R70953 and R70963); *Plestiodon leucostictus* (Hikida, 1988) from Hualien City, Hualien County (KUZ R69421 and R69424); *Plestiodon elegans* (Thompson, 1912) from Taipei City (KUZ R66354), from the Xindian District, New Taipei City (KUZ R30191, R36205), from Miaoli County (KUZ R50394, R70957 and R70964), from Yilan County, (KUZ R36552), and from Tainan City (KUZ R70090 and R70091); and *Eutropis longicaudata* (Hallowell, 1857) from Tainan City, (KUZ R70089). One *Meteterakis*-like specimen (KUZ Z2021) was obtained from a *P. chinensis* specimen (KUZ R70948).

Type locality. Taiwan, New Taipei City: Bali District, Mt. Guanyinshan.

Type host. *Plestiodon chinensis* (Gray, 1838) (Reptilia, Scincidae); site of infection: rectum and small intestine.

Diagnosis. Relatively stout body, with narrow lateral and caudal alae; lateral alae commencing from region anteri-

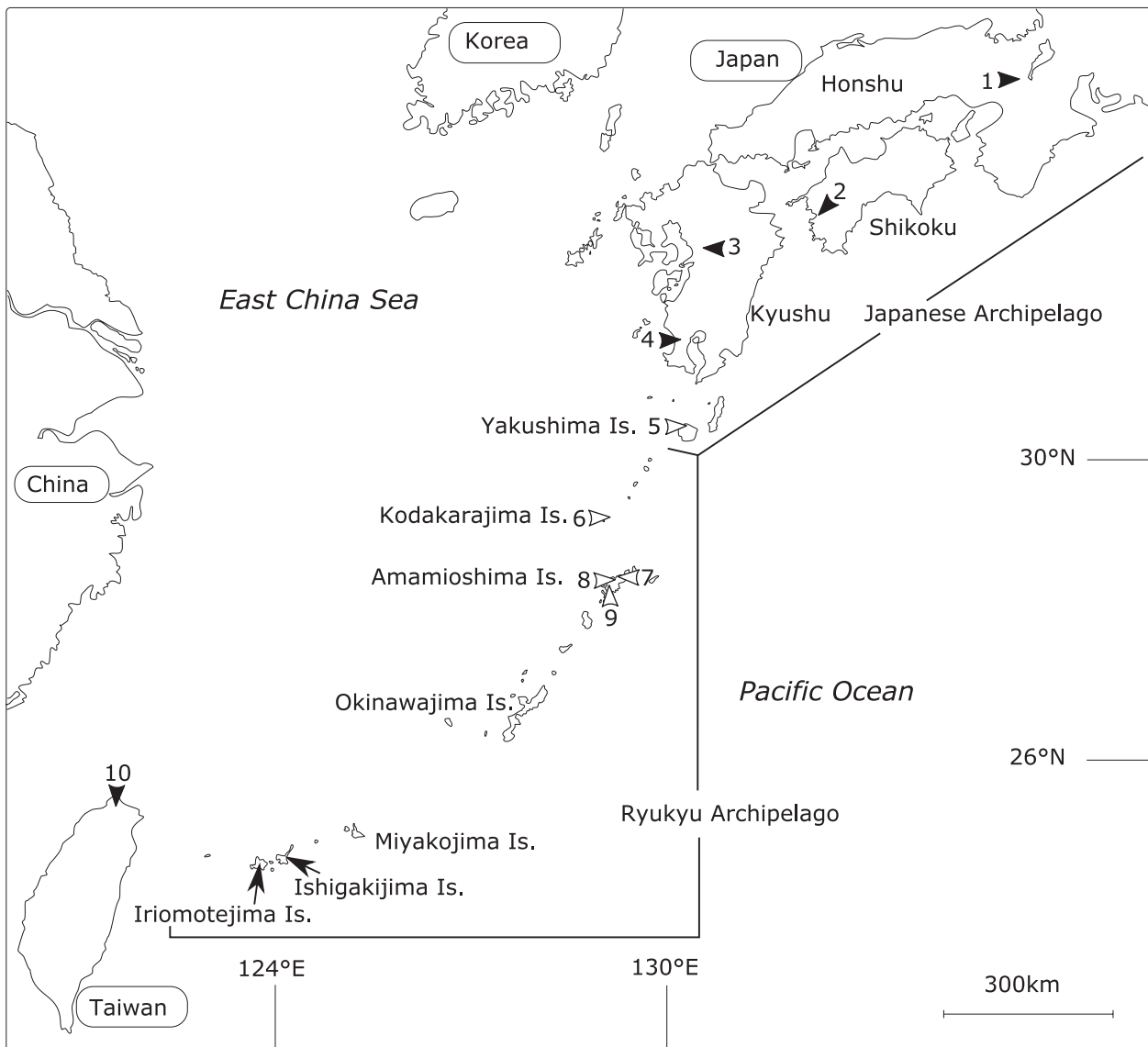


Figure 1. Map showing the known populations of *Meteterakis* Karve, 1930 inhabiting the Japanese Archipelago, the Ryukyu Archipelago and Taiwan: *Meteterakis occidentalis* sp. n. (sites 1–4), *M. amamiensis* Hasegawa, 1990 (sites 5–9; Hasegawa 1990, Sata 2015, 2018), and *Meteterakis formosensis* sp. n. (site 10). Solid arrows indicate the new species, and open arrows indicate *M. amamiensis*. Detailed site information can be found in Table 1.

or to nerve ring or front end of nerve ring in both sexes and ending at region near proximal end of spicule in male (never reaching region of precloacal sucker) or at region anterior to anus in female. Preval flap present and well developed in female. Gubernacular mass absent. Spicules with thin alae, funnel-shaped proximal ends, hyaline tips, and both proximal and distal ends bent ventrally. Right spicule, 437–510 long; left spicule 457–537 long. Each spicule with thick and long cuticular backing structures, not covered by cuticular pouch. Caudal papillae present in male, 8–13 ($N=4$) pairs with additional papillae: 12–15 ($N=4$) on right side; 9–15 ($N=5$) on left side.

Etymology. The specific name is an adjective, derived from the old name for Taiwan, which is the type locality of the new species.

Description. General. Body short and relatively stout with tapered extremities. Cephalic end with 3 lips, each lip with 2 minute apical papillae. Dorsal lip with a pair of cephalic papillae (each papilla with 2 minute papillae); each subventral lip with single papilla (each papilla with 2 minute papillae), 1 amphid and 1 smaller papillae. Flanges in inner edge of each lip unobservable. Esophagus comprise of pharynx, cylindrical portion and bulb. Bulb bearing three valves. lateral alae commencing from region anterior to nerve ring or front end of nerve ring in both sexes and ending at region near proximal end of spicule in male (never reaching region of precloacal sucker) or at region anterior to anus in female.

Male ($N=6$; KUZ Z1777–Z1779, Z1783, Z1992 and Z1993). Body length 5.03 mm (4.84–5.67 mm), maximum width 206 (151–228). Body length/body width =

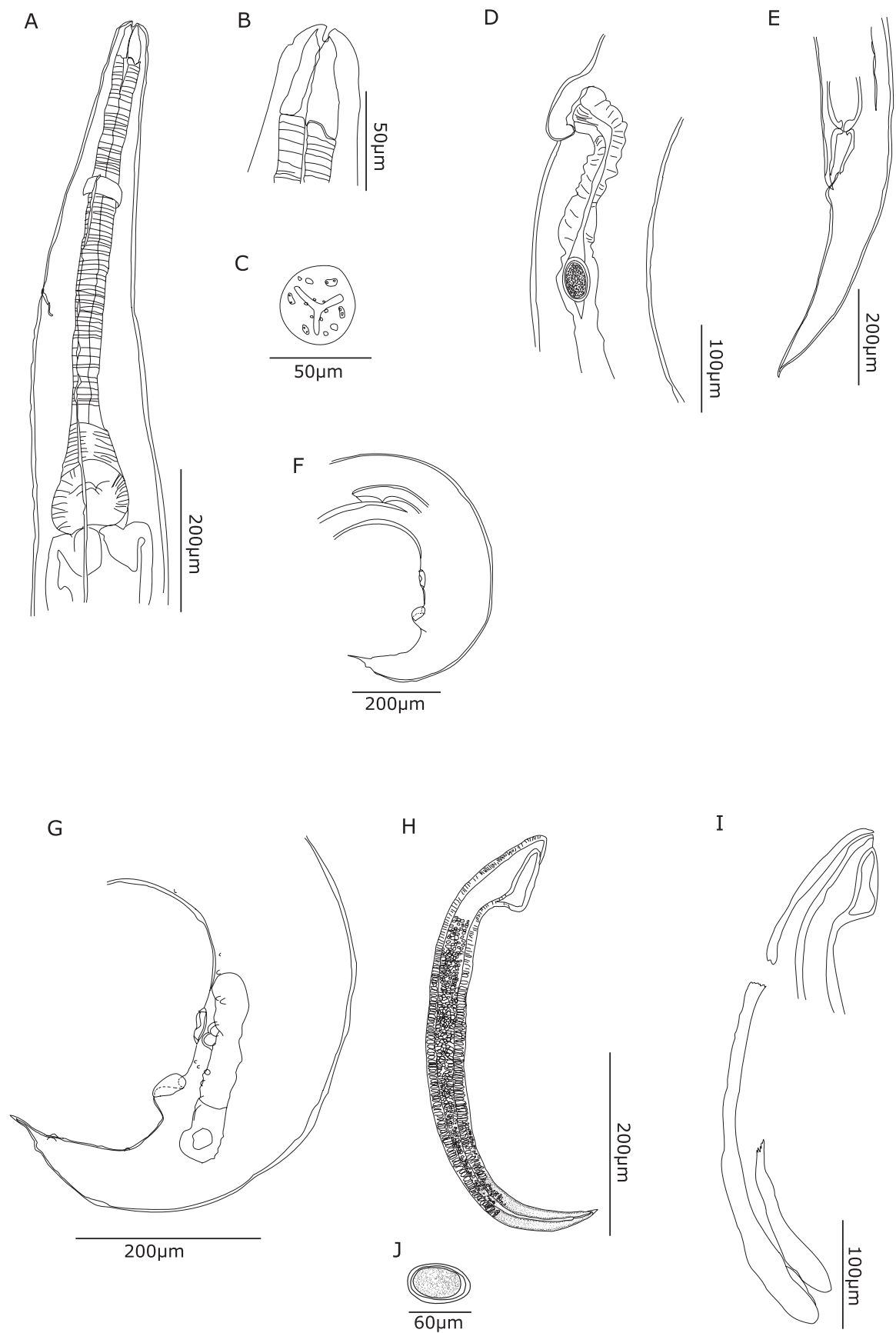


Figure 2. *Meteterakis formosensis* sp. n., holotype (KUZ Z1779: **A**, **B**, **F**, **G**), paratypes (KUZ Z1781: **D**, **J**; KUZ Z1782: **E**; KUZ Z1994: **H**, **I**; KUZ Z1995: **C**). **A** anterior region, lateral view; **B** pharynx, lateral view; **C** anterior end, apical view; **D** vulvar area of female, lateral view; **E** caudal region of female, lateral view; **F** caudal region of male, lateral view; **G** caudal papillae arrangement of male, lateral view; **H** spicule; **I** accessory of spicule; **J** egg.

Table 1. Distributions of *Meteterakis formosensis* sp. n., *Meteterakis occidentalis* sp. n., and *M. amamiensis* Hasegawa, 1990.

Species	Site #	Locality	Geographic coordinates	References
<i>M. occidentalis</i> sp. n.	1	Mt. Yoshida, Kyoto City, Kyoto Pref., JP (Honshu)	35°01'43.7"N, 135°47'09.4"E	Sata (2015); This study
<i>M. occidentalis</i> sp. n.	2	Uwajima City, Ehime Pref., JP (Shikoku)	33°13'56.8"N, 132°33'13.7"E	Sata (2018); This study
<i>M. occidentalis</i> sp. n.	3	Kumamoto City, Kumamoto Pref., JP (Kyushu)	32°47'N, 130°41'E	Sata (2015); This study
<i>M. occidentalis</i> sp. n.	4	Kagoshima City, Kagoshima Pref., JP (Kyushu)	31°35'39.76"N, 130°27'31.55"E	Sata (2015); This study
<i>M. amamiensis</i>	5	Yakushima Town, Kagoshima Pref., JP (Yakushima Island)	30°27'N, 130°29'E	Sata (2015); This study
<i>M. amamiensis</i>	6	Kodakarajima, Toshima Village, Kagoshima Pref., JP (Kodakarajima Is.)	29°13'22.6"N, 129°19'39.2"N	Sata (2018); This study
<i>M. amamiensis</i>	7	Amami City, Kagoshima Pref., JP (Amamioshima Is.)	28°24'01.3"N, 129°28'33.6"E	Sata (2015); This study
<i>M. amamiensis</i>	8	Yamato Village, Kagoshima Pref., JP (Amamioshima Is.)	28°21'30.8"N, 129°20'11.7"E	Sata (2018); This study
<i>M. amamiensis</i>	9	Mt. Yuwan, Uken Village, Kagoshima Pref., JP (Amamioshima Is.)	N/A	Hasegawa (1990)
<i>M. formosensis</i> sp. n.	10	Mt. Guanyinshan, Bali District, New Taipei City, TW	25°08'53.1"N; 121°25'47.3"E	Sata (2018); This study

24.4 (24.8–36.3). Diameter of head 46 (38–50). Total length of esophagus 724 (681–774) long with width of 36 (31–44) at cylindrical portion. Body length/esophagus length = 7.0 (7.0–7.8). Pharynx 47 (34–45) long, bulb 85 (79–95) long by 110 (91–114) wide. Grooves between lips shallow and 8.2 (7.5–9.3) long. Nerve ring and excretory pore 221 (207–243) and 396 (337–400), respectively, from cephalic end. Spicules equal or slightly different, with narrow alae, strongly chitinated, tessellated from 153 (120–141) from proximal end to distal end in right spicule (i.e. corresponding to 68.1% [68.3%–75.7%] of total length), and from 66 (95–159) to distal end in left spicule (i.e. corresponding to 86.8% [65.2%–81.9%] of total length); both proximal and distal ends bent ventrally, with wide funnel-shaped proximal ends, and pointed hyaline distal ends. Right spicule 480 (437–510) long (i.e. corresponding to 9.5% [8.0%–9.8%] of body length), left spicule 500 (457–537) (i.e. corresponding to 9.9% [8.3%–10.5%] of body length). Each spicule with thick and long cuticular backing structure, not covered by cuticular pouch. Gubernacular mass absent. Narrow caudal alae present, supported by three pairs of large papillae. Caudal papillae present, 13 (8–13) ($N=4$) pairs with additional papillae: 13 (12–15) ($N=4$) on right side; 15 (9–15) ($N=5$) on left side. Occasionally, single median papilla present. Among 13 (8–13) pairs: 1–4 pairs anterior to precloacal sucker; 2 large pairs supporting caudal alae around sucker; 1–3 small pairs around sucker; 0–2 pairs between sucker and cloaca; 1 large pair supporting caudal alae at lateral to posterior cloacal lip; 0–1 pair immediately posterior to posterior cloacal lip; and 0–2 pairs in caudal region. Precloacal sucker 47 (35–47) in diameter, 54 (26–50) from cloaca. Posterior cloacal lip developed. Tail bent ventrally, conical with pointed tip, and 304 (238–315) long. Body length/tail length = 16.6 (15.4–23.0).

Female ($N=3$; KUZ Z1780–Z1782). Body length 6.05 mm (5.56–6.30 mm), and maximum width 224 (200–

244). Body length/body width = 27.0 (25.8–27.8). Diameter of head 51 (49–52). Total length of esophagus 783 (753–806) long with width of 45 (39–50) at cylindrical portion. Body length/esophagus length = 7.7 (7.0–8.4). Pharynx 51 (38–61) long; bulb 95 (91–99) long by 108 (104–114) wide. Grooves between lips shallow and 8.5 (7.2–9.4) long. Nerve ring and excretory pore 230 (228–232) and 367 (359–372), respectively, from cephalic end. Vulva 2.71 mm (2.44–2.87 mm) from cephalic end, and located at anterior to middle of body (44.8% [43.9%–45.6%] of body length). Preclavular flap well developed. Vagina muscular running posteriorly. Tail long conical, slightly bent ventrally, and 560 (518–583) long. Body length/tail length = 10.8 (10.7–10.9). Eggs elliptical, 60 (49–68) by 41 (34–51) ($N=29$), thick shelled, containing morula stage embryos.

Occurrence. This new species was located on Mt. Guanyinshan, Bali District, New Taipei City, Taiwan (type locality) (site 10 in Fig. 1). Although a *Meteterakis*-like specimen (KUZ Z2021) with undeveloped spicules was obtained from the rectum of a *P. chinensis* specimen (KUZ R70948) from Miaoli County, Taiwan, its taxonomic account is unclear. *P. chinensis* is the only known host of this species.

Comparisons. This new species can be discriminated from almost half of the other *Meteterakis* species by the lengths of the spicules. Because *M. formosensis* sp. n. has spicules that are 437–537 μ m in length, it can be distinguished from the following eight congeners, which are diagnosed by their spicules longer than 600 μ m: *M. aurangabadensis* Deshmukh & Choudhari, 1980 (620–720 μ m), *M. karvei* Naidu & Thakare, 1981 (660–840 μ m), *M. longispiculata* (Baylis, 1929) (630–680 μ m), *M. louisii* Inglis, 1958 (970–1100 μ m), *M. singaporensis* (Sandosham, 1953) (740–960 μ m), *M. striatulus* Oshmarin & Demshin, 1972 (680 μ m), *M. vaucheri*

Adamson, 1986 (1057–1242 μm) and *M. wangi* Zhang & Zhang, 2011 (740–930 μm). This new species is also distinguishable from *M. bufonis* (Biswas & Chakravarty, 1963) (left, 270 μm ; right, 310 μm), *M. gambhiri* Gambhir et al., 2006 (220–270 μm), *M. govindi* (180–270 μm ; Karve 1930, Inglis 1958) and *M. mabuyi* (Chakravarty, 1944) (300 μm), because its spicules are longer than 400 μm . Moreover, the new species differs from *M. lyriocephali* (Crusz & Ching, 1975) because the spicules are similar in length on the left (457–537 μm) and right (437–510 μm) sides (in *M. lyriocephali*: left, 595–754 μm ; right, 340–561 μm).

In addition to the spicule length, the new species is distinguishable from the eight congeners by the following characteristics of spicules: proximal end wide, funnel-shaped and ventrally bent vs. proximal end slightly widened in *M. ishikawanae* (Hasegawa 1987) and *M. wonosoboensis* Purwaningshi, 2015 or vs. proximal end straight in *M. guptai* Gupta & Naiyer, 1993 and *M. triaculeata* (Kreis, 1933) (Inglis 1958); surface smooth, vs. rough surface in *M. saotomensis* Junker et al., 2015; and spicule alae narrow vs. wider in *M. baylisi* Inglis, 1958, *M. crombiei* Bursey et al., 2005 and *M. sinharajensis* Crusz & Ching, 1975.

Meteterakis formosensis sp. n. is distinguished from *M. lombokensis* Purwaningshi et al., 2016 by the presence of a well-developed prevulval flap in the female. Additionally, this species possesses a 35–47 μm (diameter) precloacal sucker and elliptically-shaped eggs. These characteristics can be used to discriminate this new species from *M. andamanensis* Soota & Chaturvedi, 1972, which has a 55–66 μm (diameter) precloacal sucker and spherical-shaped eggs. The number of caudal papillae (9–15) on both lateral sides of the new species can distinguish it from *M. paucipapillosa* Wang, 1980 because the latter possesses only 6 caudal papillae on both lateral sides. The new taxon is clearly distinguishable from *M. japonica* and *M. hurawensis* Bursey et al., 2017 by the absence of a gubernacular mass (Wilkie 1930, Inglis 1958, Bursey et al. 2017). *M. formosensis* sp. n. bears narrow lateral alae ending at the region near the proximal end of the spicule in the male or at the region anterior to the anus in the female. Thus, this new species differs from *M. amamiensis*, which is diagnosed by possession of the wider lateral alae ending at the precloacal region in the male, and at the region near the posterior end in the female (Hasegawa 1990).

Morphologically, *M. formosensis* sp. n. most resembles *M. occidentalis* sp. n., which is described below, but it differs from the latter species by the female having a relatively stout body (body length/body wide: 25.8–27.8 in *M. formosensis* sp. n. vs. 31.8–41.2 in *M. occidentalis* sp. n.), relatively longer tail length in the female (body length/tail length: 10.7–10.9 in *M. formosensis* sp. n. vs. 13.6–17.8 in *M. occidentalis* sp. n.), spicules with hyaline tips (lacking in *M. occidentalis* sp. n.) and well-developed backing structures for spicules (undeveloped in *M. occidentalis* sp. n.).

Meteterakis occidentalis sp. n.

<http://zoobank.org/95FED6DF-22ED-486B-8AD2-B950FEDE87B1>
Fig. 3

Meteterakis amamiensis; Sata 2015: 17 (in part); Sata 2018: figs 2, 3 (in part), table 1 (in part).

Type materials. Holotype: KUZ Z2000, whole specimen, adult male, obtained from the small intestine of a *P. japonicus* specimen (KUZ R69034), collected from Mt. Yoshida, Kyoto City, Kyoto Prefecture, Japan (35°01'43.7"N, 135°47'09.4"E; elevation 70 m) (site 1 in Fig. 1) on 16 May 2012. Paratypes: KUZ Z1996–Z1999 and Z2002–Z2008, whole specimens, four adult males and seven adult females, obtained from the rectum and small intestine of the same specimen of holotype's host and another *P. japonicus* specimen (KUZ R69036), data same as those from the holotype's host-specimen; KUZ Z2001, whole specimen, adult male, obtained from the small intestine of a *P. japonicus* specimen (KUZ R69575), collected from Ohura, Uwajima City, Ehime Prefecture, Japan (33°13'56.8"N, 132°33'13.7"E; elevation 4 m) (site 2 in Fig. 1) on 8 April 2013; KUZ Z2009, prepared slides of male spicules with the spicule pouch; Z2010, prepared slides of male spicules; KUZ Z2011 and Z2012, remaining body specimens of KUZ Z2009 and Z2010, respectively; KUZ Z2013, a section of the anterior end and a remaining body. KUZ Z2009, Z2010 and Z2013 were obtained from the rectum of a *P. japonicus* specimen (KUZ R69030), collected from same locality as the holotype's host-specimen on 9 May 2012.

Additional material. To reveal the geographic range of *M. occidentalis* sp. n., the following specimens deposited in the KUZ collection were examined: *M. amamiensis* (sensu Sata 2015): KUZ Z2014 from Kumamoto City, Kumamoto Prefecture, Japan (site 3 in Fig. 1), and KUZ Z658 and Z2022 from Kagoshima City, Kagoshima Prefecture, Japan (site 4 in Fig. 1).

Type locality. Japan, Kyoto: Kyoto, Mt. Yoshida.

Type host. *Plestiodon japonicus* (Peters, 1864) (Reptilia, Scincidae); site of infection: rectum and small intestine.

Diagnosis. Short and slender body, with narrow lateral and caudal alae; lateral alae commencing from region anterior to nerve ring in both sexes and ending at region near proximal end of spicule (never reaching region of precloacal sucker) in male or at region anterior to anus in female. Prevulval flap well developed in female. Male with well-developed posterior cloacal lip. Gubernacular mass absent. Spicules with narrow alae, funnel-shaped proximal end, and both proximal and distal ends bent ventrally. Right spicule 359–517 long, left spicule 368–538 long. Dorsal surfaces of each spicule covered by thin cuticular pouch. Caudal papillae present in male, 7–11 ($N=6$) pairs with additional papillae: 10–14 ($N=6$) on right side; 10–14 ($N=6$) on left side.

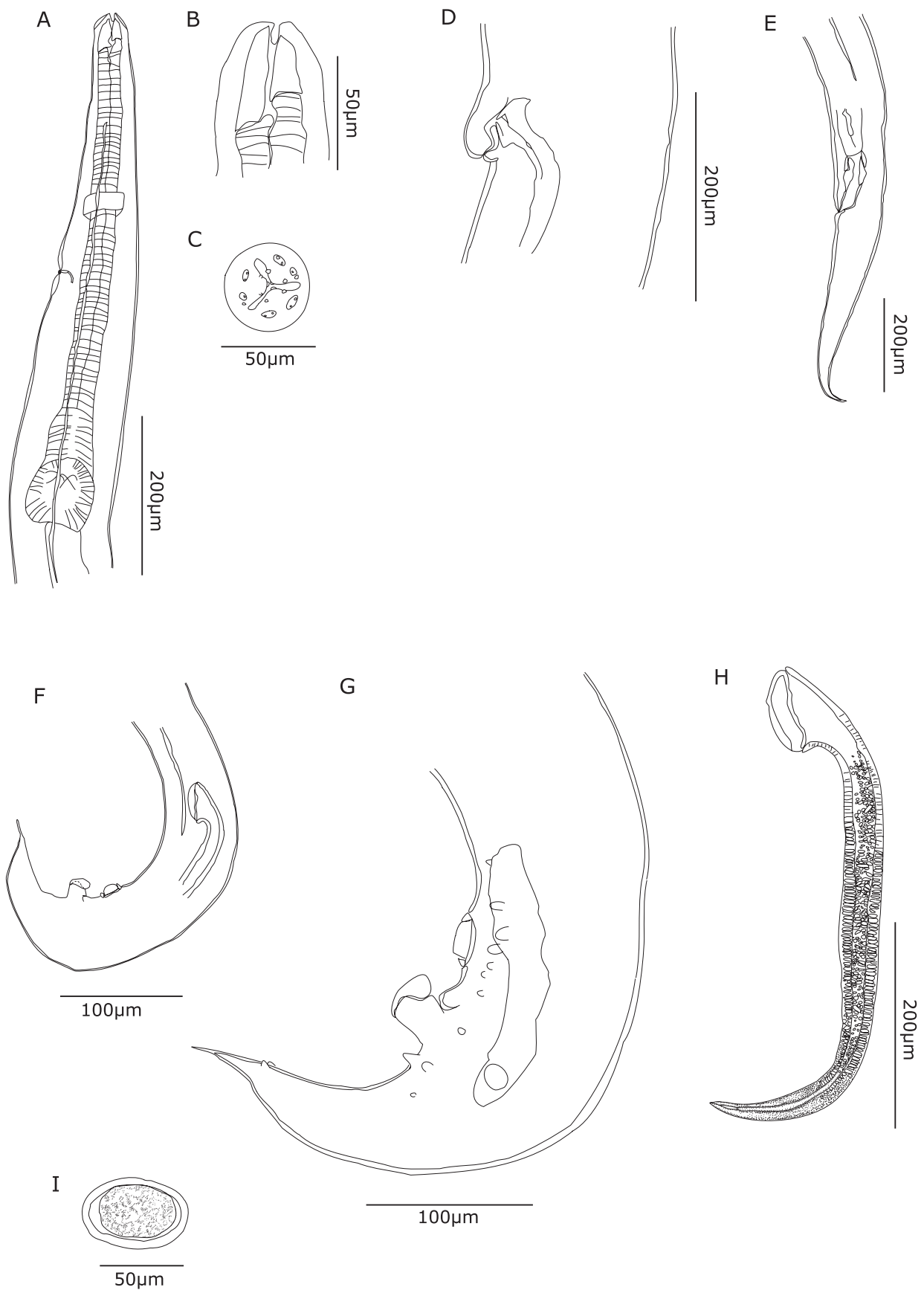


Figure 3. *Meteterakis occidentalis* sp. n., holotype (KUZ Z2000: **A**, **B**, **F**, **G**), paratypes (KUZ Z2005: **D**, **E**, **I**; KUZ Z2010: **H**; KUZ Z2013: **C**). **A** anterior region, lateral view; **B** pharynx, lateral view; **C** anterior end, apical view; **D** vulvar area of female, lateral view; **E** caudal region of female, lateral view; **F** caudal region of male, lateral view; **G** caudal papillae arrangement of male, lateral view; **H** spicule; **I** egg.

Etymology. The specific name is a Latin adjective in the nominative singular, *occidentalis* (western), referring to its distribution in the western Japanese Archipelago.

Description. *General.* Body short and slender with tapered extremities. Cephalic end with 3 lips, each lip with 2 minute apical papillae. Dorsal lip with a pair of cephalic papillae (each papilla with 2 minute papillae); each sub-ventral lip with single papilla (each papilla with 2 minute papillae), 1 amphid and 1 papilla. Inner edge of each lips with flange. Esophagus comprise of pharynx, cylindrical portion and bulb. Bulb has three valves. Narrow lateral alae present in both sexes, commencing from region anterior to nerve ring in both sexes and ending at region near proximal end of spicule (never reaching region of precloacal sucker) in male or at region anterior to anus in female.

Male ($N=6$; KUZ Z1996–Z2001). Body length 4.68 mm (4.19 mm–5.75 mm), maximum width 132 (120–163). Body length/body width = 35.5 (29.2–39.7). Diameter of head 42 (42–47). Total length of esophagus 621 (621–732) long with width of 39 (32–44) wide at cylindrical portion. Body length/esophagus length = 7.5 (6.7–7.9). Pharynx 36 (35–47) long, bulb 83 (65–97) long by 83 (84–100) wide. Grooves between lips shallow and 7.3 (7.0–13.5) long. Nerve ring and excretory pore 217 (203–241) and 328 (307–350), respectively, from cephalic end. Spicules with narrow alae, equal or slightly different, strongly chitinized, tessellated from 95 (78–104) from proximal end to distal end in right spicule (i.e. corresponding to 76.7% [76%–84%] of total length), and from 101 (43–118) to distal end in left spicule (i.e. corresponding to 76% [74.7%–91.4%] of total length), both proximal and distal ends bent ventrally, wide funnel-shaped proximal end and distal end pointed. Right spicule 408 (359–517) long (i.e. corresponding to 8.7% [8.6%–10.3%] of body length), left spicule 421 (368–538) (i.e. corresponding to 9.0% [8.3%–10.5%] of body length). Dorsal surface of each spicules covered by thin cuticular pouch. Gubernacular mass absent. Narrow caudal alae present, supported by three pairs of large papillae. Caudal papillae present, 11 (7–11) ($N=6$) pairs with additional papillae: 14 (10–12) ($N=6$) on right side; 14 (10–14) ($N=6$) on left side. Occasionally, single median papilla present. Among 11 (7–11) pairs: 1–2 pairs anterior to precloacal sucker; 2 large pairs supporting caudal alae and 0–1 small pair lateral to precloacal sucker; 0–2 pairs between posterior region of sucker and cloaca; 1 large pair supporting caudal alae at lateral to posterior cloacal lip; 0–2 pair immediately posterior to posterior cloacal lip; 1–3 pairs posterior region of tail; 0–1 pair lateral to cloaca. Precloacal sucker 36 (32–36) in diameter and 33 (23–38) from cloaca. Posterior cloacal lip developed. Tail bent ventrally, conical, with pointed tip, 258 (234–282) long. Body length/tail length = 18.1 (17.9–20.4).

Female ($N=7$; KUZ Z2002–Z2008). Body length 5.49 mm (4.85 mm–5.98 mm), maximum width 158 (145–182). Body length/body width = 34.8 (31.8–41.2). Diameter of head 48 (41–56). Total length of esophagus 731 (648–798) long with width of 37 (31–42) at cylindrical portion. Body length/esophagus length = 7.5 (6.7–8.0). Pharynx 43 (38–

52) long; bulb 94 (87–106) long by 90 (79–101) wide. Grooves between lips shallow and 10.5 (7.3–13) long. Nerve ring and excretory pore 230 (162–275) and 355 (311–388), respectively, from cephalic end. Vulva 2.36 mm (2.09–2.54 mm) from cephalic end. Vulva located at anterior to middle of body (43.0% [39.9%–47.4%] of body length). Prevulval flap well developed. Vagina muscular running posteriorly. Tail long conical, slightly bent ventrally, 353 (329–415) long, with a few specimens possessing a few small papillae. Body length/tail length = 15.6 (13.6–17.8). Eggs elliptical, 62 (52–72) by 40 (32–47) ($N=70$), thick shelled, containing morula stage embryo.

Occurrence. *Meteterakis occidentalis* sp. n. occurs in the following locations in Japan: Kyoto City, Kyoto Prefecture, Honshu (site 1 in Fig. 1); Uwajima City, Ehime Prefecture, Shikoku (site 2 in Fig. 1); Kumamoto City, Kumamoto Prefecture, Kyushu (site 3 in Fig. 1); and Kagoshima City, Kagoshima Prefecture, Kyushu (site 4 in Fig. 1). *P. japonicus* is the only known host of this species (Sata 2015, 2018).

Notes on the life cycle. Most *Meteterakis* nematodes collected from recta were adult individuals. Several larval nematodes were collected from the small intestines of the host individuals, which were inhabited by *M. occidentalis* sp. n., and a small number of ensheathed *Meteterakis* were also found from there. The rate of females having eggs in hosts' small intestines and recta were 36.2% (21/58) and 72.5% (50/69), respectively.

Comparisons. *M. occidentalis* sp. n. differs from *M. formosensis* sp. n. as discussed above. Because most of the morphological characteristics of *M. occidentalis* sp. n. are concordant with those of *M. formosensis* sp. n., this new species can be distinguished from the other congeners by the features mentioned in comparisons with *M. formosensis* sp. n. and the other *Meteterakis* species. Therefore, *M. occidentalis* sp. n. can receive the taxonomic status of a distinct species within the genus.

Discussion

Heterakidae has been classified into three subfamilies, Heterakinae, Spinicaudinae, and Meteterakinae (Inglis 1957). While the life cycles of heterakine and spinicaudine nematodes have been well documented, the life cycle of meteterakine species remains unknown (Anderson 2000). During the dissection and examination of host reptile specimens, larval nematodes were collected from the small intestines of *P. japonicus*. Because those larvae often co-occurred with adult worms of *M. occidentalis* sp. n. and/or ensheathed *Meteterakis* nematodes in the host materials, they are likely to be *M. occidentalis* sp. n. larval individuals.

Most of the *M. occidentalis* sp. n. specimens of both sexes collected from the recta were at the adult stage. Moreover, mature female of *M. occidentalis* sp. n. bearing

eggs occurred less frequently in the hosts' small intestines than in the recta, suggesting that the larvae of this species may mature in the small intestine and then migrate to the rectum to oviposit. A similar life cycle has been recorded for two spinicaudine species that are parasites in the rectum of Malagasy chameleons (Anderson 2000). The present results provide new insights into the life cycle of the meteterakine nematodes and indicates that their life cycles may resemble those of spinicaudine species.

The spicule length, which has been regarded as a useful taxonomic character of the genus *Meteterakis* (e.g. Junker et al. 2015), exhibits certain intraspecific variations. Moreover, the characteristic of spicule length sometimes overlaps with those of other congeners. In contrast, the present study revealed an obvious morphological difference between the two morphologically similar species, *M. occidentalis* sp. n. and *M. amamiensis*, in the width and ending positions of the lateral alae. Because the characteristics of the lateral alae in *M. occidentalis* sp. n. showed much less intraspecific variation, lateral alae can be used as a diagnostic character among the *Meteterakis* species. The combination of the lateral alae and other traditional taxonomic character within this genus may help discriminate the species of *Meteterakis*. However, *Meteterakis* species, which were described previously, sometimes lack morphological descriptions of their lateral alae (e.g. Inglis 1958, Gambhir et al. 2006). Therefore, the characteristics of their lateral alae should be revisited by future taxonomic revisions. Additionally, it is preferable that future descriptive studies of new *Meteterakis* species contain the characteristics of lateral alae.

Because a previous phylogenetic study (Sata 2018) did not include "*M. amamiensis*" (sensu Sata 2015) specimens from Kumamoto City, Kumamoto Prefecture, Japan (Kyushu) and Yakushima Town, Kagoshima Prefecture (Yakushima Island), Japan, their taxonomical accounts remained unclear. The present morphological observations revealed that the *Meteterakis* specimens from Kumamoto Prefecture, and Yakushima Island are *M. occidentalis* sp. n., and *M. amamiensis*, respectively, based on the characteristics of their lateral alae. Although the herpetofauna of Yakushima Island is closely related to that of Kyushu, and their compositions have genetically diverged deeply from each congeneric species on the Amamioshima Islands (Tanaka-Ueno et al. 1998, Brandley et al. 2012), *M. amamiensis* is indigenous to Yakushima and Amamioshima Islands. Thus, the geographic range of this nematode species would be obviously discordant with the host biogeography.

This discordant pattern between the endoparasites and their host species was indicated by the two unidentified *Meteterakis* species on Ishigakijima and Iriomotejima Islands (Sata 2018). Therefore, the range of each *Meteterakis* species cannot always be predicted by the host's distribution pattern. Thus, the species diversity of *Meteterakis* should be revealed by geographically exhaustive faunal and taxonomic surveys. Moreover, the taxonomic account of the unidentified *Meteterakis* on Ishigakijima and Iriomotejima Islands should be clarified by a future study using appropriate specimens of the species.

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