

Integrative taxonomy of araneomorph spiders: Breathing new life into an old science

Rivera Quiroz, F.A.

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Chapter 4

First records and a new genus of Comb-tailed spiders (Araneae: Hahniidae) from Thailand with comments on the six-eyed species of this family

F. ANDRES RIVERA-QUIROZ*1, BOOPPA PETCHARAD³ and JEREMY A. MILLER¹

¹ Understanding Evolution group, Naturalis Biodiversity Center, Darwinweg 2, 2333CR Leiden, The Netherlands

² Institute for Biology Leiden (IBL), Leiden University, Sylviusweg 72, 2333BE Leiden, The Netherlands.

³ Faculty of Science and Technology, Thammasat University, Rangsit, Pathum Thani, 12121 Thailand.

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Abstract

The family Hahniidae is reported from Thailand for the first time. The genus *Hexamatia* gen. nov. and two new species, *Hexamatia seekhaow* sp. nov. and *Hahnia ngai* sp. nov. are described and illustrated. DNA sequences are provided for all the species reported here. The phylogenetic position of the novel genus *Hexamatia* and its relation to *Hahnia* are discussed. Based on these results a new combination is proposed for *Hexamatia senaria* (Zhang, Li & Zheng 2011) = *Hahnia senaria*. Known distributions for the species *Hahnia saccata* Zhang, Li & Zheng 2011, originally described from China is expanded. A brief review and notes on the taxonomy on the six-eyed hahniids are included.

Keywords: Thai, Chiang Mai, new species, Hahniids, phylogeny

Introduction

The family Hahniidae Bertkau 1878 is relatively easy to identify due to the advanced location of the tracheal spiracle in relation to the spinnerets and the characteristic arrangement of these in more or less one transverse row (at least, in the Hahniinae) [1,2]. Other members of this family (e.g., Cicurina, Cybaeolinae) do not share this transverse disposition of the spinnerets [3,4]. The Hahniidae currently includes 351 species in 23 genera distributed worldwide [5]. The family status of Hahniidae has been confirmed by molecular phylogenies being placed within the RTA clade, closely related to Cybaeide and Dyctinidae [6,7]. However, the relations and delimitations of its genera have always been problematic. Only a few local revisions have been done, two for Nearctic species [2,8] and one for New Zealand species [9]. Beside these revisions, Lehtinen (1970) published some comparative tables including diagnostic characters of 17 extant genera (10 currently valid, [5]) and one more from Baltic amber. Presently two genera, Cicurina Menge, 1871 and Hahnia C. L. Koch, 1841, have served as "wastebin taxa" for new species descriptions, having a great morphological heterogeneity and accounting together for almost 70% of all the valid hahniid species [5]. The great heterogeneity and unclear delimitations in these and other hahniid genera are a recurrent note in new species publications [9–11].

The Hahniidae have a worldwide distribution, being more diverse in the Americas and Asia but also having a fair number of species described in Europe, Africa and Oceania [5]. In Asia, eight genera and 93 species have been recorded distributing from the Middle East to Eastern Russia and Japan. In South and South East Asia, hahniids have been reported from Hong Kong, Indonesia, Laos, Philippines, Southern China, Sri Lanka, Taiwan, and Vietnam [1,10–17]. This is the first time the Hahniidae are reported in Thailand. Here we describe a new genus and two new species for this family based in molecular and morphological data. We also report this family in Thailand for the first time. Additionally, we include a brief literature review on the rare six-eyed hahniids.

Material and Methods

The hahniid species reported here were collected in the Chiang Mai Province, Thailand, between July 16th and 28th 2018. All the specimens were captured using methods optimized for ground dwelling spiders: leaf litter sifting, Winkler extractors, pitfall traps and direct collecting on ground, among leaf litter and under rocks or logs.

Specimen habitus and other somatic characters were photographed under a Leica MI6SC Stereomicroscope equipped with a Nikon DS-Ri2 camera. Genitals were photographed using a Leica DM 2500 microscope attached to the same camera. Specimens were observed in ethanol using semi permanent slide preparations [18]. Female genitalia were dissected, digested using pancreatine solution [19], and cleared with methyl salicylate.

Table 1– GenBank accession numbers DNA sequences used for our analyses. * marks the new sequences generated for the present work.

Family	Species	COI	Н3	12s	16s	18s	28s
Agelenidae	Agelena labyrinthica	FN554797	KR074077			AY633862	AY633851
Cybaeidae	Calymmaria sp. 1	DQ628611	DQ628638			DQ628702	DQ628666
	Cryphoeca exlineae	KM840792.1	MN590107.1			MN590054.1	MN590084.1
	Cybaeus morosus	FJ263792	DQ628641			DQ628707	DQ628671
Hahniidae	Antistea brunnea	HQ580602.1	MN590134.1			MN590079.1	MN590103.1
	Cybaeolus cf. rastellus	KY017745	KY018252			KY016481	KY017117
	Cybaeolus pusillus		KY018253.1			KY016482.1	KY017118.1
	Hahnia cinerea	GU683831.1	MN590136.1			MN590081.1	MN590105.1
	Hahnia clathrata	FJ949005	FJ949043			FJ948923	
	Hahnia nava	KY270115	KY018254.1			KY016483.1	
	Hahnia ngai*	MT433973	MT445988		MT434973	MT437224	MT434975
	Hahnia ononidum	MG047916.1	MN590137.1			MN590082.1	MN590106.1
	Hahnia saccata*	MT433972		MT434903		MT437222	
	Hahnia sp. ZZ- 2016(China)	KR074066	KR074092			KR074014	
	Hahnia zhejiangensis	KR074067.1	KR074093.1			KR073991.1	KR074041.1
	Hexamatia seekhaow*	MT433971	MT445987	MT434902	MT434972	MT437221	MT434974
	Neoantistea agilis	HQ580773.1	DQ628644.1			DQ628714.1	DQ628678.1
	Neoantistea quelpartensis	JN817206.1				JN816788.1	JN816996.1

Four legs were taken from one individual of each species for DNA extraction. Six gene fragments (COI, H3, 12S, 16S, 18S and 28S) were amplified following Miller, Griswold, and Haddad [6] and Wheeler et al. [7] protocols; list of primers provided in the Supplementary Materials (SM1). Sequences were edited in Geneious Prime 2020.0.5. New sequences generated for this study were deposited in GenBank; accession numbers are reported in Table 1. All the specimens used here have been deposited in the collection of the Naturalis Biodiversity Center, Leiden, the Netherlands (RMNH. ARA.18411–RMNH.ARA.18415).

We used sequences from the three species we collected, as well as 15 other species with available sequences in Genebank. We used in total 14 species of Hahniidae, three species of Cybaeidae, and one of Agelenidae, Agelena labyrinthica Walckenaer, 1805, as an outgroup. The sequences used to test the relationships and position of the novel species within the Hahniidae are listed in Table 1. We used MAFFT v.7.450 online (https://mafft.cbrc.jp/alignment/server/) with default parameters to build the alignments. Alignments for 18S were further trimmed manually due to the size difference of some sequences. 16S and 12S were not used due to the low availability of these loci for the Hahniidae in Genbank; Table 1 only reports accession numbers of these markers for our sequences. Hahnia pusilla C. L. Koch, 1841, type species of Hahniidae, as well as two more species of Hahnia and two of Iberina had only COI sequences available in Genebank, therefore, they were not used in our final dataset. Matrix was built using COI, H3, 18S and 28S alignments in Sequence Matrix v.1.8 (http://www.ggvaidya.com/ taxondna/); matrix available in SM1. Each loci was treated as a partition and examined with jModelTest2 [20] in CIPRES [21] to get the best model fit for each; GTR+I+G was selected in all the cases. Our datasets were analyzed using MEGA X [22] for Maximum Parsimony (SPR, default values, bootstrap= 1000); RaXML [23] in CIPRES for Maximum Likelihood (GTR, bootstrap= 1000) and . MrBayes v. 3.2.6 [24] for windows for the Bayesian inference (GTR+I+G, two independent runs with one cold and three heated chains, mcmc=1,000,000 gen, samplefreq=1000, burnin=2500). The program Tracer v. 1.7.1 [25] was used to analyze the performance of our BI analyses, and Mega X to estimate the genetic distances (JC model, gamma dist., gamma parameter= 1.00; gaps data treatment= pairwise deletion) for our whole dataset.

Abbreviations in text and figures: A – Epigynal atrium; ALS – Anterior lateral spinnerets; AME – Anterior median eyes; BI – Bayesian inference; Cd – Copulatory duct; CF – Cymbial furrow; Ch – Chelicera; Co – Copulatory opening; Cy – Cymbium; E – Embolus; F – Femur; Fd – Fertilization duct; LE – lateral eyes; MA – Median apophysis; ML – Maximum Likelihood; MP – Maximim parsimony; P – Patella; PA – Patellar retrolateral apophysis; PLS – Posterior lateral spinnerets; PME – Posterior median eyes; PMS – Posterior median spinnerets; RTA – retrolateral tibial apophysis; S – Spermatheca; Sd – Spermatic duct; Ss – Secondary spermatheca; G– glands; T – Tibia Te – Tegulum.

Results

Topologies inferred by the three different phylogenetic analyses recovered nearly identical topologies (Fig. 4.1a–c). The genus *Hahnia* was homogeneously recovered as diphyletic. The clade *Hahnia* 1 was formed by six *Hahnia* species and *Hahnia* 2 by *H. ngai* and *H. saccata*, the two *Hahnia* species we captured in Thailand. *Hahnia* 1 showed high support, although the internal are not fully resolved, having moderate to weak support values in the ML and MP analyses. This clade was found as a sister

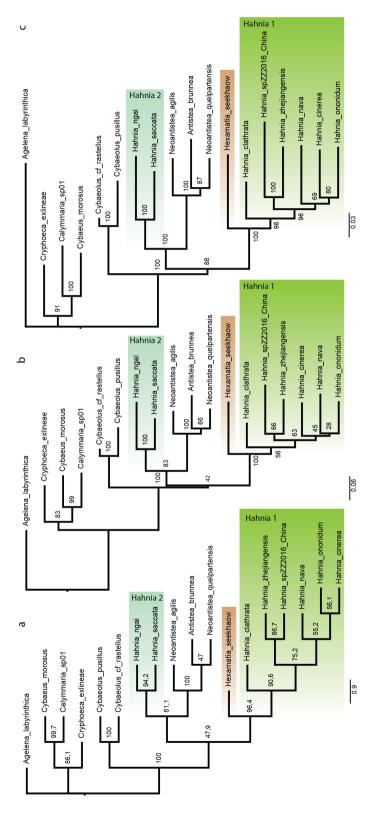


Figure 4.1.-a-c. Tree topologies obtained by different analyses. a- Maximum Parsimony most parsimonious tree, numbers at node indicate bootstrap support; b- Maximum Likelihood, numbers at node indicate bootstrap support; c-Bayesian Inference, numbers at node indicate posterior probabilities. The agelenid Agelena labyrinthica was used as an outgroup to the Cybaeodinae and Hahniidae. Fourteen species from the Hahniidae and three of Cybaeide were used. Note the diphily of Hahnia and the position of Hexamatia gen. nov. as a sister group to Hahania 1.

group to the new genus *Hexamatia* in all our trees. The clade *Hahnia* 2 appears to be more related to Antistea+Neoantistea. This branch is recovered and highly supported in all the analyses. The cluster formed by Antistea+Neoantistea is strongly supported although its internal relationships are not resolved and show weak to moderate support in the MP and ML. The three cybaeid representatives form a highly supported group that is consistently recovered as a sister to the monophyletic Hahniidae. Our BI, showed an average deviation of split frequencies under to 0.003 after 1,000,000 generations. None of the Estimated Sample size parameters fell under the commonly used threshold of 200 suggesting that our BI ran for an adequate length [26,27]. The trace plot and histograms of both runs are available in the SM1. Pairwise genetic distances for our alignment showed *Hexamatia* to have a wide range of distances with respect to Hania species. When compared to species in *Hahnia* 1, this range went from 9.5 to 25% while the distance vs. *Hahnia* 2 is found between 10.7 to 17.8%. In comparison, the distances between *Hexamatia* and Antistea+Neoantistea were higher and less variable, between 18.0 to 19.2%. See SM1 for complete distance matrix.

Taxonomy

Order Araneae Clerck, 1757 Family Hahniidae Bertkau, 1878 Genus *Hexamatia* Rivera, Petcharad & Miller gen.nov.

Type species: Hexamatia seekhaow Rivera, Petcharad & Miller sp. nov

Etymology: The genus name is formed from two Greek roots: Hexa (six) and mati (eye). Refers to the number of eyes present in this genus, one of its diagnostic characters. The gender is feminine.

Diagnosis: Distinguished from most hahniid genera by the combination of the following characters: presence of only six eyes, small body size close to 1 mm, and body pale yellow to white, lacking abdominal patterns in males, and having faint chevron lines in females ([15]: figs. 23A, B). It can be separated from other six-eyed hahniids by the following combination of characters: from *Amaloxenops* Schiapelli & Gerschman, 1958 by having a backward curved RTA without twists, and presence of PA on the pedipal patella and MA on the bulb; from *Intihuatana* Lehtinen, 1967 by having an unbifurcated RTA, a shorter and bifurcated PA, and presence of MA; and from *Scotospilus* Zhang, Li, and Pham 2013 by the comparatively short RTA, bifurcated PA and presence of MA.

Composition: *Hexamatia seekhaow* Rivera, Petcharad & Miller sp. nov., *Hexamatia senaria* (Zhang, Li, and Zheng 2011) comb. nov., based on the original description and illustrations.



Figure 4.2.–a–d. *Hexamatia seekhaow* **sp. nov**. Male: Habitus: a– ventral view; b– lateral view; c– dorsal view. Prosoma: d– anterior view. Palp: e–retrolateral view; f– ventral view. Scale bars: a, b= 0. 5 mm; d– f= 0.15 mm. CF – Cymbial furrow; Cy – Cymbium; E – Embolus; F – Femur; MA – Median apophysis; P – Patella; PA – Patellar retrolateral apophysis; RTA – Retrolateral tibial apophysis; T – Tibia.

Distribution: *Hexamatia seekhaow* sp. nov. is known from Chiang Mai, Thailand; and *Hexamatia senaria* (Zhang, Li, and Zheng 2011) from Yunnan, China (Fig. 4.8).

Hexamatia seekhaow Rivera, Petcharad & Miller sp. nov.

Fig. 4.2, 4.3

Material:

Holotype: THAILAND • 1 &; Chiang Mai, Doi Suthep National Park; 18°48.502'N, 98°53.528'E. 1409m; 24-28 July 2018; Booppa Petcharad, Jeremy Miller, F. Andres

Rivera-Quiroz Leg.; Montane evergreen forest with pine. Hand coll. among leaf litter; RMNH.ARA.18411 (four legs used for DNA extraction).

Etymology: The species epithet is a derivation of the Thai *seekhaow* (white); refers to the lack of color in the body of the holotype of this species.

Diagnosis: *Hexamatia seekhaow* sp. nov. greatly resembles *H. senaria* [15] but can be distinguished by the bifurcated PA and having a slightly shorter RTA with a blunter tip (Fig. 4.2f; Fig. 4.3d, e; [15]: figs. 21A-D; 22). Another putative difference is the presence of denticles in the distal portion of the RTA; these are not mentioned nor illustrated for *H. senaria*.

Description: Carapace yellowish-white, pale brown in the cephalic region (Fig. 4.2b, c). Legs same color as the carapace. Abdomen white without chevron pattern; oval, longer than wide (Fig. 4.2a–c). Six eyes in two triads, AME absent ALE 0.04, PME 0.02, PLE 0.02; ALE-ALE0.02, PME-PME 0.03, PME-PLE contiguous (Fig. 4.2d). Chelicerae with three promarginal and two retromarginal teeth (Fig. 4.3g). Tracheal spiracle near the middle of the abdomen (Fig. 4.2a).

Male palp: Pale brown, same color as the cephalic region (Fig. 4.2c). CF darker, almost as long as the RTA (Fig. 4.2e–f; 3b). Oval shape from ventral view (Fig. 4.2f; 4.3a). Median apophysis narrow, elongate and transparent(Fig. 4.3a, b). Embolus filiform, black and long, originating retrollaterally and coiling clockwise around the bulb (Fig. 4.2f; 4.3a, b). RTA spur-like with dark rings. Patellar apophysis short and bifid, with the longer prong hook-shaped (Fig. 4.3c).

Male: Total length 1.1, carapace 0.46 long, 0.33 wide; clypeus 0.01; Chelicera 0.2 long, 0.1 wide; Pedipalp 0.4 long; Palp bulb 0.11 wide; Leg I: femur 0.32, patella 0.13, tibia 0.26, metatarsus 0.22, tarsus 0.15; Leg II: femur 0.31, patella 0.12, tibia 0.19, metatarsus 0.19, tarsus 0.15; Leg III: femur 0.27, patella 0.08, tibia 0.16, metatarsus 0.17, tarsus 0.15; Leg IV: femur 0.34, patella 0.11, tibia 0.22, metatarsus 0.21, tarsus 0.16; leg formula IV-I-II-III; abdomen 0.45 long, 0.34 wide.

Distribution: Known from the type locality, Doi Suthep National Park, Chiang Mai, Thailand (Fig. 4.8).

Notes: See the discussion section for remarks on six-eyed species.

Genus Hahnia C. L. Koch, 1841

Hahnia (C. L. Koch, 1841): 61. Type species Hahnia pusilla C. L. Koch, 1841. Hahnia ngai Rivera, Petcharad & Miller sp. nov.

Fig. 4.4; 4.6a-c

Material:

Holotype: THAILAND • 1♀; Chiang Mai, Doi Suthep National Park; 18°48.502'N, 98°53.528'E. 1409m; 24-28 July 2018; Booppa Petcharad, Jeremy Miller, F. Andres Rivera-Quiroz Leg.; Montane evergreen forest with pine. Winkler extractor; RMNH. ARA.18415 (four legs used for DNA extraction).

Paratypes: THAILAND • 1♀; Chiang Mai, Doi Inthanon National Park; 18°35.268'N,

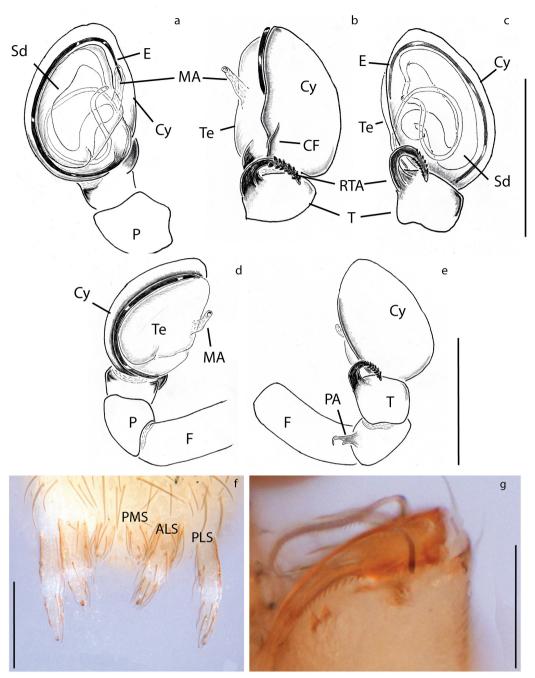


Figure 4.3.–a–d. *Hexamatia seekhaow* **sp. nov.** Male palp: a– ventral view, cleared; b– retrolateral view; c– dorso–retrolateral view, cleared; d– prolateral view; e– dorso–retrolateral view. Male spinnerets: f– ventral view. Scale bars: a–f = 0.15 mm; g = 05mm. ALS – Anterior lateral spinnerets; CF – Cymbial furrow; Cy – Cymbium; E – Embolus; F – Femur; MA – Median apophysis; P – Patella; PA – Patellar retrolateral apophysis; PLS – Posterior lateral spinnerets; PMS – Posterior median spinnerets; RTA – Retrolateral tibial apophysis; Sd – Spermatic duct; T – Tibia Te – Tegulum.

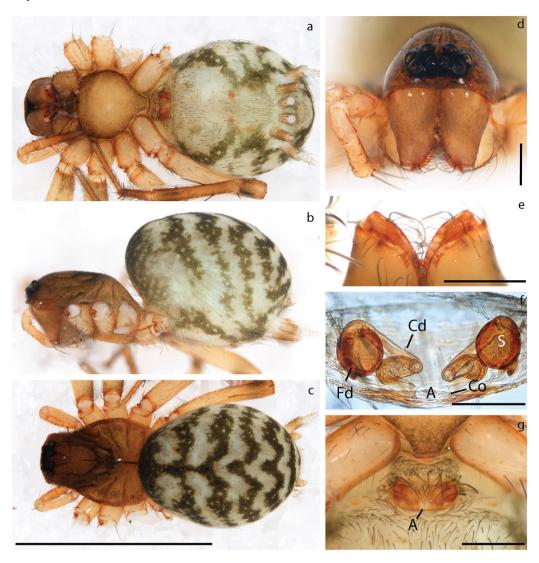


Figure 4.4.–a–c. *Hahnia ngai* **sp. nov.** Female: Habitus: a– ventral view; b– lateral view; c– dorsal view. Prosoma: d– anterior view. Chelicerae: e–posterior view. Epigynum: f– dorsal view, cleared; g– ventral view. Scale bars: a–c= 1.0 mm; d, e, g= 0.25 mm; f= 0.1 mm. A – Epigynal atrium; Cd – Copulatory duct; Co – Copulatory opening; Fd – Fertilization duct; S – Spermatheca.

98°29.240'E. 2572m; same date and collectors as holotype; Cloud forest. Winkler extractor; RMNH.ARA.18414 • 1♀same data; Hand coll.; RMNH.ARA.18413.

Etymology: The species epithet is a derivation of the Thai *ngai* (simple), in reference to the relatively simple vulva without the well-formed secondary spermathecae commonly seen in other *Hahnia* species.

Diagnosis: Hahnia ngai sp. nov. can be easily separated from other members of this

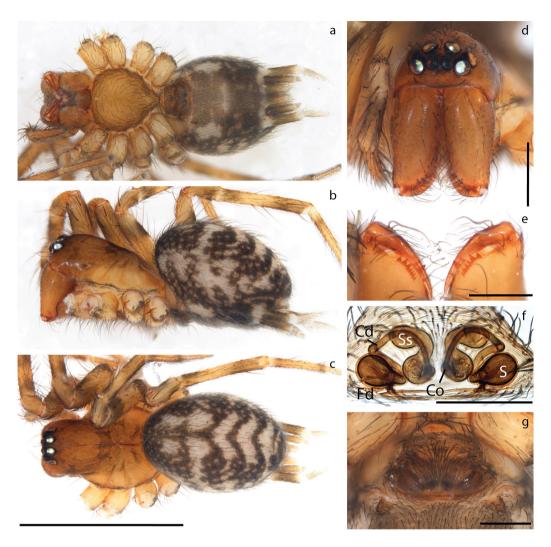


Figure 4.5.–a–d. *Hahnia saccata* **Zhang, Li & Zheng, 2011**. Female: Habitus: a– ventral view; b– lateral view; c– dorsal view. Prosoma: d– anterior view. Chelicerae: e–posterior view. Epigynum: f– dorsal view, cleared; g– ventral view. Scale bars: a–c= 1.0 mm; d= 0.50 mm; e–g = 0.25 mm. A – Epigynal atrium; Cd – Copulatory duct; Co – Copulatory opening; Fd – Fertilization duct; S – Spermatheca; Ss – Secondary spermatheca.

genus by the simplified female genitalia. Copulatory ducts show only slightly swollen areas with glandular insertions (Fig. 4.4g; 4.6b, c) but do not form a receptacle or secondary spermathecae (as seen in Fig. 4.5g; 4.6e, f).

Description: Carapace pear-shaped, reddish-brown, slightly darker in the cephalic region; smooth texture (Fig. 4.4c). AME 0.04ALE 0.06, PME 0.07, PLE 0.04; AME-AME 0.03, AME-ALE 0.02, PME-PME 0.05, PME-PLE 0.03 (Fig. 4.4d). Chelicerae with three promarginal and three retromarginal teeth (Fig. 4.4e). Legs pale brown, slightly

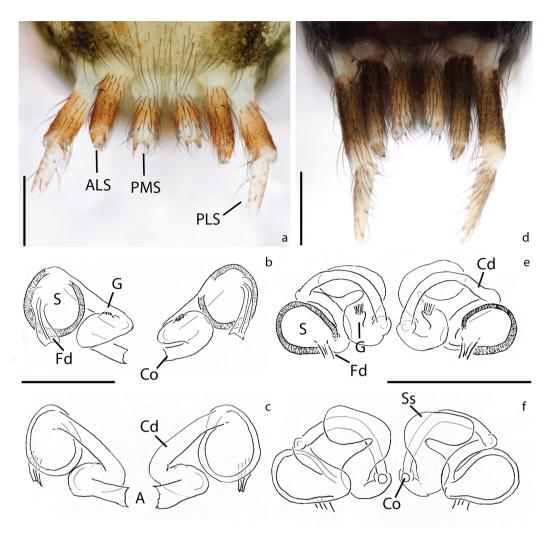


Figure 4.6.–a–f. Female spinnerets and genitals: *Hahnia ngai* sp. nov. Spinnerets. a– ventral view. Epigynum, cleared. b– dorsal view; c –ventral view. *Hahnia saccata* Zhang, Li & Zheng, 2011. Spinnerets. d– ventral view. Epigynum, cleared. e– dorsal view; f –ventral view. Scale bars: a, d– f=0.25 mm; b, c= 0.1 mm. A – Epigynal atrium; ALS – Anterior lateral spinnerets; Cd – Copulatory duct; Co – Copulatory opening; Fd – Fertilization duct; PLS – Posterior lateral spinnerets; PMS – Posterior median spinnerets; S – Spermatheca; Ss – Secondary spermatheca; G– glands.

darker on the distal segments. Abdomen dark grey with light patches forming five to six chevron bands; oval, longer than wide (Fig. 4.4c). Tracheal spiracle near the middle of the abdomen (Fig. 4.4a).

Vulva: Epigynal plate semitransparent, spermathecae well visible by transparency. Copulatory openings close together, forming a small semi-circular atrium (Fig. 4.4f, g; 4.6c). Spermatheca sub-speherical with brownish red coloration (Fig. 4.4f). Copulatory ducts very simple, slightly swollen centrally (Figs. 4.4f; 4.6b, c).

Female: Total length 2.8, carapace 1.25 long, 0.91 wide; clypeus 0.09; Chelicera 0.45

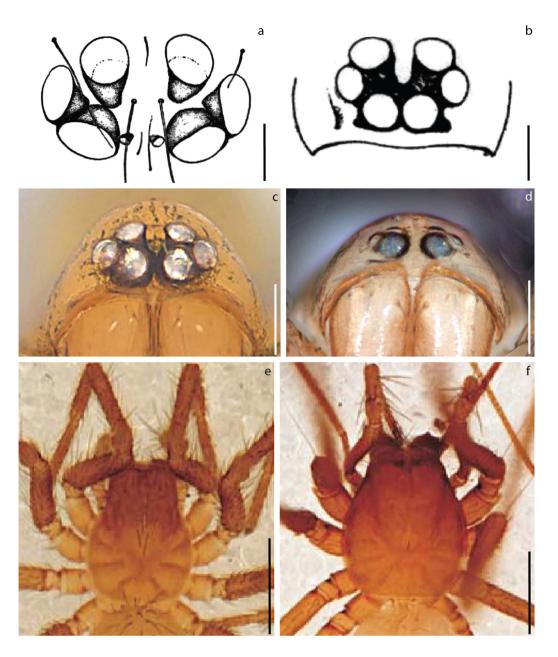


Figure 4.7.–Examples of eye reduction in the Hahniidae. Eight eyes with minute AME: a– *Alistra myops*; modified from Schiapelli and Gerschman de P. 1959. Six eyes: b– *Amaloxenops vianai*; modified from Schiapelli and Gerschman de P. 1958; c– *Scotospilus longus*; modified from Zhang, Li, and Pham 2013; d– *Hexamatia seekhaow* sp. nov. No eyes: e, f– *Iberina mazarredoi*; modified from Fernández–Pérez, Castro, and Prieto 2014. Scale bars: a–d= 0.1 mm; e–f = 0.5 mm.

long, 0.25 wide; Leg I: femur 0.95, patella 0.37, tibia 0.71, metatarsus 0.72, tarsus 0.55; Leg II: femur 0.94, patella 0.34, tibia 0.72, metatarsus 0.68, tarsus 0.55; Leg III: femur



Figure 4.8.–Map of mainland South East Asia. Showing the collecting sites of Zhang, Li & Zheng, 2011 (*Hahnia saccata* and *Hexamatia senaria*), circle; and our new hahniid specimens (*Hexamatia seekhaow* sp. nov. and *Hahnia ngai* sp. nov. and *Hahnia saccata*), square.

0.89, patella 0.33, tibia 0.63, metatarsus 0.71, tarsus 0.51; Leg IV: femur 1.12, patella 0.34, tibia 0.93, metatarsus 1.01, tarsus 0.62; leg formula IV-I-II-III; abdomen 1.65 long, 1.23 wide.

Distribution: Known from two localities in Chiang Mai, Thailand (Fig. 4.8): Doi Suthep National Park (type locality), and the neighboring Doi Inthanon National Park.

Hahnia saccata Zhang, Li & Zheng, 2011

Hahnia saccata Zhang, Li & Zheng, 2011: 16, figs. 14A-E, 15A-H, 16A-G. Fig. 4.5; 4.6d–f

Collected material: THAILAND • 2 ♀; Chiang Mai, Doi Suthep National Park; 18°48.780'N, 98°55.928'E. 643m; 25-28 July 2018; Booppa Petcharad, Jeremy Miller,

F. Andres Rivera-Quiroz Leg.; Dipterocarpus forest. Hand coll.; RMNH.ARA.18412 (four legs of one specimen used for DNA extraction).

Description: Carapace pear-shaped, reddish-brown, slightly darker in cephalic region; smooth texture (Fig. 4.5c). AME 0.06, ALE 0.11, PME 0.08, PLE 0.05; AME-AME 0.02, AME-ALE 0.01, PME-PME 0.06, PME-PLE 0.04 (Fig. 4.5d). Chelicerae with three promarginal and seven retromarginal teeth (Fig. 4.5e). Legs color similar to carapace, darker on the proximal and distal part of each segment. Abdomen dark grey with light patches forming five to six chevron bands; oval, longer than wide (Fig. 4.5c). Tracheal spiracle near middle of abdomen (Fig. 4.5a).

Vulva: Epigynal plate dark. Copulatory openings close together but not forming an atrium (Fig. 4.5g; 4.6f). Spermatheca sub-speherical with brown coloration (Fig. 4.5f). Copulatory ducts forming a secondary spermatheca (Figs. 4.5f; 4.6e, f).

Female: Total length 3.20, carapace 1.45 long, 1.04 wide; clypeus 0.10; Chelicera 0.70 long, 0.33 wide; Leg I: femur 1.22, patella 0.46, tibia 1.13, metatarsus 0.92, tarsus 0.63; Leg II: femur 1.12, patella 0.45, tibia 0.90, metatarsus 0.81, tarsus 0.61; Leg III: femur 0.98, patella 0.41, tibia 0.75, metatarsus 0.80, tarsus 0.49; Leg IV: femur 1.31, patella 0.45, tibia 1.12, metatarsus 1.03, tarsus 0.65; leg formula IV-I-II-III; abdomen 1.73 long, 1.20 wide.

Distribution: Known from the Menglun Nature Reserve, Yunnan, China (type locality), and Doi Suthep National Park, Chiang Mai, Thailand (present work) (Fig. 4.8).

Discussion

The Hahniidae, especially the Hahniinae have traditionally been seen as an easily diagnosable group in part due to the transversal comb-shaped position of the spinnerets; although their position as a family has changed overtime, being initially considered a subfamily of the Agelenidae ([1,8,29], among others) and Dictynidae ([1,4,30] among others). Currently, the monophyly of the family is largely recognized, and its relations have been indirectly tested as part of broad scoped phylogenetic studies [6,7]. However, the relations between its genera have never been phylogenetically tested. Although our data did not include representatives of all the known hahniid genera, we found some consistent and well supported results with the 14 hahniid species and four loci we analyzed. The position of the new genus *Hexamatia* as a sister group to the core of *Hahnia* species in our study is confidently recovered in all our topologies. We consider that this, plus the morphological differences between the new genus and *Hahnia* (presence of six eyes, small size close to 1mm and almost complete lack of coloration and abdominal patterns) are sufficient to consider it outside of the *Hahnia* 1 group, and as a genus of its own; proposing also a new combination for *Hexamatia senaria* (Zhang, Li, and Zheng 2011) comb. nov. Although we were not able to test the relationships between Hexamatia and other six eyed Hahniids like Amaloxenops [1,31], Intihuatana antarctica [32], and Scotospilus [10]; clear morphological differences could be observed in somatic

and genital characters like body size, coloration, size and shape of RTA and PA, and the presence of MA (see *Hexamatia* gen. nov. diagnosis). The clade *Hahnia* 2 formed by H. *saccata* and H. *ngai* was found to be closely related to Antistea+Neoantistea in our analyses (Fig. 4.1a–c); suggesting that these species might be misplaced in *Hahnia*. However, these and many other Asian hahniids require a broader revision and more comprehensive phylogeny to fully resolve their relations within this family. Therefore, *H. ngai* and *H. saccata* remain in *Hahnia*; in the case of the later, as it was originally described by Zhang, Li, and Zheng [15].

Eye reduction in the Hahniidae- This phenomenon appears to be rare in hahniid spiders. Most known species of this family have eight eyes; still, some instances of eye reduction have been documented in at least six genera. Modifications in the eyes range from size reduction of AME and lack of AME, to complete absence of eyes [1]. The evolution of this phenomenon in this family has never been studied, and the relations of the eye-reduced species are largely unknown. Even their taxonomy has been constantly a subject of debate [1,32–34]. Size reduction of the AME (Fig. 4.7a) is relatively common being observed in several species of the following genera: Alistra [1,9.35], Amaloxenops [32–34], Hahnia ([1,36], among others), and Neohahnia [1,37,38]. Reduction in number of eyes (Fig. 4.7b-d) is much rarer being documented only in a few species: Amaloxenops vianai [1,31], Hexamatia senaria [15] Hexamatia seekhaw n.sp., Intihuatana antarctica [32], Scotospilus longus [10], and two unpublished species documented in a revision of South American hahniids [34]; a quick examination of the illustrations and descriptions of these species suggest that they are not closely related. Finally, complete lack of eyes (Fig. 4.7e-f) has only been reported in the genus Iberina [39,40]. This wide range in the degree of eye reduction and broad geographical spread of this phenomenon suggest that eyes are a very plastic character and the loss or reduction might have evolved independently several times within this family. Nevertheless, a more comprehensive phylogeny of the Hahniidae is necessary to test this hypothesis

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Supplementary Material

List of primers used in our study, aligned matrix in nexus format, trace plot and histograms from the BI analysis, and pairwise genetic distance for our dataset. (Included in the original publication in the European Journal of Taxonomy)