

Morphology and Genetic Diversity of Golden Silk Orb-weaver (*Nephila*) Siders in Thailand

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Spiders play a pivotal role in ecosystems, serving as predators to regulate populations of small organisms. They commonly exhibit body color polymorphism which could be due to a combination of environmental factors, adaptation, or genetic drift from population subdivision. This polymorphism, in turn, could affect their behavior and prey capture efficiency. Certain morphological traits may be unique to specific subregions, suggesting possible subspecies classification. In this study, we examined morphological polymorphism in the giant wood spider, *Nephila pilipes*, in Thailand, particularly focusing on leg variation and genetic variation at the *cytochrome c oxidase I* (*COI*) gene. We used partial sequence of the *COI* gene for our samples and constructed a phylogenetic tree by including other species. We explored the relationship between morphological polymorphism, *COI* genotype, and geographic distribution. Our findings categorized female *N. pilipes* into seven types based on legs I and IV, with no correlation to geographic region. The *N. pilipes* specimens formed two clades, each containing all leg types and collection regions, consistent with low genetic differentiation within the species. *Trichonephila antipodiana* specimens formed three clades while all *Argyrodes flavescens* specimens were grouped into a monophyletic clade. Additionally, *Nephila kuhli*, previously suggested to be a melanic morph of *N. pilipes*, was also included in the *N. pilipes* cluster, with a low genetic distance. Our results suggest that the leg variants in *N. pilipes* represent polymorphism within the species rather than distinct biospecies.

Keywords: *Nephila pilipes*, *COI*, Polymorphism, Leg I, Leg IV, Tufts of setae

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BACKGROUND

Spiders exhibit a remarkable global distribution, thriving in diverse habitats from arid deserts to frigid tundra, and occupying niches ranging from aerial spaces to underwater environments. As generalist predators, they exert significant ecological influence by regulating populations of arthropods, pests, and small vertebrates. In spiders, color polymorphism is a key adaptation that is driven by a complex interplay of factors, including predator avoidance, prey attraction, sexual selection (differentiation of behavior, size, or color between sexes), migration, and genetic drift (Rao et al. 2015). Given the vast diversity of color morphs in spiders, it has been suggested that species identification through color morphology would be challenging (Gaikwad et al. 2017).

This phenomenon is particularly evident in golden silk orb-weaver spiders, who are renowned for their extreme sexual size dimorphism where females can be up to 500 times heavier than males (Kuntner et al. 2019; Turk et al. 2020). Their large, robust, golden aerial orb webs have been shown to enhance prey capture, particularly of flying insects, compared to colorless webs. Importantly, body coloration directly impacts survival and foraging success. For instance, Rao et al. (2015) demonstrated that white morphs of *Verrucosa arenata* displayed superior body condition, even though yellow morphs attracted more prey. Similarly, Tso et al. (2002, 2004) reported that melanic morphs of *Nephila pilipes* attracted fewer prey compared to the typical ones.

Spiders in the family Nephilidae Simon, 1894, originally comprised of four genera: *Nephila* Leach, 1815, *Clitaetra* Simon, 1889, *Herennia* Thorell, 1877, and *Nephilengys* L. Koch, 1872. This family was reclassified and placed in the family Araneidae by Wunderlich in 2004 but was later resurrected as the subfamily Nephilinae by Kuntner et al., (2019), resulting in the creation of three new ranks: *Trichonephila* Dahl, 1911 (divided from *Nephila*), *Indoetra* (Kuntner 2006) (from *Clitaetra*), and *Nephilingis* (Kuntner et al. 2013) (from *Nephilengys*). Consequently, the family Araneidae, subfamily Nephilinae now consists of seven genera (Kuntner et al. 2019). According to the World Spider Catalog (<http://wsc.nmbe.ch/>, accessed on 25th June 2025), the number of species in the genus *Nephila* was reduced from 22 to 10, with 12 becoming members of the genus *Trichonephila*. Although all seven genera of Nephilinae are known as golden silk orb-weaving spiders, only *Nephila* and *Trichonephila* truly produce golden silk.

Six species of Nephilinae spiders have been found in Thailand: *N. pilipes* (Fabricius, 1793) (giant wood spider), *Nephila kuhli* (Doleschall, 1859), *Trichonephila antipodiana* (Walckenaer, 1841) (batik golden silk orb-weaver), *Trichonephila clavata* (Koch, 1878), *Nephilengys malabarensis* (Walckenaer, 1841), and *Herennia multipuncta* (Thorell, 1877). Among these species, *N. pilipes* has the greatest distribution across Asia and Australia. The common *N. pilipes* characteristics are as follows: 1) Small white-grey setae on carapace, and black setae arranged in a triangular shape on both sides of the foveal groove (Harvey et al. 2007). 2) Yellowish-black abdomen, which features a transverse white band on the dorso-basal margin (Fig. 1A), and two longitudinal yellow stripes across the abdomen. 3) Yellow spots scattered on the ventral abdomen (Tso et al. 2002, 2004; Harvey et al. 2007). 4) Black legs with yellow intersegmental membranes. This form is known as the yellow or typical morph (Tso et al. 2002 2004; Sankaran et al. 2020).

Some *N. pilipes* specimens vary from the common color pattern. For example, populations from eastern Australia, New Guinea, and Vanuatu, as discussed by Harvey et al. (2007), lack dorsal yellow stripes or have indistinct yellow markings on the abdomen. Similarly, some unique features have been observed in Thailand, such as extra yellow color on the leg segments and the presence of tufts of setae on the femur, tibia, and metatarsus. Previous research studying the hunting efficiency of *N. pilipes* revealed that yellow markings on the legs made spiders more distinct from the background vegetation and allowing them to attract more prey at night (Chuang et al. 2007; Fan et al. 2009). Therefore, the *N. pilipes* individuals exhibiting special patterns may increase their hunting efficiency. Additionally, these characteristics vary between individuals within the same region, indicating a high degree of polymorphism. One possibility is that the various forms of *N. pilipes* could be linked to subspecies differentiation.

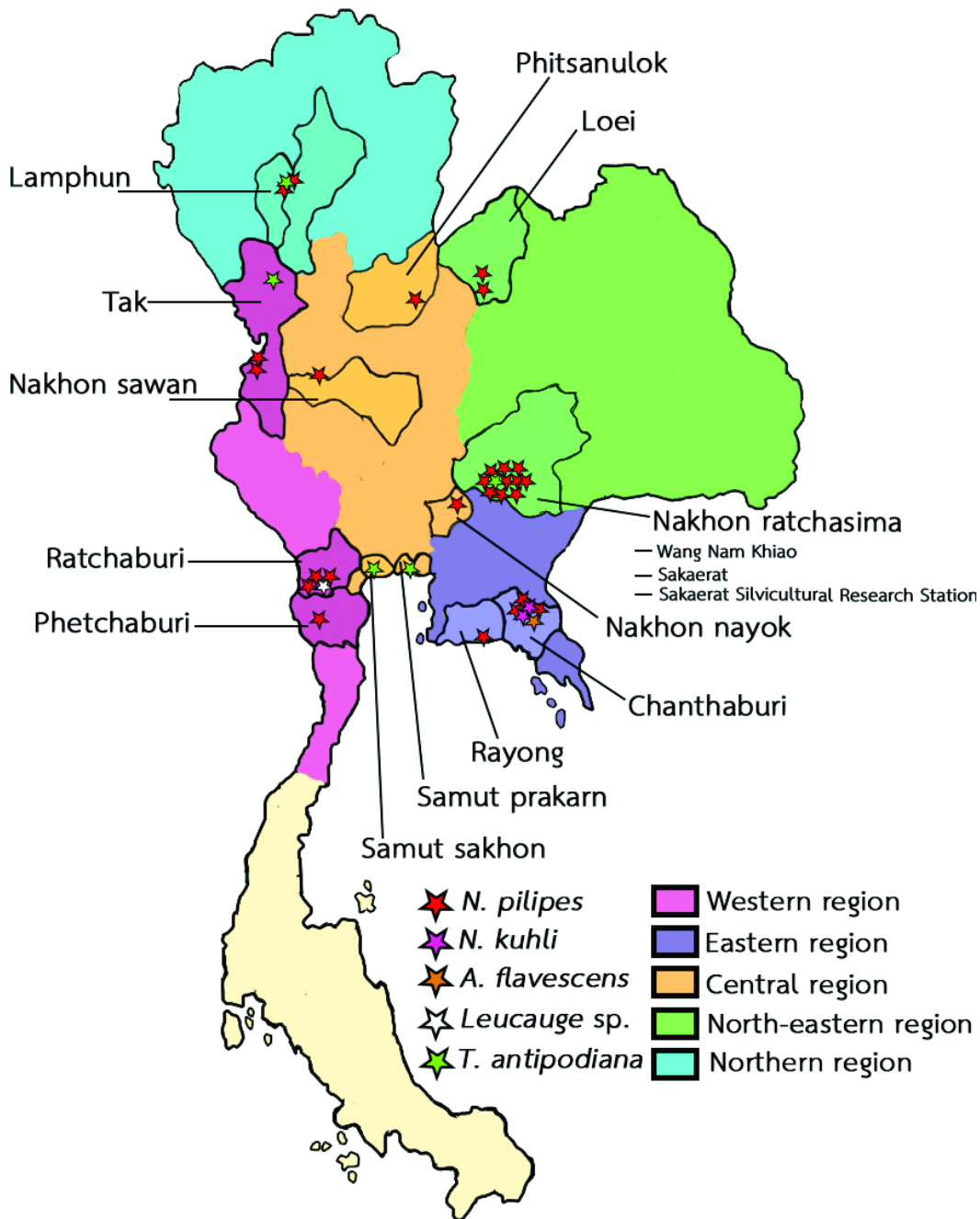


Fig. 1. Spider sampling locations in Thailand. Province names are given in the map. Colors of the stars represent spider species collected in this study.

Species and subspecies delimitation in *Nephila* can be controversial. *Nephila pilipes* and *N. kuhli* (previously known as *N. kuhlii*) are considered distinct species. Excluding body color, they appear identical. A previous study labeled dark variant of *N. pilipes* as the black or melanic morph (Tso et al. 2002, 2004; Rao et al. 2015; Sankaran et al. 2020). Sankaran et al. (2020) also described their '*N. kuhlii*' specimen as a black morph of *N. pilipes*, leading to ambiguity about whether the melanic morph is genuinely *N. kuhli*. Moreover, according to Harvey et al. (2007), color patterns from detailed examinations of their specimens did not support any morphological characteristics that would justify recognizing a separate species or subspecies in most cases. The

exceptions were for specimens from Papua New Guinea and West Papua, which differed in sternal color pattern and carapaceal projections. Nested Clade Phylogeographic Analysis (NCPA) by Su et al. (2007) suggested no significant association between genetic variation and geographic distance in the Asian continent haplotypes, again providing little support for recognizing separate species or subspecies.

This study investigated the morphological polymorphism in *N. pilipes*, aiming to elucidate potential subspecies distinctions through morphological character analysis and DNA barcoding of specimens collected across diverse regions of Thailand. We focused on the larger, more character-rich females. Males are much smaller and predominantly orange coloration patterns, creating challenges for visual identification, so the body coloration have been neglected and mainly focused on genetics. Its primary function of such bright coloration has been hypothesized to attract prey, and thereby enhance female survival. Furthermore, selective pressures have favored reduced male size likely to minimize intraspecific competition (Danielson-François et al. 2012; Zhang et al. 2022). *Argyrodes flavescens* (O. Pickard-Cambridge, 1880), a co-evolving kleptoparasites of *N. pilipes* that frequently steals prey and prompts web relocation, was also included in our study. While it often lays egg sacs on the host's web and damages the host's web, its body coloration can help the host increase prey attraction rates (Zhang et al. 2022) and thus their distribution might also be related to the host distribution. We sequenced the cytochrome *c* oxidase subunit I (*COI*) gene, a widely used DNA barcode in arthropod species identification, including arachnids (Gaikwad et al. 2017; Tyagi et al. 2019), to analyze genetic variation and construct haplotype networks. Finally, we explored the correlation between morphological diversity and genetic distance to determine whether the observed variations reflect phenotypic plasticity, geographic differentiation, or subspecies divergence.

MATERIALS AND METHODS

Sample collection

Our sampling efforts spanned most regions of Thailand, with the exception of the southern provinces due to safety concerns (Fig. 1). Golden silk orb-weaver spiders have been reported primarily inhabit rainforests, thriving in environments with high humidity and consistent rainfall (Harvey et al. 2007; Su et al. 2007). However, in Thailand, they can be found in various types of habitats, including dry evergreen forest, peat swamp forest, deciduous dipterocarp forest, mixed deciduous forest, Pine forest, and mangrove forests. Notably, mangrove forests were specifically

associated with *T. antipodiana*. We collected 48 specimens. This included 31 females and six males of *N. pilipes* (Fig. 2A and 2D); four females and one male of *T. antipodiana* (Fig. 2B and 1E); five *A. flavescens*, a kleptoparasitic orb-weaver; and one *Leucauge* sp. as an outgroup (Table 1).

Although we observed one male of *N. kuhli* (Fig. 2F) that resembled male *N. pilipes*, we could not include it in our analysis as we failed to capture it. All collected specimens were preserved in 95% ethanol prior to morphological analysis and DNA extraction.

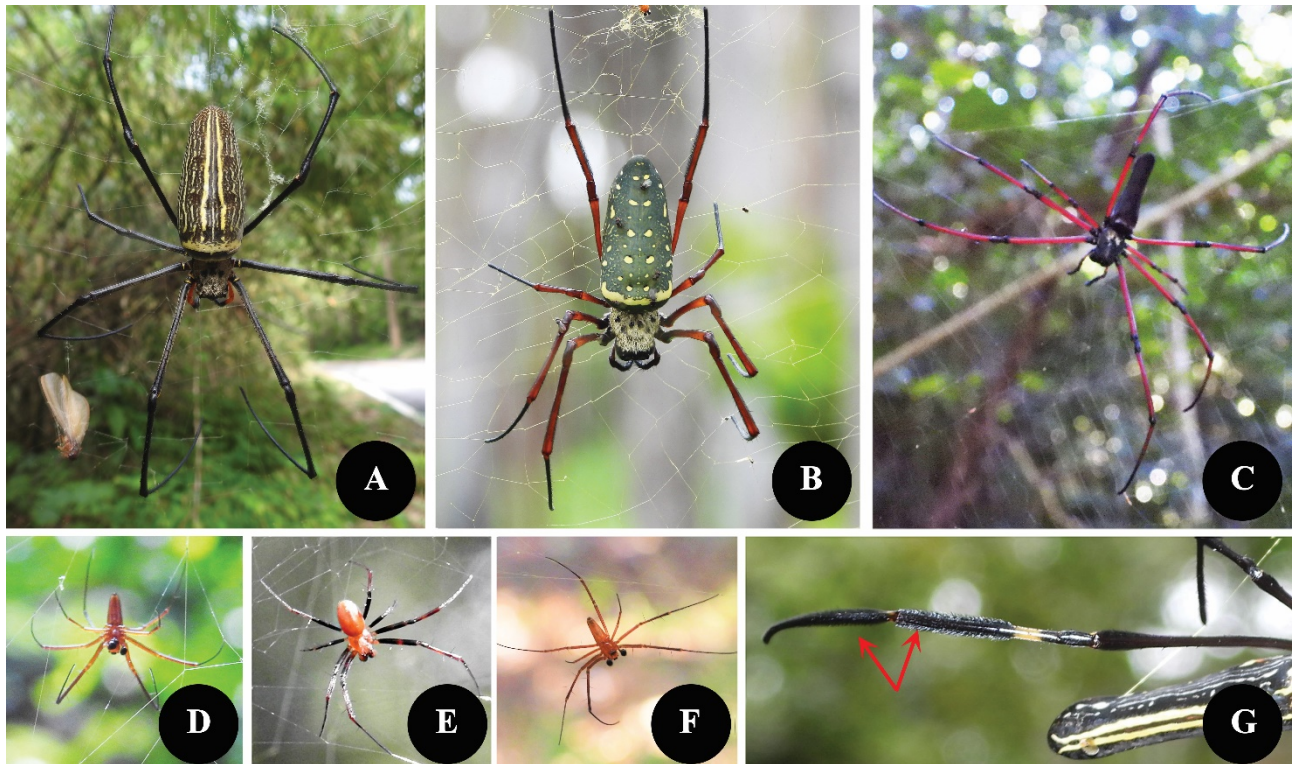


Fig. 2. Spiders in the subfamily Nephilinae, genus *Nephila* and *Trichonephila*, Thailand. (A) *Nephila pilipes*, giant wood spider. (B) *Trichonephila antipodiana*, batik golden silk spider. (C) *Nephila kuhli*, a black wood spider. (D-F) Adult male of three species as above. (G) Tufts of setae (red arrows) on tibia IV and metatarsus IV of *N. pilipes*.

Table 1. Number of specimens categorized by species and sampling location. Forest type of specimen habitat indicated by letters: (De), Dry evergreen forest; (Ps), Peat swamp forest; (Dd), Deciduous dipterocarp forest; (Md), Mixed deciduous forest; (P), Pine forest; (M), Mangrove forest.

Collection site	Species			
	<i>N. pilipes</i>	<i>A. flavescens</i>	<i>T. antipodiana</i>	<i>Leucauge</i> sp.
Phitsanulok	2 ^{De}	-	-	-
Nakhon Nayok	2 ^{De}	-	-	-
Phetchaburi	1 ^{De}	-	-	-
Rayong	1 ^{Ps}	-	-	-
Tak	2 ^{De}	-	1 ^{Dd}	-
Nakhon Sawan	1 ^{Dd}	-	-	-
Ratchaburi	5 ^{Md}	-	-	1 ^{De}
Chanthaburi	8 ^{De}	2 ^{De}	-	-
Loei	2 ^P	-	-	-

Lamphun	1 ^P , 1 ^{De}	-	1 ^P	-
Nakhon Ratchasima	2 ^{Dd} , 1 ^{Md} , 8 ^{De}	3 ^{Dd}	1 ^{Dd}	-
Samut Sakhon	-	-	1 ^M	-
Samut Prakarn	-	-	1 ^M	-
Total	37	5	5	1

Specimen data

Thailand • 1♀1♂; Phitsanulok, Nong Mae Na; 11 Jun. 2019; Fah Lertkulvanich leg; Nepi01 and Nepi01m • 1♀1♂; Nakhon Nayok, Sarika; 14°19'43.4"N 101°15'45.6"E; 15 Jun. 2019; Nepi02 and Nepi02m • 1♀ Phetchaburi, Kaeng Krachan; 18 Jun. 2019; Nepi03 • 1♀; Rayong; 12°38'59.6"N 101°32'41.5"E; 22 Jun. 2019; Nepi04 • 1♀ Samut Prakan, Bang Kachao; 23 Jun. 2019; Nepi01a • 2♀; Tak, Mae La Mung; 28 Jul. 2019; Nepi05,06 • 1♀; Tak, Thung Krachoe; 14 Dec. 2019; Nepi02a • 1♀; Nakhon Sawan; Mae Lei; 15 Sep. 2019; Nepi07 • 3♀; Ratchaburi, Yang Hak; 13°14'55"N 99°30'41.6"E; 21 Nov. 2019; Nepi08, 10, and 11 • 3♂; same data as for preceding; Nepi08m, Nepi10m, Nepi11m ; • 1♀; same data as for preceding; Leu01 • 1♀; same data as for preceding; Para1101 • 6♀; Chanthaburi, Sai Khao; 24 Nov. 2019; Nepi12 to Nepi17 • 1♂; same data as for preceding; Nepi13m • 1♀ same data as for preceding; Para1201 • 2♀; Loei, Si Than; 2 Dec. 2019; Nepi18, 19 • 1♀; Lamphun, Tha Pla Duk; 18 Jul. 2020; Nepi20 • 1♀ same data as for preceding; 19 Jul. 2020; Nepi03a • 1♀ Samut Sakhon, Bang Ya Phraek ; 5 Jul. 2020; Nepi04a • 11♀; Nakhon Ratchasima, Udomsap; 15 Aug. 2020; Nepi21 to 31 • 1♀; same data as for preceding; Nepi05am • 1♀; same data as for preceding; 15 Aug. 2020; Para2101.

DNA extraction, amplification, purification, and sequencing

Genomic DNA was extracted from legs using the FavorPrep™ Tissue Genomic DNA extraction Mini kit (Favorgen Inc., Taiwan) and the concentration was measured with a Nanodrop spectrophotometer. The mitochondrial DNA marker, cytochrome *c* oxidase subunit I (*COI*), was amplified by using a universal primer pair for *COI*, LCO-1490: 5'-GCTCAACAAATCATCATAAAGATATTGG-3' and HCO-2198: 5'-TAAACTTCAGGGTGACC AAAAATCA-3' (Folmer et al. 1994). PCR reactions were performed in a total volume of 100 µl, each containing 0.2 mM of dNTPs, 1x of reaction buffer, 2 mM of MgCl₂, 0.25 U of *Taq* polymerase, 0.5 µM of each primer (LCO and HCO), and 50–100 ng of spider DNA. Amplifications were performed using an Eppendorf™ PCR Mastercycler Nexus with the following step: 94°C for 3 min for an initiation denaturation; followed by 40 cycles of 95°C for 30 sec, 45°C for 30 sec, and 72°C for 30 sec; then 72 °C for 5 min; and a final cool down step to 20°C. PCR

products were assayed by electrophoresis on 1% agarose gels at 120 V for 30 min, then visualized under UV light after being stained with 0.5 µg/ml ethidium bromide. Target DNA fragments were isolated and purified using the FavorPrep™ Gel/PCR Purification kit (Favorgen Inc., Taiwan) and dissolved in 40 µl elution buffer. The purified PCR products were sent to Macrogen, Inc (Korea) for sequencing. NCBI accession numbers for *COI* sequences determined in this study are in Table S3.

Morphological analysis

We examined the morphology of *N. pilipes* by visually observing all body parts, adhering to the taxonomic classifications established by Harvey et al. (2007) and “Spiders of the World” (Jocqué 2007). The carapace size was used to determine the growth stage of each specimen. We documented specific characteristics using a Nikon P1000 camera, employing the focus stacking technique. Legs were the primary morphological feature emphasized in this analysis due to their distinct and clear characteristics compared to other body parts. We excluded genitalia and mandibles from our observations.

Data analysis of phylogenetic relationships

All sample sequences were edited in BioEdit, a sequence alignment editor software (Hall et al. 1999) prior to molecular identification via the Basic Local Alignment Search Tool-Nucleotide (BLAST-N) on the NCBI website (<http://blast.ncbi.nlm.nih.gov/>). All studied sequences (Table S1) were combined with the highest-scoring GenBank sequences, including those from *Nephilengys*, *Herennia*, and other *Nephila* and *Trichonephila* species absent from Thailand (Table S1). Sequences were aligned using Mega-X software (Molecular Evolutionary Genetic Analysis Version 10.0.5) (Kumar et al., 2018), with a gap opening penalty of 15.00. Phylogenetic trees were constructed via the Maximum Likelihood method, utilizing the General Time Reversible (GTR) model, incorporating a Gamma Distribution with Invariant Sites (G + I), a choice substantiated by its optimal BIC/AIC values. Five discrete Gamma Categories were employed. Partial deletion at 95% site coverage was used for the treatment of gaps and missing data. The bootstrap method was replicated 1,000 times. *A. flavescens* (Theridiidae) and *Leucauge sp.* (Tetragnathidae) served as the outgroup taxa.

Relationship between haplotypes

The haplotype network was constructed using POPART (Leigh and Bryant, 2015) employing median-joining networks to assess relationships among haplotypes.

RESULTS

Morphology of *N. pilipes*

Though *N. pilipes* exhibited largely consistent traits, we observed variation in a few key body parts, specifically the presence or absence of tufts of setae, or the segmental yellow coloration on the legs, and the pattern of white-grey setal on the foveal groove. A thorough grasp of arachnid morphology is essential for any analysis to differentiate between meaningful and trivial data. This study, therefore, focused on the most visually distinguishable regions: the cephalothorax, abdomen, and legs.

Cephalothorax: Carapace black (Fig. 3A), slightly longer than wide, and lacks a foramen surrounding the chelicerae. It is typically covered with white-grey setae, except for two distinct triangular spots at the dorso-medial area. The eyes are arranged in two rows (4:4) and lack brownish rings. The foveal groove does not display W-shaped white-grey setae; these are unclear and vary, and sometimes appearing as non-setae shapes, *e.g.*, T-shaped. Middle part contains a pair of extremely small, rounded protuberances known as dorso-medial horns. The chelicerae are black, usually with two rows of teeth (variations in cheliceral boss, fang furrow, and teeth were not documented). The maxilla (Fig. 3D) and labium are black and longer than broad, the clypeus is black, and the sternum is black, cordate, not pitted, and extends between coxae IV.

Abdomen: Dorsal black, featuring a prominent pair of yellow continuous stripes that runs from the anterior to the posterior and an additional horizontal stripe at the anterior, which are unique characteristics of *N. pilipes*. Three pairs of sigillae are visible dorsally (Fig. 3C). Ventrally, the surface is embellished with spreading yellow spots. The two book lungs display a striking yellow (red-brown in ethanol) color on either side of the epigastric furrow. An unpaired sigilla is located posterior to the epigastric furrow, with three further pairs of sigillae situated between the unpaired sigilla and the spinnerets (data not shown). The posterior portion overhangs the orange spinnerets (Fig. 3E), with the tracheal spiracle close to them. Among the observed specimens, the most notable visual variation was the spreading yellow spots on the ventral surface.

Pedipalps: The maxillae are black without a basal spur (Fig. 3D); the trochanter and femur are yellow; the patella is orange in the wild (yellow in ethanol); the tibia is orange on the proximal side connecting to the patella and black on the distal side; the tarsi are black and contain tufts of setae. Specimens Nepi14 and Nepi15 from Chanthaburi, and Nepi29 and Nepi30 from the Sakaerat Silvicultural Research Station, exhibited black pedipalps that turned scarlet in ethanol (details in the

Type of specimen section). These specimens were identified as the melanic morph (Tso et al. 2002, 2004; Harvey et al. 2007; Rao et al. 2015; Sankaran et al. 2020). An additional variant was observed in Nepi17, which had black tibia, patellae, and tarsi on the dorsal surface.

Legs: The coxae are black with yellow-brown margins on the ventro-lateral side (Fig. 3G); the trochanter is not notched; all other segments are black; the intersegmental membranes are bright yellow; tibiae and metatarsi lack both long and short curved spines and trichobothria; tarsi are shorter than metatarsi and lack trichobothria. There is no ventral comb of serrated hairs; legs lack prolateral scopulae. Three claws with teeth on the paired claws aligned longitudinally. The legs displaying the clearest distinguishable features upon visual inspection, contained specific characteristics like tufts of setae on certain segments, such as the femur, tibia, and metatarsi, or yellow coloring at the middle of the tibia. Legs I and IV exhibited the most variation, whereas legs II and III were the least Variable. Consequently, legs were selected as the primary focus for morphological characterization, utilizing these distinct features to classify the specimens.

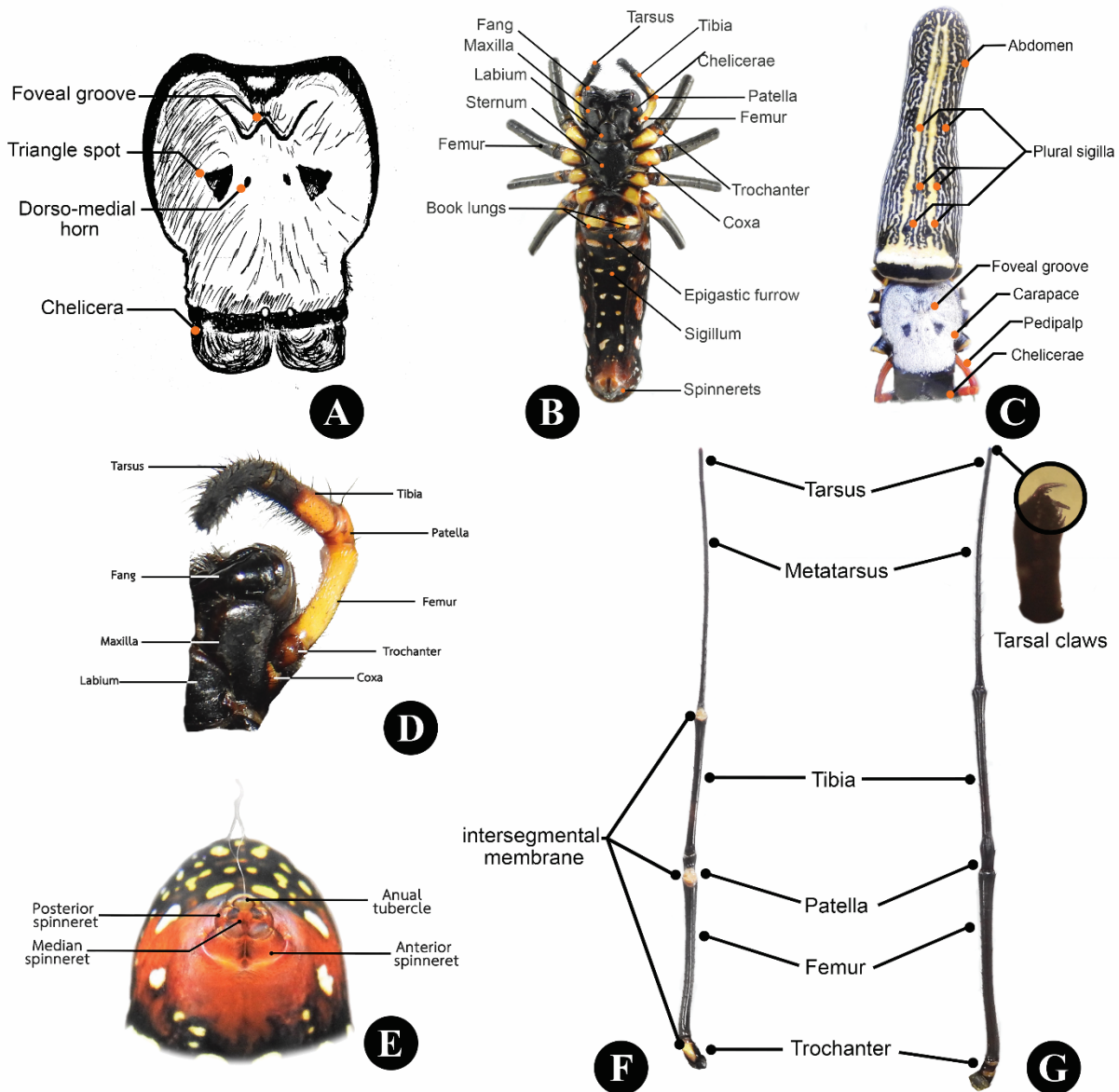


Fig. 3. Morphology of female *N. pilipes*. (A) Dorsal cephalothorax. (B) Ventral. (C) Dorsal. (D) Pedipalp. (E) Spinnerets. (F–G) Dorsal and ventral leg.

N. pilipes male description

The carapace of the male *N. pilipes* is orange and lacks setae (Fig. 4A–B); the dorsal medial horn is absent, and the fovea is not clearly visible. The abdomen is orange with dark brown stripes dorsally (Fig. 4B). Sigillae are indistinct. Legs are orange, except for the tibia, tarsus, and metatarsus, which are dark brown. All six males examined shared similar characteristics, with no distinguishable variation.

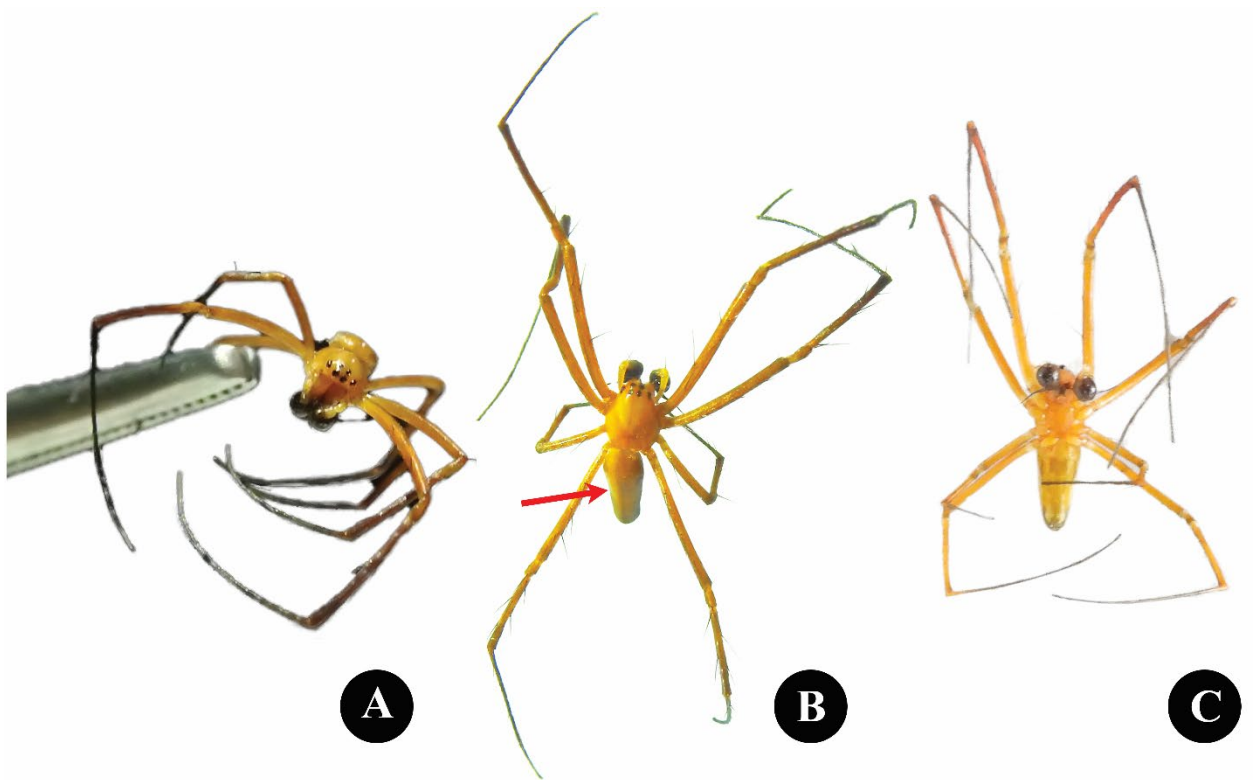


Fig. 4. An example male of *N. pilipes*. (A) Frontal. (B) Dorsal, red arrow shows a very dark brown color running at longitudinally across the dorsal abdomen. (C) Ventral.

Morph of *N. pilipes*, classified by Legs I and IV

Out of 37 *N. pilipes* specimens collected from the wild (31 females and six males), Legs I and IV were categorized into five types based on characteristic similarities. The differentiation between types has been summarized in figure 5 and table 2.

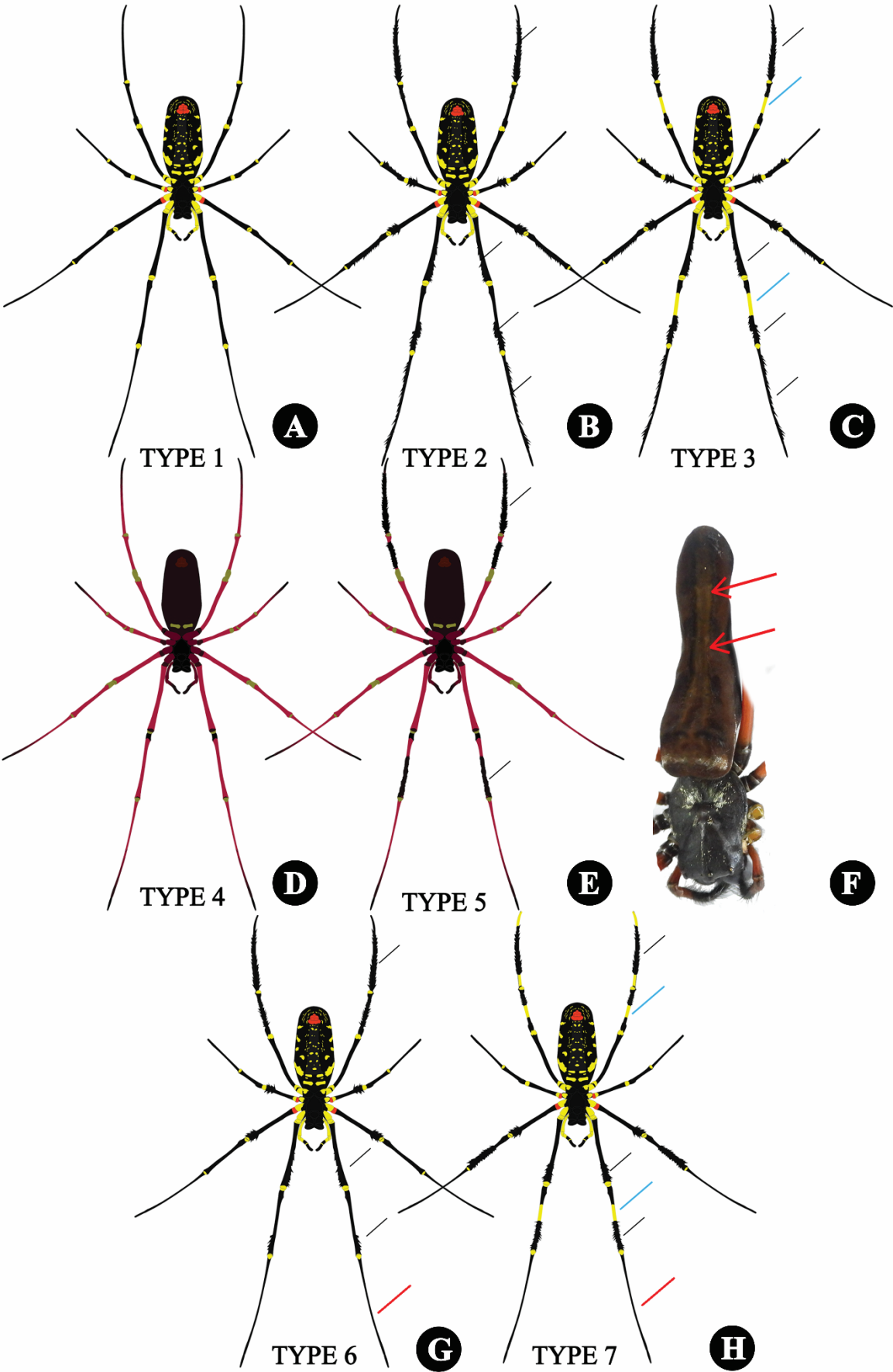


Fig. 5. Types of *N. pilipes* in Thailand, grouped by legs I and IV. (A) Type 1. (B) Type 2. (C) Type 3. (D) Type 4. (E) Type 5. (F) Pair of yellow stripes of melanic morph after being kept in alcohol.

(G) Type 6 (H) Type 7. Black lines = tufts of setae, blue lines = yellow color on mid-tibia, red lines = features absent. Pictures are not representative of the actual size of spiders.

Table 2. Summary of the seven types *N. pilipes*, categorized by features on Legs I and IV

Type	Leg I			Leg IV			Carapace (mm)		Stage
	Leg type	Tufts of setae	Yellow color on mid-tibia	Leg type	Tufts of setae	Yellow color on mid-tibia	width	length	
1	a			a			7.5–10.7	8.6–11.5	Adult/ subadult
2	b	✓		b	✓		4.8–8.9	5–8.8	Adult/ subadult
3	c	✓	✓	c	✓	✓	6.0–7.2	6.7–8.2	
4	e			d			9.6–9.9	10.6–11.5	
5	e	✓		e	✓		4.6	5.7	
6	d	✓ (absent on metatarsus)		b	✓		8.3	9.2	Subadult
7	c	✓ (absent on metatarsus)	✓	c	✓	✓	4.3	5.0	

Leg I

Leg I was divided into five types: Type a (Fig. 6A): no of tufts of setae on all segments. Type b (Fig. 6B): presence of tufts of setae on the femur, tibia, and metatarsus. Type c (Fig. 6C): same as Type b with a yellow coloration at the mid-tibia. Type d (Fig. 6D): same as Type b but absence of tufts of setae on the metatarsus. Type e (Fig. 6E): All segments are scarlet; the intersegment membrane color is difficult to discern and absence of tufts of setae.

Leg IV

Leg IV showed features similar to Leg I, with the main difference being its length. However, variations were noted in two of the 31 female specimens analyzed (Nepi13 and Nepi19), suggesting that Leg IV, in addition to Leg I, may be significant for grouping the specimens. Leg IV was also divided into five different groups: Type a (Fig. 6F): same as Leg I Type a. Type b (Fig. 6G): presence of tufts of setae, same as Leg I Type b. Type c (Fig. 6H): presence of tufts of setae and yellow coloration, same as Leg I Type c. Type d (Fig. 6D): scarlet coloration, same as Leg I Type e. Type e (Fig. 6J): same as Type d with presence of tufts of setae on the tibia and metatarsus.

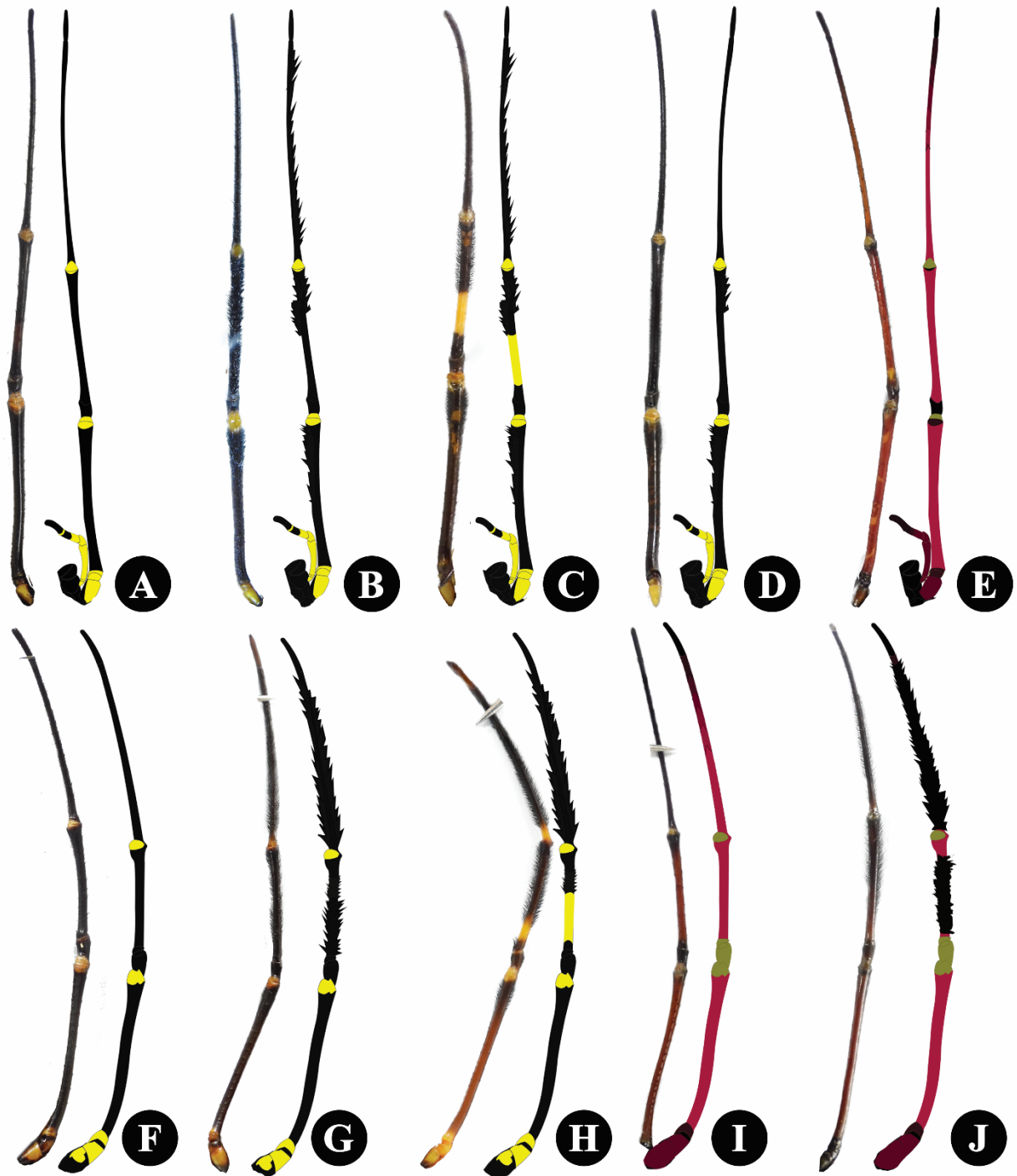


Fig. 6. Leg I (A–E) and IV (F–J) of *N. pilipes*, classified by special characteristics. (A), (F) Type a, no tufts of setae. (B, (G) Type b, presence of tufts of setae. (C), (H) Type c, presence of tufts of setae and tibia has a yellow color at the middle of segment. (D) Type d, presence of tufts of setae except metatarsus. (E), (I) Type e, all segments are scarlet, no tufts of setae. (J) Type e, all segments are scarlet and contain tufts of setae.

Description of each type of specimen

Specimens were first assigned a growth stage based on carapace size (Fig. 7). Then specimens were classified into seven types (summarized in Fig. 8) based solely on the

characteristics of Legs I and IV, disregarding other morphological variation. Type 1 lacks tufts of setae (Fig. 8A); Type 2 possesses tufts of setae (Fig. 8B); Type 3 have tufts of setae and a yellow coloration on the mid-tibia of Legs I and IV (Fig. 8C); Type 4 is characterized by a scarlet form (Fig. 8D); Type 5 is similar to Type 4 but includes tufts of setae (Fig. 8E); Type 6 is akin to Type 3 but lacks tufts of setae on Leg I (Fig. 8G); and Type 7 is similar to Type 2 but without tufts of setae on Leg I (Fig. 8H).

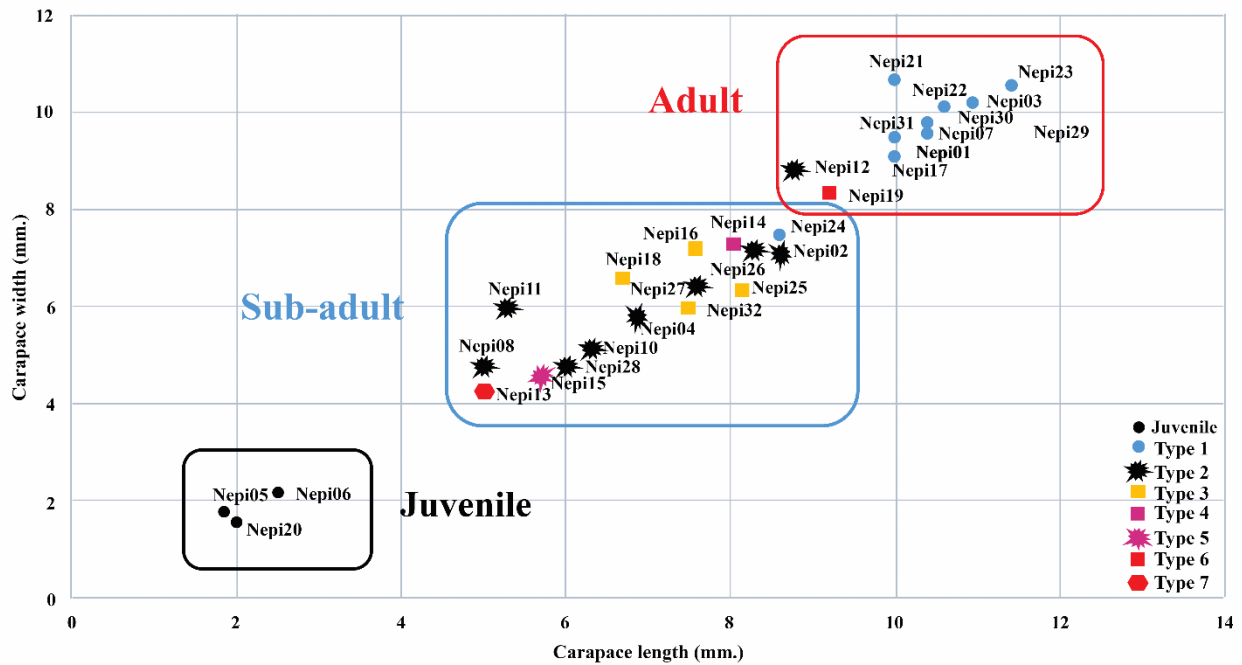


Fig. 7. Scatter plot of *N. pilipes* carapace sizes to identify the growth stages and range of morph types.

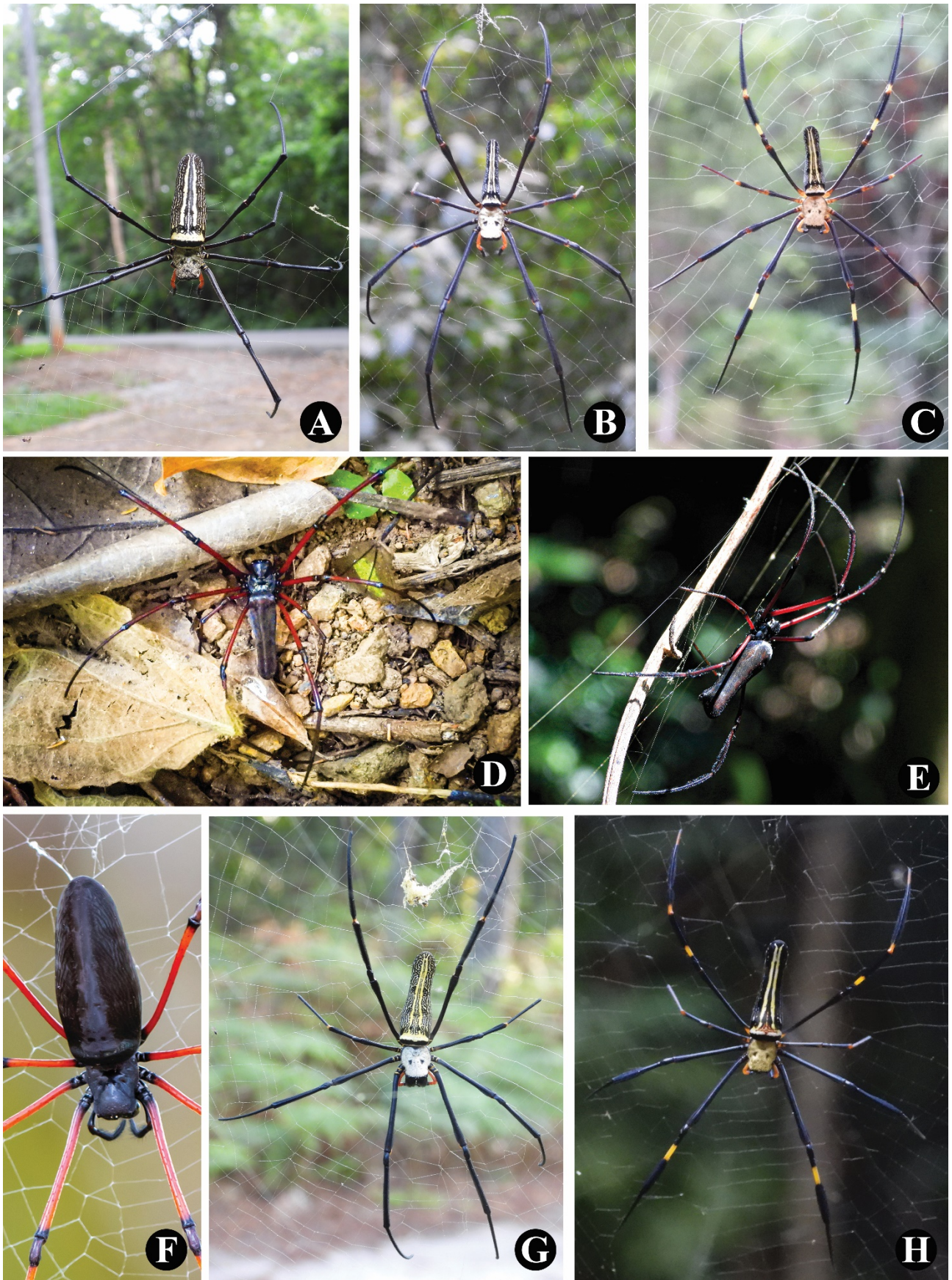


Fig. 8. Types of *N. pilipes* in native habitats, Thailand, grouped by leg type. (A) Type 1. (B) Type 2. (C) Type 3. (D) Type 4. (E) Type 5. (F) Dorsal cephalothorax and abdomen of Type 4 and 5. (G) Type 6. (H) Type 7.

Type 1, typical – all segments lack tufts of setae (Legs Type I-a, IV-a)

Specimen codes: Nepi01, Nepi03, Nepi07, Nepi17, Nepi21–24, and Nepi31. Cephalothorax and abdomen are typical. All leg segments lack tufts of setae; Leg I (Type a) and Leg IV (Type a) figure 6A. This is the most common type in the wild, typically found at the full-adult stage (carapace width 7.5–10.7 mm and length 8.6–11.5 mm). It has been suggested that *N. pilipes* with special features are immature and may develop these characteristics upon maturation. The Nepi 24 is the only one still in subadult stage.

Type 2, tufts of setae on legs (Legs Type I-b, IV-b)

Specimen codes: Nepi02, Nepi04, Nepi08, Nepi10, Nepi11, Nepi12, Nepi26, Nepi27, and Nepi28. Main body typical form. The legs have tufts of setae on the femur, tibia, and metatarsus of Leg I (Type b) and Leg IV (Type b) (Fig. 6B). This type is common but less so than Type 1 with most determined to be at the subadult stage; only Nepi12 was an adult (carapace width 8.9 mm and length 8.8 mm). Some specimens exhibited an orange tarsus (Nepi08, Nepi10, Nepi11).

Type 3, tufts of setae, and yellow color at middle of tibia (Legs Type I-c, IV-c)

Specimen codes: Nepi16, Nepi18, Nepi25, and Nepi32. Main body typical form. Legs have tufts of setae like Type 2, and a distinct yellow color is present at the mid-tibia on Legs I (Type c) and IV (Type c) (Fig. 6C). This type is rarer, with the black-yellow tibia making it more noticeable and easier to identify. Some specimens lacked tufts of setae on the metatarsus of Leg I (Nepi13), suggesting that the metatarsus normally has few tufts of setae, which might have been lost during specimen preservation.

Type 4, all black, no tufts of setae (Legs Type I-e, IV-d)

Specimen codes: Nepi14, Nepi29, and Nepi30. Fig. 6D, carapace and abdomen are black, the former lacking white-grey setae and the latter devoid of yellow stripes. Legs are scarlet in nature or dark brown in ethanol without tufts of setae on any segment (Leg I (Type d), and Leg IV (Type d)). This type is the rarest, found in abundant forest. It shares the same characteristics with Type 1, including body size, carapace features, leg and abdomen patterns, and stage of growth, but differs in the complete black coloration of the body. The absence of visible yellow stripes on the dorsal surface of the abdomen in this type was noted, with hints of yellow stripes emerging when specimens were placed in 95% ethanol (Fig. 6F). This suggests that Types 1 and 4 might be variants influenced by different alleles or environmental factors.

Type 5, all black with tufts of setae (Legs Type I-e, IV-e)

Specimen code: Nepi15. Fig. 6E, subadult stage, shares the same features as Type 4 but is distinguished by tufts of setae present on the tibia and metatarsus of Leg IV (Type e), but absent on Leg I (Type e).

Type 6, tufts of setae on legs, except metatarsus of leg I (Legs Type I-d, IV-b)

Specimen code: Nepi19. Fig. 6G, shares the same characteristics as Type 2 but leg I (Type d) lack the tufts of setae on the metatarsus while leg IV is Type b

Type 7, tufts of setae, and yellow color at middle of tibia (Legs Type I-c, IV-c)

Specimen code: Nepi13. Fig. 6H, similar to Type 3 (Leg I-c and IV-c), with the primary difference being the absence of tufts of setae on the metatarsus of Leg I.

BLAST-N result on partial *COI* gene of spiders

The BLAST-N analysis of the 640–670 bp *COI* sequences from the specimens revealed significantly similar sequences (E-value = 0), with query coverages ranging from 88–100%, and identities 98.10–100% (Table S2). The three best-hit sequences from GenBank (KF433551.1, KF433557.1, and JN032337.1) were found using all our 37 *N. pilipes* sequences as queries. These sequences were not associated with a specific collection area. One sequence of *T. antipodiana* from Sakaerat (AB17972.1) was the best-hit for our five *T. antipodiana* sequences (Nepi01a, Nepi02a, Nepi03a, Nepi04a, and Nepi05am). Three sequences of *A. flavescens* (MK392924.1, MK392923.1, and MK392925.1) matched with our kleptoparasitic specimens (Table S3). Finally, outgroup, *Leucauge* sp. (MK057511.1) was most similar to our *Leucauge* sp. (Leu09).

Sequence analyses and phylogenetic tree

Genetic differences, measured by *p*-distance, varied both within and between species (Table S4). *N. pilipes* showed low genetic divergence (0–4%), while *T. antipodiana* from Singapore (HQ441924.1) was notably distinct from the others (7–8%). The maximum likelihood phylogenetic tree from 640–670 bp of mtDNA *COI* revealed that all 37 sequences from this study, 13 from GenBank, and one *N. kuhli* clustered into three clades, with three subclades within Clade 1 (Fig. 9). Inferences of clades are summarized in table 3. Clade 1A and 1C were the largest, encompassing all *N. pilipes* specimens from our study and nearly every known *N. pilipes* type. Clade 1A included 20 specimens across six types (Type 1–4 and 6–7) with Nepi06, Nepi32, and KF433551.1 slightly separated within this subclade. Clade 1B contained only one GenBank sample from Townsville, Australia (KF433551.1). Clade 1C was composed of Types 1–5. However, these three subclades are separated by low bootstrap support (46–89). Clade 2 comprised only two species, from Duganagete and Banahaw, Philippines (DQ779256.1 and DQ779251.1). Clade 3 was composed of a *N. pilipes* specimen from Mackay, Australia (DQ779229.1) and *N. kuhli* from India (*N. kuhlii*_MN341002.1). Interestingly, *N. kuhlii* (MN341002.1) formed a polyphyletic clade with *N. pilipes* in Clade 3 and showed high similarity (average identity 98.24%) to our specimens. Lastly, Clade 4 consisted of

only one *N. pilipes* sample from Indonesia (KC849088.1). Clades 1B, 2, 3, and 4 contained only samples from GenBank and thus their morphologies are of unknown type. In addition, specimens collected from the same region were distributed across both Clade 1A and Clade 1C. For *T. antipodiana*, the samples clustered into three clades (Fig. 10 and 11B). Clade 1 is composed of all specimens from Thailand, KC849080.1 from Malaysia and HQ441926.1 from Bali, Indonesia (the latter two were both 99.34% similarity to our specimens). Clade 2 has only HQ441925.1 from the Philippines (average identity 98.55% to our samples). Clade 3 from Singapore was more different from the others (identity 92.74%). Specimen Nepi03a from Lamphun shared 98.77–99.70% identity to the others in the clade. Despite this close genetic relationship, Nepi03a presented a unique morphology. Typically, *T. antipodiana* in Thailand has yellowish green and orange or scarlet legs called yellowish-green morph (Low et al. 2018), but Nepi03a possessed a pale grey abdomen without yellowish green coloration (Fig. 12).

Table 3. Inferences of clades, collection sites, and type of specimen (labeled in Fig. 9 and 10)

Clade	Description	Type of specimen
<i>Nephila pilipes</i>		
1A	Thailand (Northern, North-eastern, Eastern, Central, and Western) China	1, 2, 3, 4, 6, 7, unknown
1B	Australia (Townsville)	Unknown
1C	Thailand (North-eastern, Central, Eastern, Northern and Western), Japan (Ihaya and Okinawa), Taiwan, Indonesia (Bali)	1, 2, 3, 4, 5, unknown
2	Philippine (Dugangete and Banahaw)	Unknown
3	Australia (Mackay), India (as <i>N. kuhli</i>)	Unknown
4	Indonesia	Unknown
<i>Trichonephila antipodiana</i>		
1	Thailand (Northern, Central, Western, and North-eastern) Malaysia, and Indonesia (Bali)	Yellowish-green, white gray
2	Philippines (Luzon)	Unknown
3	Singapore	Expected to be yellowish-green

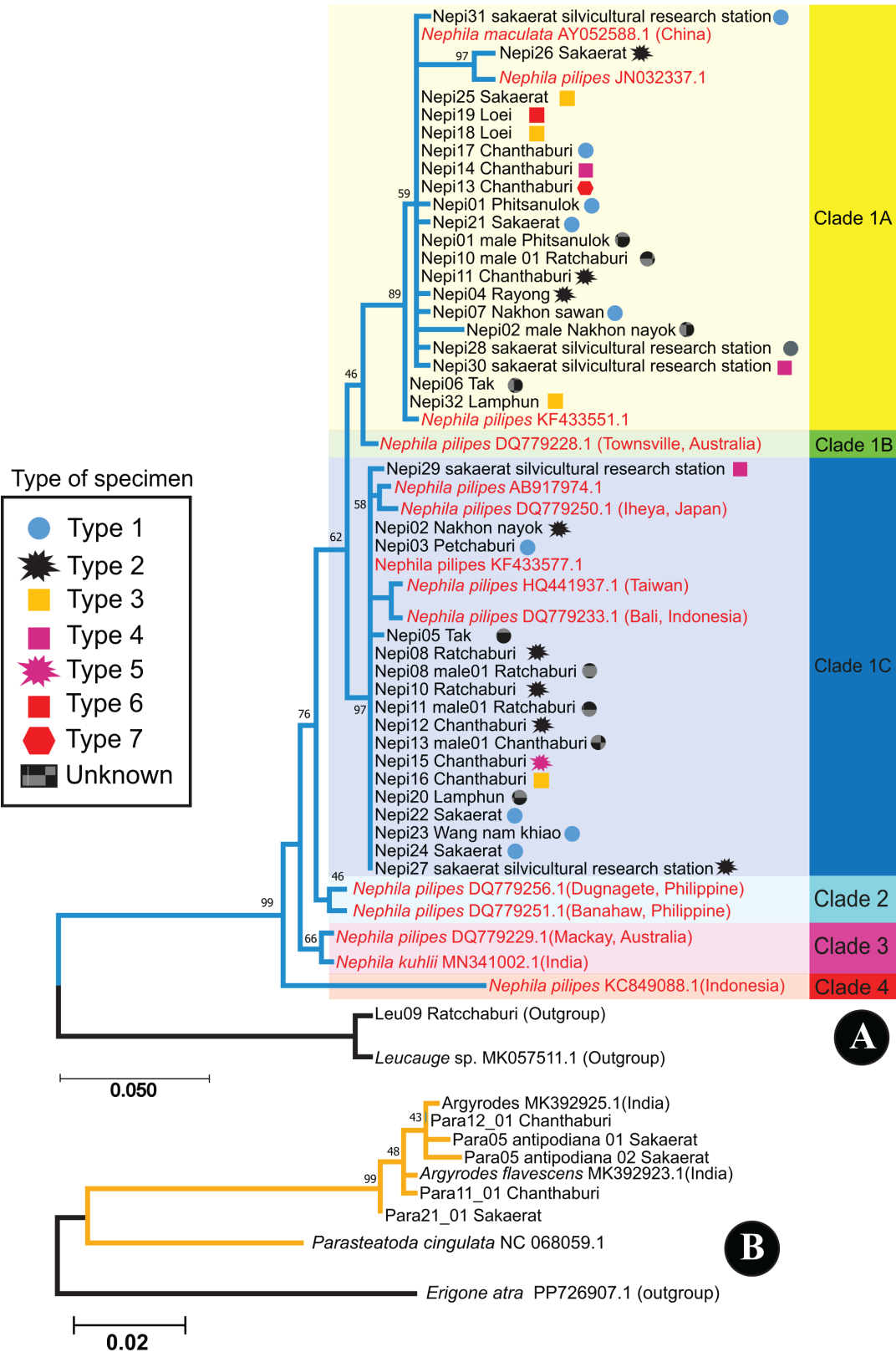


Fig. 9. (A) Maximum likelihood phylogenetic tree of *N. pilipes* with some best hit sequences from GenBank (red text) and (B) *Argyrodes flavescens*. Blue lines = *N. pilipes* clade, black line = outgroup.

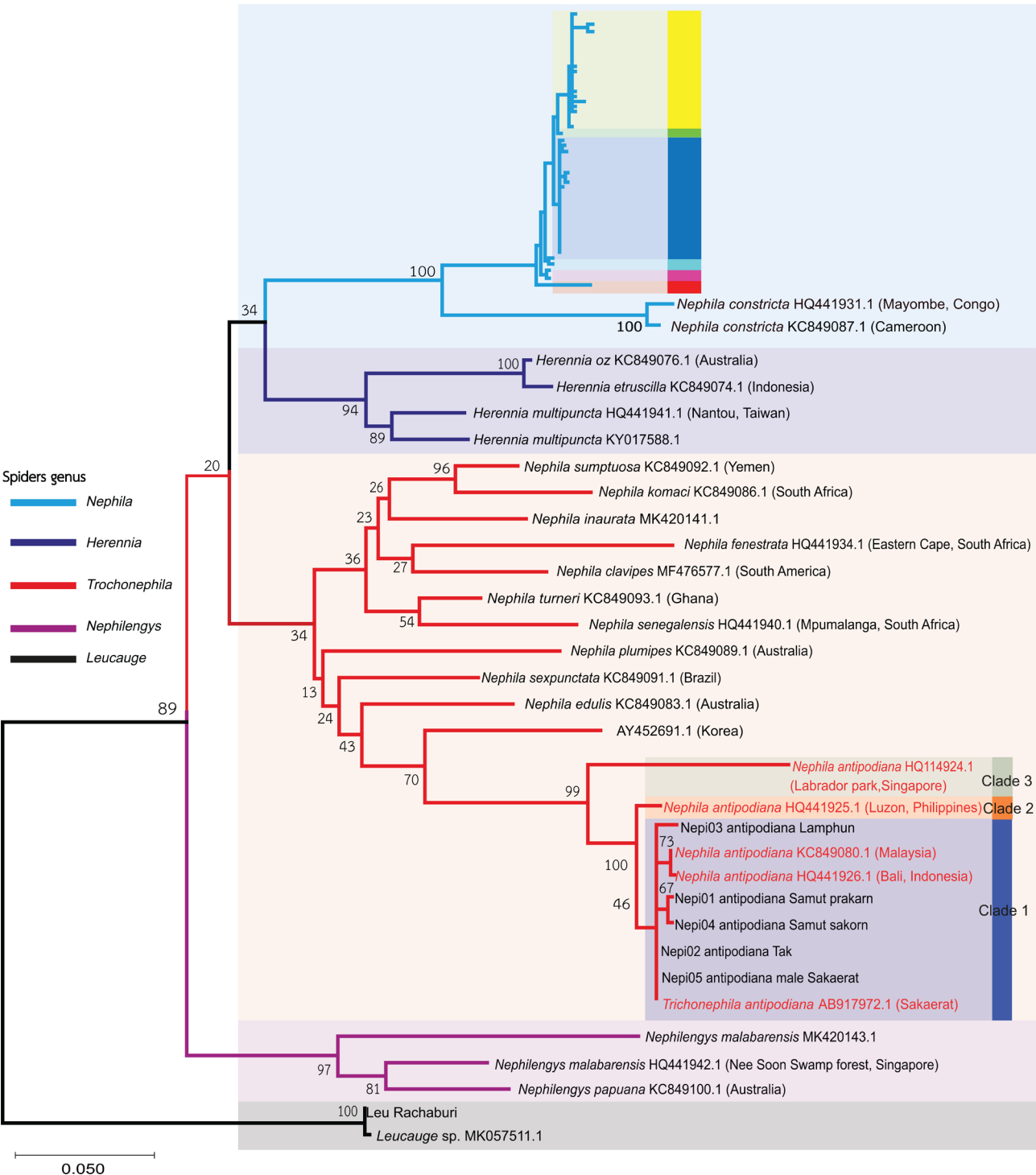


Fig. 10. Maximum-likelihood tree of Nephilid spiders. Colors of the branches represent specific taxa groups. Outgroup species were *Leucauge* sp.

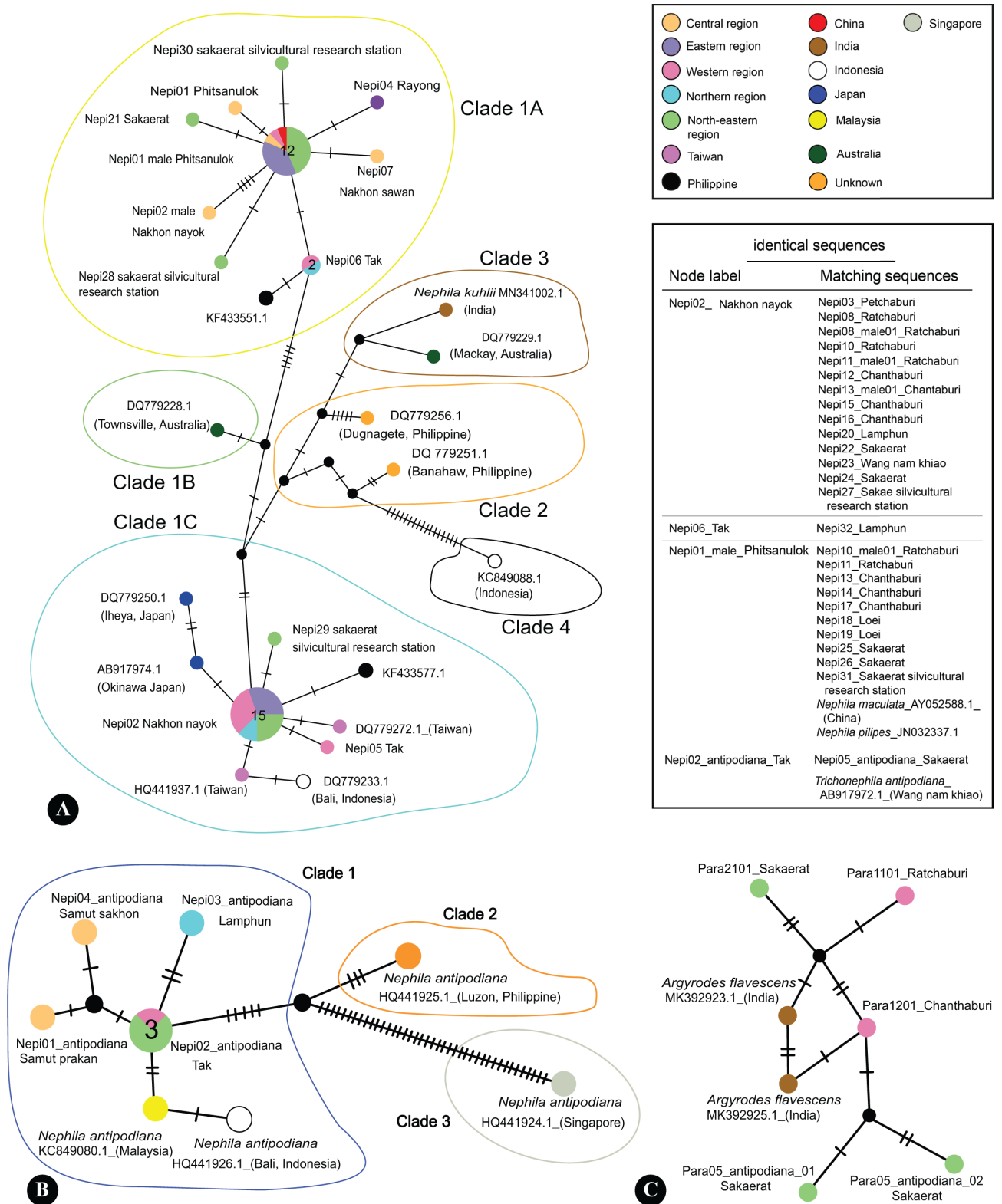


Fig. 11. Haplotype network of golden orb-weaver spiders and their kleptoparasite. (A) *N. pilipes*. (B) *T. antipodiana* (C) *A. flavescens*. The top right box shows the geographical origins of the specimens, represented by colors. The middle right box shows specimens that are not shown at specific nodes because they have identical sequences to the node label specimen. Black circles are hypothetical haplotypes to display connections between haplotypes in this study.

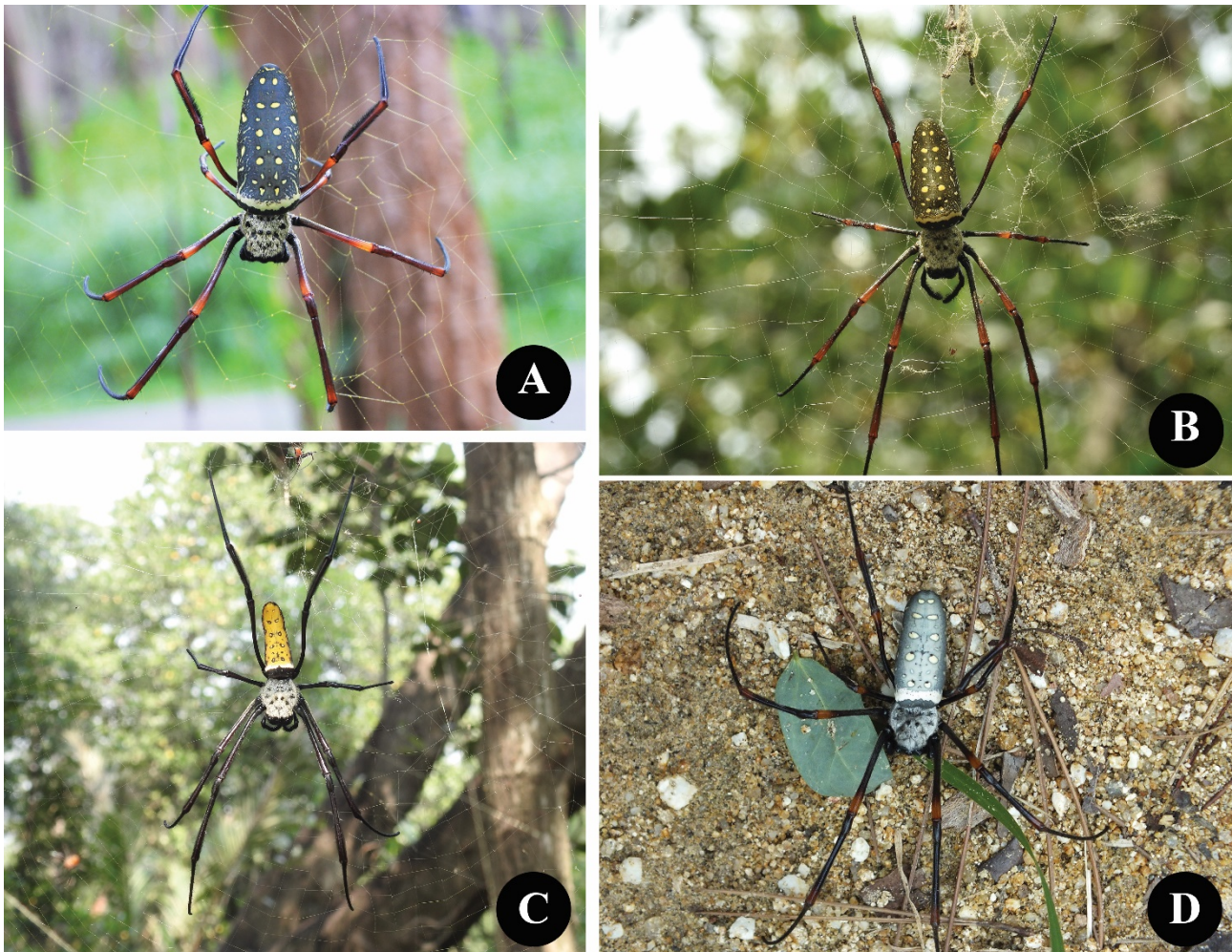


Fig. 12. Polymorphism of *T. antipodiana* somatic coloration. (A–B) Yellowish-green is the most common morph. (C) Bright yellow abdomen (not used in this study). (D) White-grey abdomen (Nepi03a) from Lamphun, pine forest. Noted that all four types possessed the same color on the ventral abdomen.

Haplotype network analysis

Haplotype network analysis from mitochondrial sequences revealed groupings into clades consistent with the phylogenetic tree findings. *N. pilipes* formed six mitochondrial groups (Fig. 11A): Clade 1 was divided into three subclades: 1A with the Central, Eastern, Northern, and North-eastern regions of Thailand, plus a China sample (AY052588.1); 1B was restricted to DQ779228.1 from Australia; 1C encompassed the Central, Western, Eastern, Northern, North-eastern regions of Thailand, and sequences from Japan, Taiwan, and Indonesia. Clade 2 contained *N. kuhlii* MN341002.1 from India and DQ779229.1 from Australia. Clade 3 comprised two sequences from the Philippines. Clade 4 contained only KC849088.1 from Indonesia, distinct from Clade 5 by 17 mutational steps. Importantly, there was no correlation between clades and geographical distribution or morphological types. Identical sequences formed haplotype clade nodes (Fig. 11 top right).

T. antipodiana (Fig. 11B), specimens from Thailand, Malaysia (KC849080.1), and Indonesia (HQ441926.1) formed Clade 1, including Nepi03a having a white-grey morph. Clade 2 consisted solely of a sequence from the Philippines (HQ441925.1). Clade 3 contained a sequence from Singapore, significantly diverging from the main clade with 45 mutation events and identities of 92.55–92.87% compared to Clade 1.

The haplotype network of the kleptoparasite *Argyrodes flavescens* (Fig. 11C) was analyzed to determine any geographical or host species correlations. Due to limited specimen data, with differences of only one or two mutation steps and no apparent geographical or host species correlation, the network remained undivided into groups.

DISCUSSION

Polymorphism in *N. pilipes*

N. pilipes demonstrates significant polymorphism, with the least variation in the cephalothorax, and the most at the ventral abdominal spots and in the leg patterns. Additionally, at the gross level, we observed differences in body size which is most simply explained by the individual's stage of development, *i.e.*, juvenile, subadult or adult. Spider growth stage is presumably influenced by food availability and other environmental factors (Wolfgang, 1987), thus the body sizes of our samples likely reflect a combination of these factors and age. At more detailed levels, we found at least variation in the cephalothorax, and the most at the ventral abdominal spots and in the leg patterns.

During this study, we noticed that some morphological features were harder to characterized, less reliable, or both. For example, the color of the setae on the cephalothorax of *Nephila* often changed upon preservation in alcohol. The most common color was white-grey which changed to yellow in ethanol. However, some specimens changed to green, orange, brown or black hues. Interestingly, in *T. antipodiana* cephalothorax color remained stable upon preservation, with the reason for inter-genus difference unknown (data not shown). Another inconsistent character was the triangular spots found on cephalothorax. They varied in size, with no specific identifiable pattern. One possibility for the inconsistency of the pattern could be due to sample manipulation because vigorous scratching during handling could reduce the carapace setae color (Fig. 13). However, variations in triangular spots shape were already observed in *N. pilipes* prior to collection (Fig. 13C and E), indicating a potential genetic basis, phenotypic plasticity, or both. Thus, these

features were not used for *N. pilipes*. differentiation. Future research with diverse populations is needed to further validate these findings.

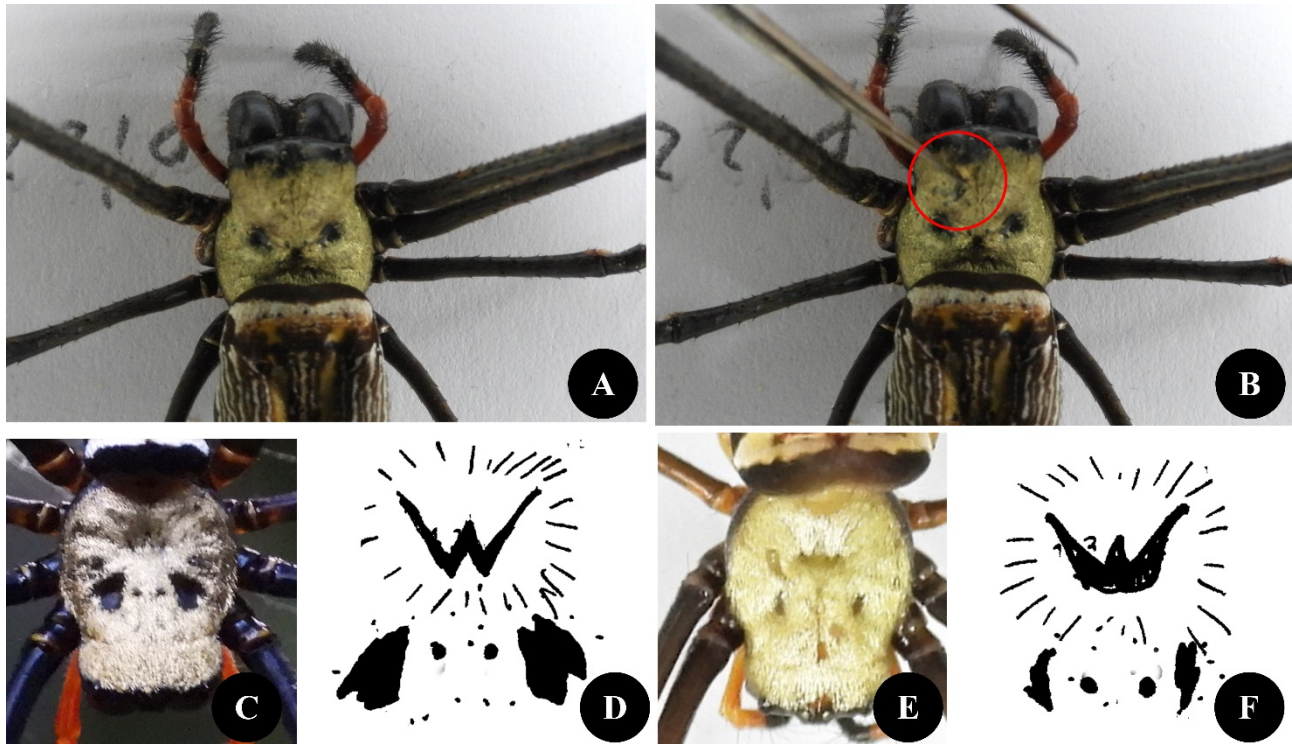


Fig. 13. Variation of triangular shapes on cephalothorax of *N. pilipes*. (A–B) A white-grey setae covered on carapace had faded out by strong scratching with forceps (indicated by red circle). (C–F) An example of different triangular shapes on cephalothorax; (C–D) Nepi12 as a dark-wide spot, and (E–F) Nepi13 as a thin and small spot.

In contrast to the preceding inconsistent characters, we found that legs I and IV have the most distinct variations, making them crucial for our specimen classification. Although both pairs were very similar, some specimens exhibited different features between legs I and IV, as noted in Type 6 and Type 7 (Nepi13 and Nepi19, respectively). As a result, specimens were categorized into seven types. If these leg pairs had been identical, the classification would have reduced to five. We did not consider Legs II and III for classification due to their low variation, and the absence of yellow color on mid-tibia in Type 3.

The carapace size also provided insights into developmental stages. Type 1 with a carapace length and width 7.5–10 mm indicates the adult or late subadult stage. Whereas those in Types 2, 3, and 5 with smaller carapaces (4.3–8.0 mm width, 5–8.6 mm length), likely represent juvenile and subadult forms. It is possible that characteristics like the tufts of setae and black-yellow coloration on the mid-tibia, that are present in juveniles, might disappear in adulthood. Naturally, a spider in the juvenile stage undergoes character changes upon reaching adulthood, particularly in the sex organs (Wolfgang, 1987), potentially causing misclassification (Gaikwad et al. 2017). Our genetic and phylogenetic studies did not support the idea that these morphological differences (tufts of setae

and tibia coloration) correspond to subspecies divisions. However, definitive conclusions require a more comprehensive understanding of the life cycle and body development of *N. pilipes*.

Our hypothesis regarding the color polymorphism in spiders suggests that environmental factors play a significant role. Previous research by Tso et al. (2002, 2004) showed that the typical morph of *N. pilipes* was more effective at capturing insects than the melanic morph. This is likely because the yellow leg joint spots enhance visual contrast, camouflaging the spider from distant prey (Rao et al. 2015). In our study, Types 3 and 7 exhibited yellow coloration on the mid-tibia. While not the dominant phenotype, this coloration might offer short-term foraging advantages, particularly at night (Chuang et al. 2007). The research on *Verrucosa arenata* (Walckenaer, 1841) by Rao et al. (2015) found that the yellow morph might attract more insects but could also increase predator visibility. Although high-signaling coloration can attract prey, it also attracts predators like hymenopterans (Fan, Yang, and Tso, 2009). Therefore, Types 3 and 7 could offer advantages to the preadult spiders by increasing the prey attraction rate and thereby allowing them to mature faster (Rao et al. 2015). Then upon reaching adulthood, the conspicuous yellow markings disappear, yielding the typical Type 1 morph.

N. kuhli* and melanic morph of *N. pilipes

According to Tso et al. (2002 2004), Harvey et al. (2007), and Sankaran et al. (2020), *N. pilipes* exhibits a dark-colored form termed the melanic morph. Our Type 4 (Nepi14, Nepi29, and Nepi30) and Type 5 (Nepi15) may represent this melanic variant. Except for the somatic coloration on the abdomen, legs, and prosoma, the melanic morph would be identical to the typical morph in all other aspects (Sankaran et al., 2020). This observation leads to the inference that Type 4 and Type 5 represent the black morphs of Type 1 and Type 2 respectively. Furthermore, the maximum likelihood phylogenetic tree and haplotype network analysis clustered *N. kuhli* MN341002 into *N. pilipes* (clade 3) with high identity levels (97.31–98.58%). Therefore, our data support the hypothesis that *N. kuhli* represents a form of polymorphism within *N. pilipes*, suggesting that their classification needs reevaluation.

Correlation between polymorphism, geographic regions, and genetic differentiation

The maximum likelihood analysis of our 37 specimens of *N. pilipes* and 13 GenBank sequences revealed a single main clade, further divided into two primary groups (Clade 1A and C). Importantly, we found no clear correlation between observed polymorphism and genetic clusters, as

nearly all specimen types were distributed across both groups. Similarly, no distinct subclusters emerged based on the geographical regions of our Thai specimens.

While our Thai samples showed no genetic-geographic correlation, we did observe some geographic variation among specimens from other countries. Most of these clustered outside our main clades, forming distinct groups: Clade 1B (Australia), Clade 2 (Philippines), Clade 3 (Australia and India), and Clade 4 (Indonesia). However, a few samples from China, Bali, Taiwan, and Japan were found within Clade 1A and C, exhibiting slightly more genetic distance from the Thai specimens

This finding aligns with Su et al. (2007), who reported that *N. pilipes* from Okinawa, Japan to Bali, Indonesia share a common haplotype with low molecular divergence, showing no significant association between genetic variation and geographic distance. Their phylogeographic analysis also supports our result that *N. pilipes* from Southeast Asia and Southern China form a homogeneous population with low molecular divergence distinct from international clades (Clade 1B, 2, 3, and 4).

Su et al. (2007) proposed that the Quaternary interglacial periods (2.6 million years ago), marked by northward expansion of tropical rainforests, facilitating the widespread distribution of ancestral *N. pilipes* across Australia, Asia, and India. This is consistent with our observation of Australian and Indian specimens clustering within the same clade (Fig. 9, Clade 3). Conversely, glacial periods (typically occurring every 100,000 years) led to the reduction of tropical rainforests, replacement by pine forests or savannas, and rising sea levels, creating geographic barriers, likely resulted in the distinct genetic groupings seen in Fig. 9 clades 1B, 2, and 3. Nonetheless, specimens from Bali, Taiwan, and Japan within Clade 1C suggest an inconsistency in the impact of these barriers. This discrepancy can be explained by aerial dispersal mechanisms like ballooning behavior, which allow spiders to transverse vast distances and mitigate the effects of geographic barriers on their distribution (Kraus 2002; Lee et al. 2004, 2015; Harvey et al. 2007; Su et al. 2007; Rao et al. 2015; Bartoletti et al. 2018).

*N. pilipes*_KC849088.1 from Indonesia (Clade 4) was identified as the most distantly related, as it differs from the Thai specimens through more than 20 mutational steps and exhibited the lowest percent identity with other specimens (95.64–96.54%). This specimen might represent a rare haplotype or possibly a subspecies. However, due to the lack of morphological information on this specimen and the absence of reported subspecies for *N. pilipes*, this remains speculative. Furthermore, two specimens from Australia clustered into different clades, indicating significant genetic variation within the Australian population. This aligns with the morphological polymorphism observed in the Eastern Australian population, which is characterized by a gray abdomen lacking yellow markings, and clearly visible plural sigillae, contrasting with the typical

morphology of populations from Northern, North-western, and Western Australia. The distinct characteristics of the Eastern Australian type may represent a unique form of *N. pilipes*, but the association of the two specimens in the tree with these polymorphisms remains unverified due to the lack of morphological data linked to these sequences. Besides aquatic barriers, high altitudes represent another geographic barrier, as observed in related species like *Trichonephila clavipes* in the Western Andean Cordillera, where populations are differentiated from other South American groups based on the results of a haplotype network analysis using *COI* (Bartoletti et al. 2018). Similarly, the Himalayan mountains partially isolate *N. pilipes* in India from the main Asian continent (Su et al. 2007). However, in Thailand, the absence of such high-altitude regions supports our finding of no significant geographic association. Furthermore, not only *N. pilipes* but also *Araneus diadematus* (Clerck 1757) showed no correlation between geographic and genetic distances, despite the presence of various ecophenotypes, such as color variants at different locations. This is consistent with the absence of notable differences in their genital structures (Kraus 2002).

Among *T. antipodiana* specimens, Nepi01a, Nepi02a, Nepi04a, and Nepi05a exhibited a yellowish-green morph (Fig. 12A and 12B), whereas Nepi03a was unique in being white-grey (Fig. 12D). Despite these color differences, they clustered together genetically, though Nepi03a showed slightly more genetic variation (Fig. 10). The link between this minor difference and the white-gray morph is unclear, further studies with more samples are necessary. Notably, the abdomen color of all the specimens changed to yellow after 24 hours of preservation, highlighting the importance of recording morphs pre-preservation. Our Thai specimens grouped genetically with samples from Malaysian (KC849080.1) and Indonesian (HQ441926.1). In contrast, specimens from Luzon, Philippines, and Labrador Park, Singapore, exhibited extremely high genetic variation (identities 98.74% and 92.74% respectively), and were separated by numerous mutational steps. The significant genetic divergence, especially in the Singapore haplotype, is puzzling given the lack of obvious geographical barriers. We hypothesize these highly divergent populations from the Philippines and Singapore may represent undescribed subspecies of *T. antipodiana*, necessitating further research.

Possible subspecies of *N. pilipes* in Thailand

While no *N. pilipes* subspecies have been officially reported in Thailand, the existence of *N. pilipes annulipes* Thorell, 1881 (West Papua), and *N. pilipes jalorensis* Simon, 1901 (Malaysia, now a synonym) (Harvey et al. 2007), and the phenotypic polymorphism in our specimens led us to hypothesize that some might belong to these subspecies.

However, our results indicated minimal genetic variation among Thai specimens (97.80–100% identity) (Table S2 and S3). This low genetic variation, coupled with the absence of significant geographic barriers within Thailand, can be attributed to the aerial dispersal via ballooning (Lee et al. 2004). This behavior, common in Araneomorphae spiders, e.g., *Tetragnatha* colonizing Hawaii from North America (Turk et al. 2020), facilitates extensive gene flow over thousands of kilometers (Su et al. 2007; Bartoletti et al. 2018). The inability to distinguish *Araneus diadematus* from geographically distant populations (Kraus 2002) further supports that long-distance airborne travel makes *N. pilipes* subspecies unlikely to emerge. Despite this, the significant genetic differences observed in some Indonesian specimens (KC849088.1) compared to others suggest that the future identification of *N. pilipes* subspecies remains a possibility.

COI DNA barcode for studying golden orb-weaving spider variation

The golden orb-weaving spider exhibits extreme sexual dimorphism, with males being hundreds of times smaller and 500 times lighter than females. Males also generally lack distinct variation. Consequently, morphological studies have primarily focused on females. However, the *COI* gene can effectively identify both inter- and intraspecific variation, including subspecies (Voronova 2014), making it valuable for discerning differences even in males if sufficient associated female DNA data are available.

In this study, *COI* sequencing clarified the phylogeography of Nephilinae species, revealing species distribution history linked to geographic changes. For instance, Nephilid spiders from China showed similarities to those from other Asian countries, reflecting the interconnectedness of continental populations from India to the Sunda shelf (Su et al. 2007). In contrast, sequences from isolated regions like Australia, Indonesia, the Philippines, and India differed from the main Asian continent. Without morphological data, it remains unclear if these isolated specimens represent distinct subspecies. Future research should prioritize collecting more samples from broader geographic areas to aid subspecies identification and integrate historical climate change data for a more comprehensive understanding of *Nephila* phylogeography.

CONCLUSIONS

This study is the first to describe morphological variation of leg types and to assess genetic variability in *Nephila* in Thailand. The results revealed that polymorphism in *Nephila* leg types does not correlate with genetic-geographic variation. This information would be valuable for taxonomists

and ecologists in species identification and differentiation of ecotypes. Studies on genetic variation such as this one are the first step to facilitate future phylogenetic and genotype-to-phenotype research on arachnids.

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Competing interests: All authors declare that they have no conflict of interest.

Availability of data and materials: DNA sequences generated in the study have been deposited in the NCBI database.

Consent for publication: Not applicable.

Ethics approval consent to participate: The experimental protocols were approved by the Animal Experiment Committee of the Kasetsart University (Approval No. ACKU68-SCI-021). Spiders were anesthetized on ice before preserving in 95% ethanol and transported to the laboratory for DNA extraction and morphological analysis.

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Supplementary materials

Table S1. GenBank accession numbers of spider *COI* gene sequences used in this study.

(download)

Table S2. Overall percent identities of all our specimen sequences compared to GenBank sequences. (download)

Table S3. Best BLAST-N result of partial *COI* mtDNA sequence of each specimen. (download)

Table S4. Genetic divergence (*p*-distance) within *N. pilipes* and *T. antipodiana*. (download)