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

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Naturalis Biodiversity Center
Leiden University
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Front cover image: Computer drawing of the face of the spider *Teutamus politus* Thorell, 1890 (Araneae, Liocranidae). F. Andrés Rivera-Quiroz 2019.

Back cover image: Circular phylogenetic tree of the family Symphytognathidae (Araneae, Araneomorphae). F. Andrés Rivera-Quiroz 2020.

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Chapter 1

General Introduction

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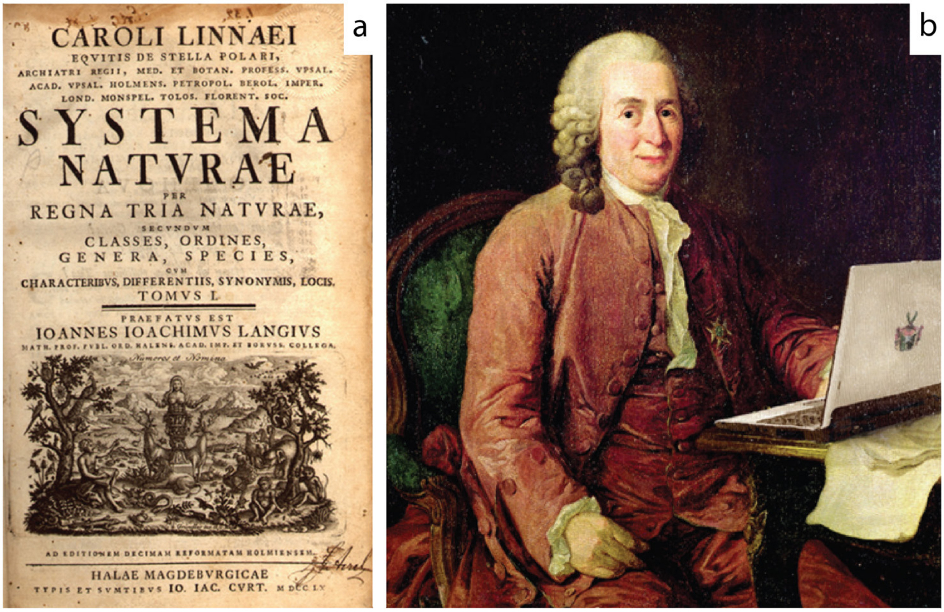
Taxonomy: what it is, what are its problems and the proposed solutions

Taxonomy is the science and practice of the classification and description of things; in biology, it encompasses the description, identification, nomenclature, and classification of organisms based on their inferred relationships [1–3]; this is the general notion of what taxonomy is and what a taxonomist does. However, in the context of biology, taxonomy has a much broader background that involves all sort of biological knowledge, from the formal naming of groups of organisms (e.g. species) to the temporal and spatial distributions of those groups, their morphology, anatomy, behavior, molecular information, among many other data. The science of taxonomy is currently in a state of transition, where content within publications is being digitally mobilized and incorporated into an interconnected knowledge network.

Throughout its more than two centuries of existence, taxonomy has provided a nomenclatural and relational framework to all the biological disciplines setting the foundations for discovering and understanding biodiversity. Nevertheless, this foundational role can often be confounded with a trivial and expendable one, or even be considered as a mere "service provider" [4, 5] to other fields of science. This perception of simplicity in the taxonomic endeavor, together with the realization of the massive number of undescribed —and unknown— taxa [6–12] and the human impact imposed on ecosystems lead to a crisis commonly known as the *taxonomic impediment* [6, 8, 13–15].

This taxonomic impediment (or impediments?) has become symptomatic in many ways, namely: the reduction of the taxonomic workforce and university curricula; the reduction of funding and economic support; and the view of taxonomy as an obsolete science [4, 9, 14–22]. An unintended consequence has also been the reduction in the literature outlets, leaving fewer venues for the publication of fundamental science. This problem has been highlighted by the recent decision to exclude the taxonomic megajournal Zootaxa from widely used science evaluation metrics (although this decision was subsequently reversed in the wake of reactions from the taxonomic community) [23–25].

This taxonomic impediment was noted by governments during the late 1990's and some agencies like the American National Science Foundation (NSF) created programs to enhance taxonomic training and research for future generations. That was the case of the Partnership for Enhancing Expertise in Taxonomy (PEET) [14, 26–28]. Similar projects were created in other countries like a federal directive for training specialists in cladistics in Brazil [19] and the Darwin Initiative in the UK. These programs looked to revive taxonomy, increase the workforce (especially in little known and neglected taxa), and stimulate the production and sharing of information on the internet, making taxonomic knowledge more accessible. A survey of the PEET impact in the creation of



CAROLI LINNAEI
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Figure 1.1.-"*Linnaeus in the information age*". a) The tenth edition of Carl Linnaeus' *Systema Naturae* published in 1758. Modified from Pyle [43]. b) Depicts an anachronistic portrait of Linnaeus —considered the father of Taxonomy— as a *techie*, using modern technology to develop his science. Modified from Godfray [5].

taxonomic careers showed that although PEET trainees greatly impacted scientific production in their respective fields, there were common concerns about funding continuity and the opportunity of finding steady jobs in taxonomy [28]. This same work emphasized the need for multidisciplinary training that, in addition to traditional taxonomic skills, also developed competence in the use of molecular techniques, data management, information dissemination, etc., as well as capabilities for integrating taxonomy with biogeography, ecology, ethology, etc.

It is clear then that taxonomy needs to change to improve taxonomy-based products and keep up with modern, faster data-driven science. Essential elements for 21st century taxonomy include universal access to primary source information [29–31] and the incorporation of new technologies to facilitate better description, analysis, understanding and conservation of biodiversity (Fig. 1.1). In this context, Godfray and Knapp [17] summarized the major changes that taxonomy has gone through in recent decades, transforming from a purely descriptive discipline to a hypothesis-driven science. This transition has been driven by a "philosophical makeover" that incorporated phylogenetic systematic ideas to test relationships between taxa, along with advances in DNA sequencing that have made vast amounts of molecular data available. Similarly, Godfray [5, 30] and Wheeler et al. [15] have articulated the need to build a more robust online taxonomic infrastructure to overcome some common problems in taxonomy, such as the publication of isolated descriptions, and limitations in data and image sharing and publication. The first decade of this century saw a healthy and necessary debate concerning the significance of taxonomy, its role among the modern biological science, and the epistemological, philosophical and technical reforms needed for taxonomy to stay relevant in the future [5, 8, 9, 15, 17, 18, 20, 21, 29, 30, 32–42]

"Taxonomy as a team sport"

Taxonomy is often understood as the individual efforts of taxonomists working in isolation; however, the current state of taxonomic science and the pace of the loss of biodiversity demand taxonomy become a group effort that greatly takes advantage of the internet and its capabilities to accelerate the rate and quality of its production and distribution [35]. The taxonomy of a group of organisms typically arises from the growing collection of publications in the primary taxonomic literature of that taxon, and does not reside in a single publication or institution. As such, it tends to be scattered among multiple journals, with contributions by various authors; this has made taxonomic literature a (usually) poorly defined integral of the accumulation of literature [30] with a tendency to be unorganized and fragmentary. Godfray [30] suggested the implementation of a unitary model of taxonomy where one group or institution would be in charge of the administration of current taxonomy for a given group. Spider taxonomy, together with a few other examples like ants [44, 45], lepidopterans [46], dipterans [47], orthopterans [48] and fishes [49], are probably the finest working examples of this authoritative "unitary taxonomy" [30, 31, 50] model.

The taxonomy of spiders is probably one of the best curated and polished, being under constant revision since the mid-20th century, and currently being administered by the World Spider Catalog [51]. The indexation and scrutiny of spider taxonomic literature began in the 1940's by Pierre Bonnet (University of Toulouse, France) and Carl Friedrich Roewer (Bremen, Germany). Afterwards, the Italian arachnologist Paolo M. Brignoli (University of Aquila, Italy) started filling the gaps in the aforementioned works and publishing periodic catalog supplements. This idea was subsequently picked up by the American arachnologist Norman I. Platnick (American Museum of Natural History, New York) who published three catalog supplements between 1989 and 1998 [52–54]. After this, the online version of the World Spider Catalog (WSC) was established and handled by Platnick from 2000 to 2014 [55]. In 2014, the WSC was transformed into a relational database administered by the Natural History Museum Bern (Switzerland) [51]. This catalog provides a fully searchable database that grants access to information on the more than 48,700 currently valid spider species and their synonyms. The WSC website also keeps a fully accessible library with more than 15,400 documents on spider taxonomy that dates back from the current year to Carl Clerck's species descriptions from 1757, the only accepted spider descriptions previous to the Linnean era (ICZN: Article 3.1) [56, 57].

Improving access, dissemination and usability of information

Economics is the study of the production, distribution, and consumption of goods and services. If the scientific community is working to modernize the production, distribution, and consumption of taxonomic information, then perhaps we should look to economics for guidance. But economics is dominated by the study of physical goods, whose value is largely driven by their scarcity: rare things tend to be more valuable than common things. In contrast to physical goods information becomes more valuable as it becomes more accessible. So to increase the value of taxonomy, we should work to increase accessibility to biodiversity knowledge [58]. Current technologies allow storing and sharing data at an unprecedented pace, which in turn, allows the transformation of old printed literature (e. g. the Biodiversity Heritage Library [59]), and direct publication of new literature in electronic PDF format. This permits world-wide access to an amount of knowledge that was previously reserved to a few great libraries [15], recovering centuries of aggregated data that might otherwise just be forsaken on a library book shelf or at the back of a researcher's drawer. Nevertheless, it is not the best format for exchanging and querying data generating what has been dubbed the " PDF Black Box" [60]. In this context, the Swiss organization Plazi [61] has created software that semiautomatically detects and extracts taxonomic treatments, pieces of text that link specimen data (e.g. collection locality and date, collector, number of specimens, developmental stage, among others) to a taxon name [62–64].

The Plazi initiative [65–67] has greatly benefited from electronic access to taxonomic literature in PDF format and has focused on mining and mobilizing biodiversity and taxonomic data contained in legacy and newly produced taxonomic literature. This can be done in two ways: a retrospective approach that transforms a taxonomic document in PDF format into a sematically categorized document in XML format that allows specimen data to be extracted; and a prospective approach where data is directly produced in XML format as has been implemented by some journals (e. g. Pensoft's ZooKeys and Biodiversity Data Journal, the European Journal of Taxonomy, among others) [58] (Fig. 1.2). These taxonomic treatments are then stored and available in Plazi's Treatment Bank where the specimen data can be directly gathered and analyzed [68]. Also, a main characteristic of this repository is that these specimen records are also contributed to the Global Biodiversity Information Facility [69], where they are aggregated with other sources of data such as collection specimen records and observations networks (among other sources), forming an unparalleled resource for discovering, analyzing and explaining broad biodiversity patterns [63–65, 68].

In this thesis, we explored the application of these principals in **Chapter 2**, focusing on the extraction of data from legacy taxonomic literature and its application for inferring

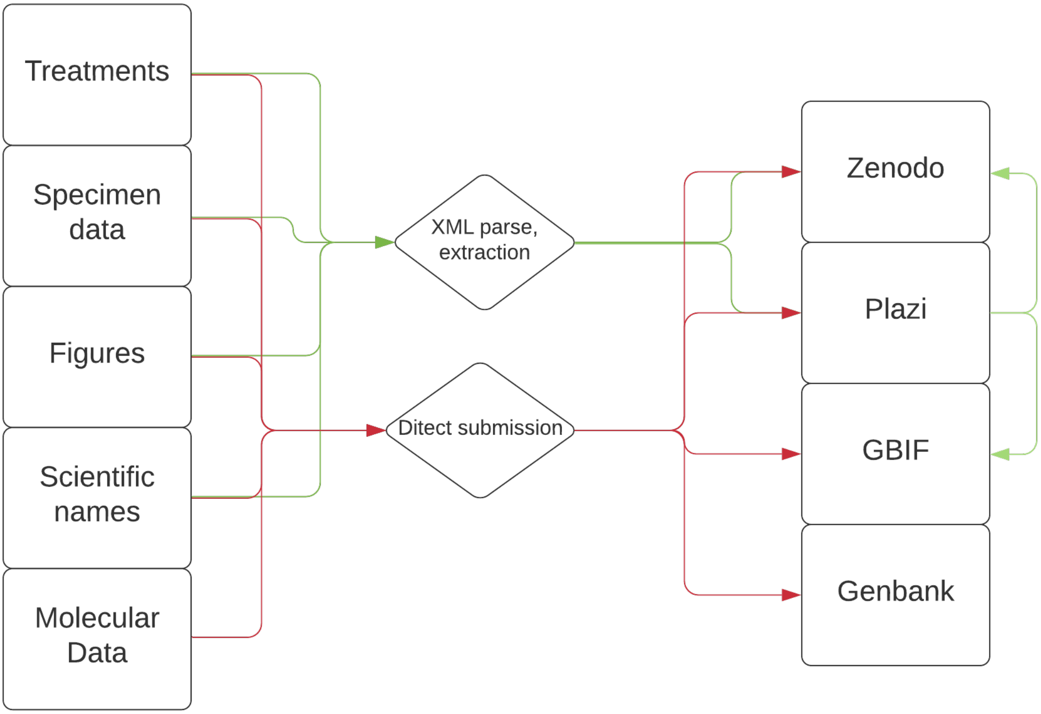


Figure 1.2.-The "Plazi approach". Schematic view of the retrospective (green) and prospective (red) data mining from taxonomic literature. Extracted data can then be displayed and analyzed in specialized repositories [61, 70] and databases [61, 69, 71].

phenological patterns that allow optimizing specimen sampling in the field (see also the fieldwork section below). Also, we used taxonomic literature obtained from the WSC to assess the rare phenomenon of genital asymmetry (GA) in spiders (**Chapter 3**, see the *Teutamus politus* section below). This chapter used taxonomic literature as an image repository observing and comparing the incidence and biological background of GA, giving a hypothetical classification based on previous work in spiders and insects GA [72–80] and drawing a preliminary evolutionary scenario for each type of asymmetry we observed [81].

The integrative taxonomy

Integrative Taxonomy was defined by Dayrat [4] as the complementary delimitation of species based on their phylogeography, morphology, genetics, ecology, behavior, etc. Although the term, as minted by Dayrat [4] failed to be the proposed "new paradigm" in the exercise and understanding of the taxonomic science and was even dubbed as potentially misleading and detrimental by Valdecasas et al. [82] due to its *guidelines* on the taxonomic nomenclature, there are some concepts that are worth recovering

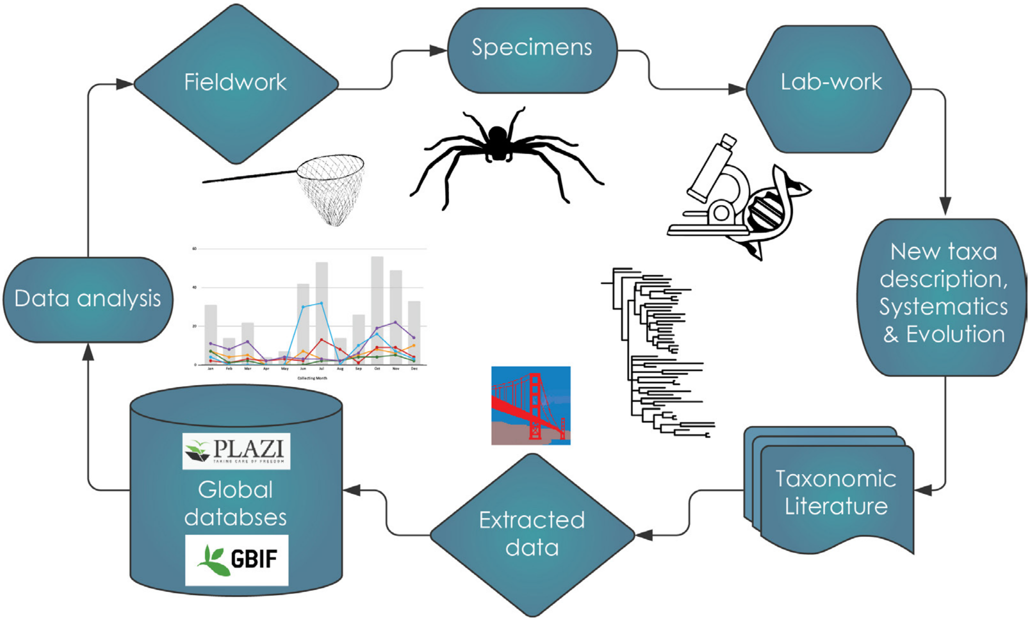


Figure 1.3.- The integrative taxonomic pipeline. Schematic workflow of the taxonomic process used in this thesis. It includes the use of the WSC [51] as a taxonomic library; the extraction of data from taxonomic literature [65, 66]; the use of these and other specimen data for biodiversity analyses; the use of molecular data (some of it from Genbank [71]) for building the phylogenies and a thorough documentation with different imaging techniques of the specimens' morphology.

and incorporating, whenever possible, in the common practice of taxonomy. Taxonomy has generally entrusted this species delimitation on morphology due to both practical and historical reasons. Nevertheless, as mentioned before, the current taxonomic crisis requires the inclusion of new technologies and techniques that favor faster and more reliable taxa descriptions; integration means multidisciplinarity [4, 83].

This multidisciplinarity has become more and more common in some fields by the integration of: morphological data (e.g. photographs, SEM, 3D imaging, CT-scans, geometric morphometrics, among others), molecular data, geographical information (e.g. collection localities and the use of GIS), and other specimen based data (e.g. number and sex of specimens, collection dates, etc.) that allow testing the species hypotheses in different and more robust approaches [4, 82, 84–86]. Pyle [43] visually exemplifies the enormous advances on species documentation by using these modern technologies. The integrative perspective, besides generating pure taxonomic information —hypotheses of classification and relations—, also contributes with molecular data, high resolution images of specimens and their relevant characters, and other kinds of data to global databases [86–92]. This accessible data can be downloaded and tested independently or incorporated to new taxonomic and systematic studies in an easy and flexible way; this can help to expedite the testing of phylogenetic hypotheses and rapidly generate new datasets and inferences with more explanatory power.

In this context, **Chapters 4** and **5** are examples of the implementation of this integrative taxonomy (Fig. 1.3). Both chapters involve the description of new taxa and also, first reports of these families for Thailand. **Chapter 4** describes two new species and one new genus of the family Hahniidae based on molecular and morphological evidence; **Chapter 5** describes three new species of the family Symphytognathidae, employing molecular and morphological evidence; and making use of micro CT-scans and 3D modeling to circumscribe the genus *Crassignatha* and clearly illustrate and discuss the genital characters of the males of this and other related symphytognathid genera. Although 3D modeling had been used before to study spider genitalia [93–95], ours are, to the best of our knowledge, the smallest spider palps that have ever been 3D modeled, being only 0.2 mm in width.

Fieldwork

The process of how we selected the locations where fieldwork was conducted is explained in detail in **Chapter 2**. Here we cover some generalities of the new specimen collection, where and how it was conducted. Southeast Asia (SEA) is one of the most diverse areas on the planet being represented by several conservation hotspots, of which the Indo-Burma and the Sundaland Hotspots are the most extensive and diverse [96, 97] (Fig. 1.4). The Indo-Burma hotspot is largely distributed over continental SEA; this region is characterized by socio-political instability, high population density and a fragmentary nature of its territory. This, in turn, generates a scattered taxonomy of most groups that makes it difficult to describe and assess its biodiversity [96, 98, 99]. Still, studies on vascular plants and terrestrial vertebrates show the Indo-Burma hotspot to be one of the richest and highest in endemism, but also one of the most threatened hotspots [96, 100].

This uniqueness and magnitude of its biodiversity is also reflected, albeit understudied, in invertebrates. The arachnofauna is mostly known from isolated species descriptions, although a few genus, family and broader revisions have been published [101–103]. Especially interesting to us was the case of the liocranid spiders, and from this family, the species *Teutamus politus* Thorell, 1890 (see *T. politus* section below, and **Chapters 2** and **3**) and its close relatives in the so called *Teutamus* group (TG). While planning our fieldwork, we wanted to improve the probabilities of capturing adult specimens of *T. politus* while also sampling other relatives of the TG. Due to the lack of data on these spiders' phenology, we used data from taxonomic literature to select the localities and times with a higher incidence of liocranid spiders based on the number of adults reported in taxonomic literature. Taking into account this information we decided to collect from July to August 2018 in the three provinces with most specimens reported in Thailand. We covered a variety of habitats in each place (Fig. 1.5) ranging from cloud forests at the top of the highest mountain in the country, to temperate pine, oak and *Dipterocarpus* forests down to tropical vegetations. We also sampled in secondary forests and rubber and oil palm plantation to observe the anthropogenic impact on the populations of these spiders.

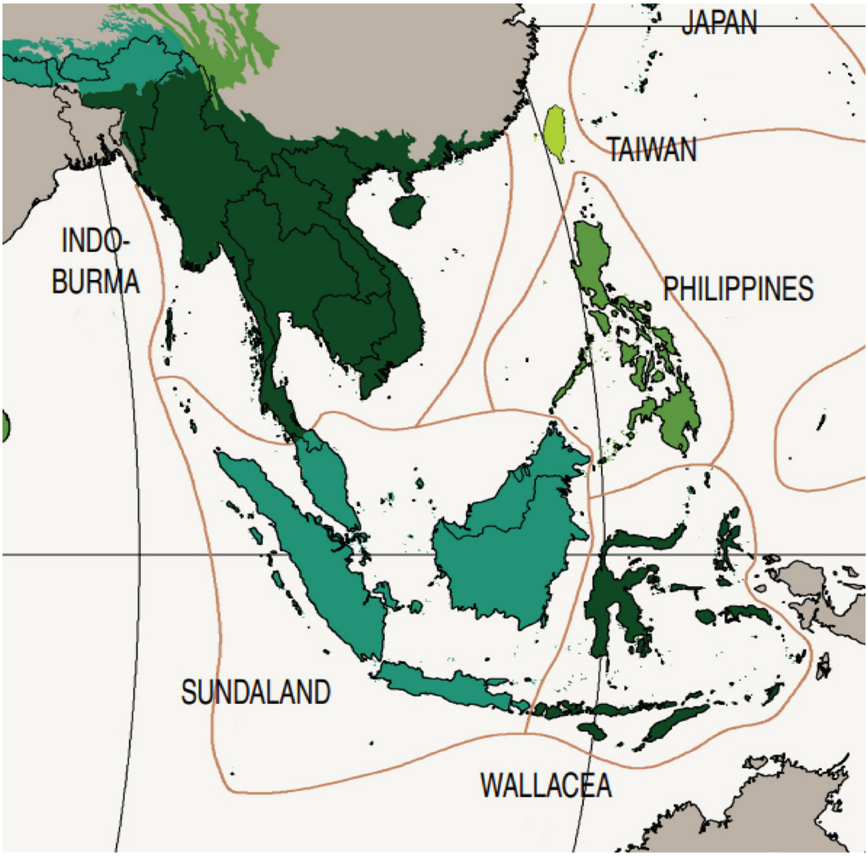
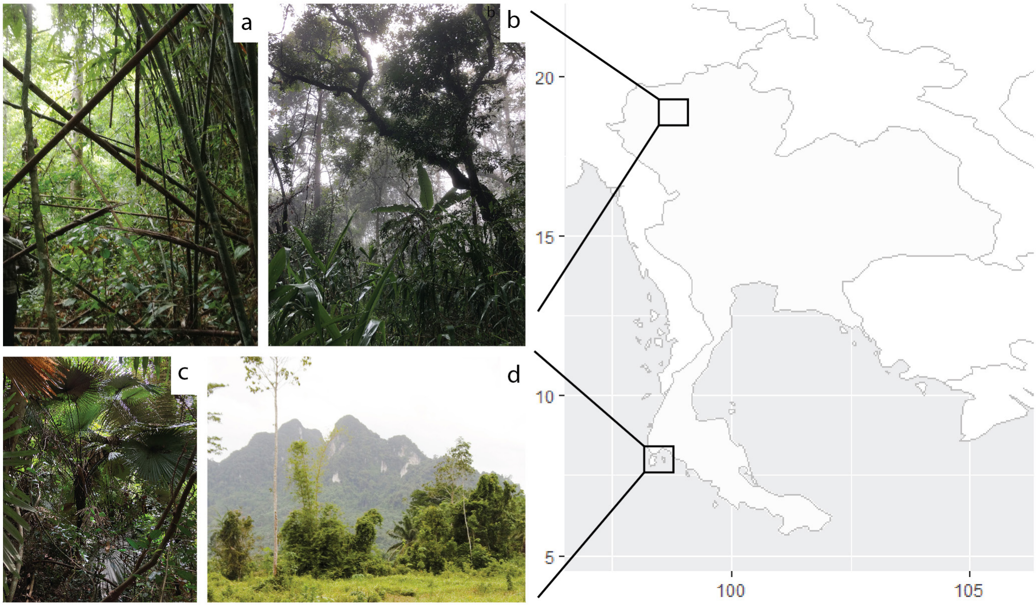


Figure 1.4.-Map of the Southeast Asian Hotspots. Modified from Mittermeier et al. [96].

Figure 1.5.- Some of the localities sampled in Thailand. a, b) Chiang Mai: a- bamboo forest in the Pha Daeng National Park. b- Cloud forest in Doi Inthanon National Park, the highest mountain in the country. c, d) Phuket and Krabi: c- a patch of *Kerrodoxia elegans* palm tree in Ton Sai. d. Secondary forest near Than Bok Khorani National Park. (Next page, top) ►

Figure 1.6.- Collecting methods. a) Winkler traps hung outside Doi Suthep National Park headquarters. b, c) Pitfall traps at a *Dipterocarpus* sp. forest near Pha Daeng National Park. d) Active search in sifted leaf litter at Doi Suthep National Park. e) Active search in cryptic habitats (i. e. under rocks, logs, bark, among leaf litter, etc) near Than Bok Khorani National Park. (Next page, bottom) ►

Also, we knew based on literature that most liocranid adult specimens were collected using pitfall traps and leaf litter sifting [101, 103–105]. Therefore, we used a combination of collecting methods (Fig. 1.6) that target ground spiders [106–108]. This allowed us to have a better representation of the liocranid species present in each site and also to capture a wide array of ground dwelling arthropods, including several families of spiders. While referring to records in taxonomic literature when planning field work is a common practice, very few have made such records persistently available to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all using FAIR [67, 109] standards in the way we did for the present work. More information about this fieldwork, data extraction and applications can be found in **Chapter 2** of this thesis. Specimens collected during this field trip were used to observe and document the genital asymmetries in *T. politus* (**Chapter 3**), and to describe new species and build molecular phylogenies of the families Hahniidae and Symphytognathidae (**Chapters 4 and 5**).

Teutamus politus

Fig. 1.7

This species is a central part of this thesis, together with other related taxa of the *Teutamus* group, of the Chapters 2 and 3.Very little is known about the biology of this interesting species. It was first described in 1890 as the type species for the genus *Teutamus*. Its description was based on one female specimen from “Pulo Pinang”, nowadays Malaysia [110]. But it wasn’t until 2001 that their name resurfaced among many others in an impressive piece of taxonomic literature on South East Asian ground spiders by the Dutch arachnologist Christa Deeleman-Reinhold [101]. This seminal work encouraged research on this and other related spider groups in the region [103–105, 111–113]. Deeleman-Reinhold [101] described six new species of this genus, observing relevant information about the habitat and distribution of these species. She made the first description of the male of *T. politus* and noted the asymmetric genital opening in the female. Dankittipakul et al. [104] significantly increased the knowledge of the genus *Teutamus* by describing 17 new species. They reported many new specimens of *T. politus* increasing the available information about their geographic and temporal distribution. These studies greatly advanced knowledge about the taxonomy, and geographical distribution of *T. politus* and other related spiders. However, their biology is still a mystery.

Teutamus species are known to inhabit the leaf litter of dark humid undisturbed rainforests, but have also been reported wandering on the forest floor in daytime. Most species were found to have limited distribution ranges, being sampled in just one locality without known overlapping distributions [101]. *T. politus* is the notable exception by having a relatively wider distribution that extends from Malaysia to the south of Thailand [101, 104].This species became interesting to us due to the clearly asymmetric female genitalia. This character had been noted and illustrated before (Fig. 1.7d) in the female and had even been used as a diagnostic character of the species but correlations to changes in male morphology and other implications like courtship and mating behavior (Fig. 1.1c) had never been studied. More information about this can be found in the third chapter of this thesis. While sampling specimens of this species our attention was

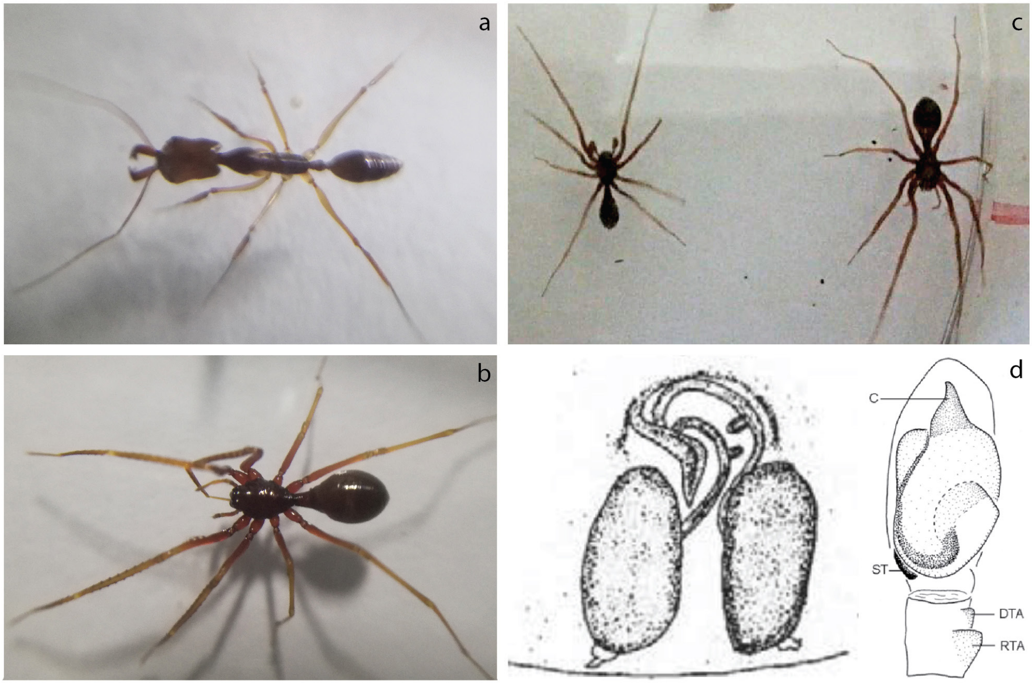

Figure 1.7.- ***Teutamus politus.*** a, b) Comparision of an ant *Odontomachus* sp. and a female of *T. politus*; exemplifies the phenomenon of ant mimicry in this spider species. Our sampling also showed them to be myrmecophylic, living on top or close to the *Odontomachus* sp. nests. c) Size comparison of male and female during the behavioral experiments. d) Taxonomic illustrations of the genitalia of this species (female epigynum —left—, and male pedipalp —right—). Modified from Deeleman-Reinhold [101]; and Dankittipakul et al. [104].

drawn to the relation they had with ants of the genus *Odontomachus* (Fig 7a). Most of the *T. politus* specimens we caught were close to or on top of these ant nests. *T. politus* bears some morphological similarities to the ants (Figs. 1.7a, b). Although they had been mentioned to be "vaguely ant-mimicking" before [101], this is the first time that this kind of spider-ant relationship has been observed in this genus. Unfortunately we were not able to make more detailed observations of the nature of the relationship. Nevertheless, similar interactions have been well documented in other spiders ranging from *myrmecophyly* —living in close relation to ants— to *myrmecomorphy* —morphologically mimicking the ants— [114–118].

The present thesis aims to show the value of generating new taxonomic content in a way that allows for rigorous testing of phylogenetic hypotheses, and boosts the impact and relevance of taxonomic work by incorporating high resolution images, molecules and specimen data. Although taxonomy is a venerable science, it holds large quantities of useful data that can help elucidate broad patterns in biological evolution, biogeography, and ecology, among other disciplines. All of these might eventually help understanding, protecting and preserving our endangered biodiversity. We are sitting on a mine whose precious data patiently awaits to be extracted.

Thesis outline

Legacy literature data extraction and application

Chapter 2: Mining data from legacy taxonomic literature and application for sampling spiders of the *Teutamus* group (Araneae; Liocranidae) in Southeast Asia. *Scientific Reports* 10, 15787. doi:10.1038/s41598-020-72549-8.

The use of taxonomic literature to infer evolutionary patterns

Chapter 3: Imperfect and askew: A review of asymmetric genitalia in araneomorph spiders (Araneae: Araneomorphae). *PLoS One* 15:6, e0220354: 1-26. doi:10.1371/journal.pone.0220354.

New taxa description in an integrative approach

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. *European Journal of Taxonomy* 724, 51-69. doi: 10.5852/ejt.2020.724.1157

Chapter 5: First records and three new species of the family Symphytognathidae (Arachnida: Araneae) from Thailand, and the circumscription of the genus *Crassignatha* Wunderlich, 1995. *ZooKeys* 1012, 21–53. doi.org/10.3897/zookeys.1012.57047

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Chapter 2

Mining data from legacy taxonomic literature and application for sampling spiders of the *Teutamus* group (Araneae; Liocranidae) in Southeast Asia

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Abstract

Taxonomic literature contains information about virtually every known species on Earth. In many cases, all that is known about a taxon is contained in this kind of literature, particularly for the most diverse and understudied groups. Taxonomic publications in the aggregate have documented a vast amount of specimen data. Among other things, these data constitute evidence of the existence of a particular taxon within a spatial and temporal context. When knowledge about a particular taxonomic group is rudimentary, investigators motivated to contribute new knowledge can use legacy records to guide them in their search for new specimens in the field. However, these legacy data are in the form of unstructured text, making it difficult to extract and analyze without a human interpreter. Here, we used a combination of semi-automatic tools to extract and categorize specimen data from taxonomic literature of one family of ground spiders (Liocranidae). We tested the application of these data on fieldwork optimization, using the relative abundance of adult specimens reported in literature as a proxy to find the best times and places for collecting the species (*Teutamus politus*) and its relatives (*Teutamus* group, TG) within Southeast Asia. Based on these analyses we decided to collect in three provinces in Thailand during the months of June and August. With our approach, we were able to collect more specimens of *T. politus* (188 specimens, 95 adults) than all the previous records in literature combined (102 specimens). Our approach was also effective for sampling other representatives of the TG, yielding at least one representative of every TG genus previously reported for Thailand. In total, our samples contributed 231 specimens (134 adults) to the 351 specimens previously reported in the literature for this country. Our results exemplify one application of mined literature data that allows investigators to more efficiently allocate effort and resources for the study of neglected, endangered, or interesting taxa and geographic areas. Furthermore, the integrative workflow demonstrated here shares specimen data with global online resources like Plazi and GBIF, meaning that others can freely reuse these data and contribute to them in the future. The contributions of the present study represent an increase of more than 35% on the taxonomic coverage of the TG in GBIF based on the number of species. Also, our extracted data represents 72% of the occurrences now available through GBIF for the TG and more than 85% of occurrences of *T. politus*. Taxonomic literature is a key source of undigitized biodiversity data for taxonomic groups that are underrepresented in the current biodiversity data sphere. Mobilizing these data is key to understanding and protecting some of the less well-known domains of biodiversity.

Introduction

In the aggregate, traditional taxonomic publications can be thought of as a repository that has accumulated vast amounts of biological data linked to specific taxonomic names. These units of taxonomic knowledge, information linked to a name within a publication, are known as taxonomic treatments [1–3]. This makes taxonomic literature not only crucial for the exchange and growth of biodiversity knowledge, but also capable of being used to detect and understand larger biodiversity patterns with historical perspective.

In recent years, great efforts have gone into the digitization of legacy taxonomic literature [4–6]. This combined with digital publications have greatly improved access to taxonomic literature. Nevertheless, although easy to share, PDF publications still have most biodiversity data embedded in strings of text making them less dynamic and difficult or impossible to read and analyze without a human interpreter [7]. This difficulty to access and use core specimen data is what we define as PDF prison [8]. Recently developed tools allow text in PDF documents to be interpreted and categorized in XML format (mark-up) allowing information to be mobilized, aggregated and reanalyzed [9–12]. Plazi Treatment Bank [8,13,14], is a project dedicated to creating a comprehensive compendium of taxonomic and biological data extracted from primary literature [15]. This platform permits mined treatment data to be accessed, queried, compared, and reused in a customized way. The strategy for data extraction can be prospective: where journals generate new data in XML format that can be uploaded directly to repositories (as has been implemented by Zookeys [2] and EJT [8,13]). or retrospective: where data is mined from legacy taxonomic literature [3,11–13] through a process called semantic enhancement [9,13]. This retrospective approach is more complicated and time consuming since the semi-automatic process of text recognition and tagging needs to be checked by a human operator [3,15]. However, it can provide useful information by extracting, integrating and using biodiversity data contained in the hundreds of years of accumulated taxonomic literature. Data integration is achieved by linking records from Plazi treatment bank to the Global Biodiversity Information Facility (GBIF) [8,16] where they are aggregated with other type of records, mainly natural history institution specimen collections and observation data based on GBIF’s taxonomic backbone [17].

Here we combined several of these cybertaxonomic tools to test the data extraction process and its potential application on the design and planning of an expedition to collect fresh material in the field. We targeted the ground spider *Teutamus politus* Thorell 1890 and its relatives from the so called *Teutamus* group (TG) (Araneae, Liocranidae) [18]. This group of spiders is mostly distributed in Southeast Asia [19–23] and is composed of seven genera: *Jacaena*, *Koppe*, *Oedignatha*, *Sesieutes*, *Sphingius*, *Sudharmia* and *Teutamus* [18]. These spiders have been cataloged in the family Liocranidae; however, their phylogenetic relationships, biology and evolution are still poorly understood[18,24]. Therefore, collection of fresh specimens of the target taxa was necessary for building a molecular phylogeny of the TG. The species *T. politus*, besides being the type species of the genus *Teutamus*, is an example of the extremely rare phenomenon of directional genital asymmetry [25]. For this reason, the collection of live adult specimens was crucial to study, document, and test the behavioral implications of their abnormal genital morphology.

Our study aimed to highlight the importance of making biodiversity data contained within taxonomic treatments accessible and reusable in accordance with the FAIR data principles [26]. This approach can help bridge gaps and focus efforts in the study of particularly interesting taxa or geographic regions. The usability of taxonomic literature data, potential applications, and its limitations and biases are discussed.

Material and Methods

Literature data extraction– We accessed all taxonomic literature of the family Liocranidae available in the World Spider Catalog [27]. We selected 55 publications that contained taxonomic treatments of the family Liocranidae [19–23,28–80] (for full list, see Supplementary Table 1). We selected and processed all publications that provided taxonomic treatments with specimen data and usable geographical references. Publications written in a language other than English were not processed since OCR parsing, as implemented by the programs used here, has mostly been developed in this language. From the marked-up documents, 21 contained information on members of the TG and two on the species *T. politus*. We used the program GoldenGATE Imagine V.3 (GGI; http://plazi.org/resources/treatmentbank/goldengate-editor/) to semantically enhance PDF documents, allowing atomization and categorization of data. In some cases, ABBYY FineReader V. 11 was used first to extract and correct text from the PDF document using optical character recognition (ORC) and text editing functions. Once the PDF documents were marked and revised, we used GoldenGATE to upload the files to Plazi's TreatmentBank [14].

Data analysis– We used Plazi Treatment Collection Statistics tool (http://tb.plazi.org/GgServer/srsStats) to download all the information relevant to our study in an excel spreadsheet to facilitate fine-grained management and analysis, largely following the approach described by Miller et al. [12]. We used these specimen based data to create profiles of the TG species allowing us to visualize where and when these taxa had been collected. Also, we used the GBIF occurrence search tool (https://www.gbif.org/occurrence/search) to look for records on our relevant TG taxa. The specific datasets we used can be found in the Data Accessibility section.

Site selection– Literature data were used to design our field collection in a way that allowed us to optimize the collection of adult specimens of our target taxa in Southeast Asia (SEA). We explored the number of specimens of the TG reported per country, province and location whenever possible. We favored those locations with a higher representation of genera from the TG but also those where *T. politus* had been reported. Finally, we analyzed the total number of adult specimens collected per month for both the TG species and *T. politus* in order to increase the chances of finding adult spiders. Based on this, we decided to sample in three provinces in Thailand between July 16 and August 12, 2018.

Sampling– Following the results of our literature analysis, we prioritized collections in national parks and protected areas. Precise geographical coordinates and specific habitat information was scarce or missing altogether in most taxonomic treatments. Therefore, we further divided each site in four different vegetation types (collecting sites details in the Supplementary Table 2) allowing us to cover a wide range of available habitats. We combined pitfall traps, Winkler extractors (for soil arthropods; www.entowinkler.at), and direct collecting targeting ground spiders. A mixture of propylene-glycol and ethanol was used in the pitfalls to avoid excessive evaporation and help with DNA preservation [81]; all specimens were collected and stored in 96% ethanol. All liocranid spiders were identified to species level. Juvenile spiders were assigned to a species only when they were at a pre-adult or late juvenile instar

Results

Literature data analysis– Data extracted from 55 analyzed publications represent in total 23 genera and ca. 160 species of the family Liocranidae with ca. 3000 specimens collected worldwide (Fig. 2.1a). A visual summary of the data extraction process and data display in Plazi's Treatment Bank and GBIF can be found in Supplementary Figure 2.1. These include treatments of all currently valid genera and 90 species of the TG based on 1,309 specimens; out of 137 currently valid species [27]. The TG was mostly distributed in East and Southeast Asia (Fig. 2.1b) with the exception of two species of the genus *Oedignatha* found in the Seychelles. Within SEA, six genera of the TG have a broad distribution being reported from India and the southern region of mainland Asia to the Malay Archipelago (Fig. 2.1c-e, g-h). Two exceptions are *Jacaena* that has not been reported south of Thailand (Fig. 2.1f) and Sudaharmia that has only been reported within Indonesia (Fig. 2.1i). Indonesia (Six genera, 386 specimens), Thailand (Five, 351) and Malaysia (Four, 212) were the countries with a highest richness and abundance of TG spiders accounting for 72.5% of all the TG records (Fig. 2.2a). Thailand was the country that combined most occurrences of the TG genera and *T. politus* having 66% of all the known specimens of this species reported in literature. Within Thailand, the best sampled province is Chiang Mai accounting for 35% of all the TG specimen records for the country. Other relatively well known provinces were Krabi, Nakhon Ratchasima and Phuket, adding up to 30% of the country records (Fig. 2.2a). Chiang Mai had reports of four TG genera and 11 species, Krabi and Phuket had relatively less representation of the TG; however, these two provinces had 66 of the 68 specimens of *T. politus* recorded for the country.

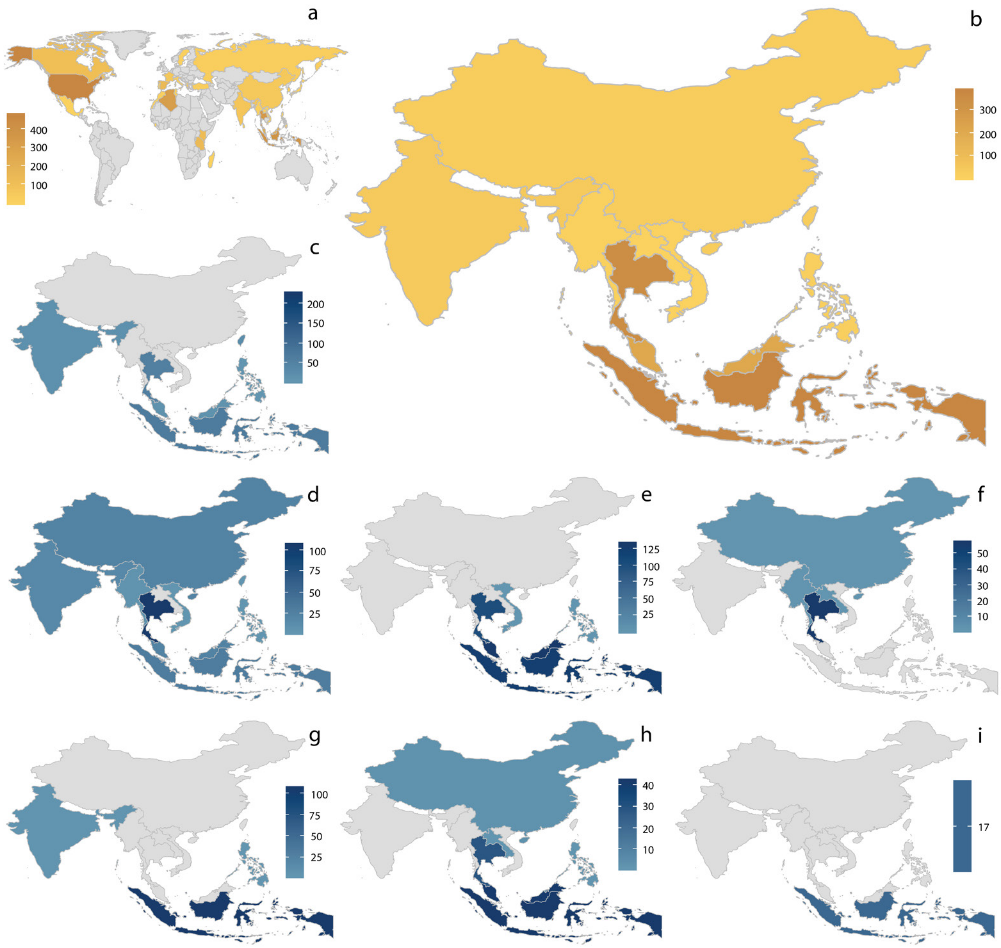

Figure 2.1.–Maps of liocranid spiders distribution. Based on geographic data extracted from taxonomic literature using Plazi's retrospective workflow (see Supplementary Table 1 for the whole set of documents used). Maps generated in RStudio [82–84]. a) Family: Liocranidae worldwide. b) Family Liocranidae in Southeast Asia (SEA). c) Genus: *Oedignatha*. d) *Sphingius*. e) *Teutamus*. f) *Jacaena*. g) *Koppe*. h) *Sesieutes*. i) Sudaharmia. Brown shades represent family distribution and blue shades represent genus distributions. Color intensity corresponds to numbers of specimens per country.

The majority of species treatments that we semantically enhanced contained collecting dates that allowed us to plot temporal distribution of the group within Thailand. Most specimens were collected between 1980 and 2009. These dates together with collecting locations allowed us to plot the known temporal and geographic distribution of our target taxon (Fig. 2.2b). For instance, most collecting is concentrated between May and December, with February and March being the least represented months. Similarly, Indonesia, Malaysia and Thailand are the best sampled countries in Southeast Asia.

Figure 2.2.–Distribution of the *Teutamus* group in Southeast Asia according to taxonomic literature. Based on data extracted from 23 studies [19–23,28–30,39,42,50,56,59,61,65,68,73–75,77–80] using Plazi's retrospective workflow. a) Proportion of specimens reported per country, with detail of provinces in Thailand. b) Temporal and spatial distribution of collections for the past 40 years. ● = Indonesia, ▲=Malaysia, ⊗=Thailand, ♦ =Philippines, ⊠=Vietnam.

From an historical perspective, Indonesia was clearly the most sampled area during the 80s and Malaysia during the 90s, with more heterogeneous and international records appearing during the 2000s.Total monthly abundances suggest that adults of the TG are mostly found in between June and July, and October to January (Fig. 2.3a). A more detailed visualization at genus level shows that most TG genera have similar seasonal variations, with the exception of *Teutamus* that is most common between June and July (Fig. 2.3a). The species *T. politus* has adults reported mostly between June and July, and some specimens from September to December but none have been recorded between January and May (Fig. 2.3b).

Fieldwork– Our sampling produced 134 adult liocranid specimens from the following genera: *Jacaena* (3), *Oedignatha* (32), *Sesieutes* (3), *Sphingius* (1), *Teutamus* (95) (Table 1). Some juvenile specimens of *Oedignatha* and *Teutamus* could be matched to adults in the same sample and assigned to the same species adding up to a total of 229

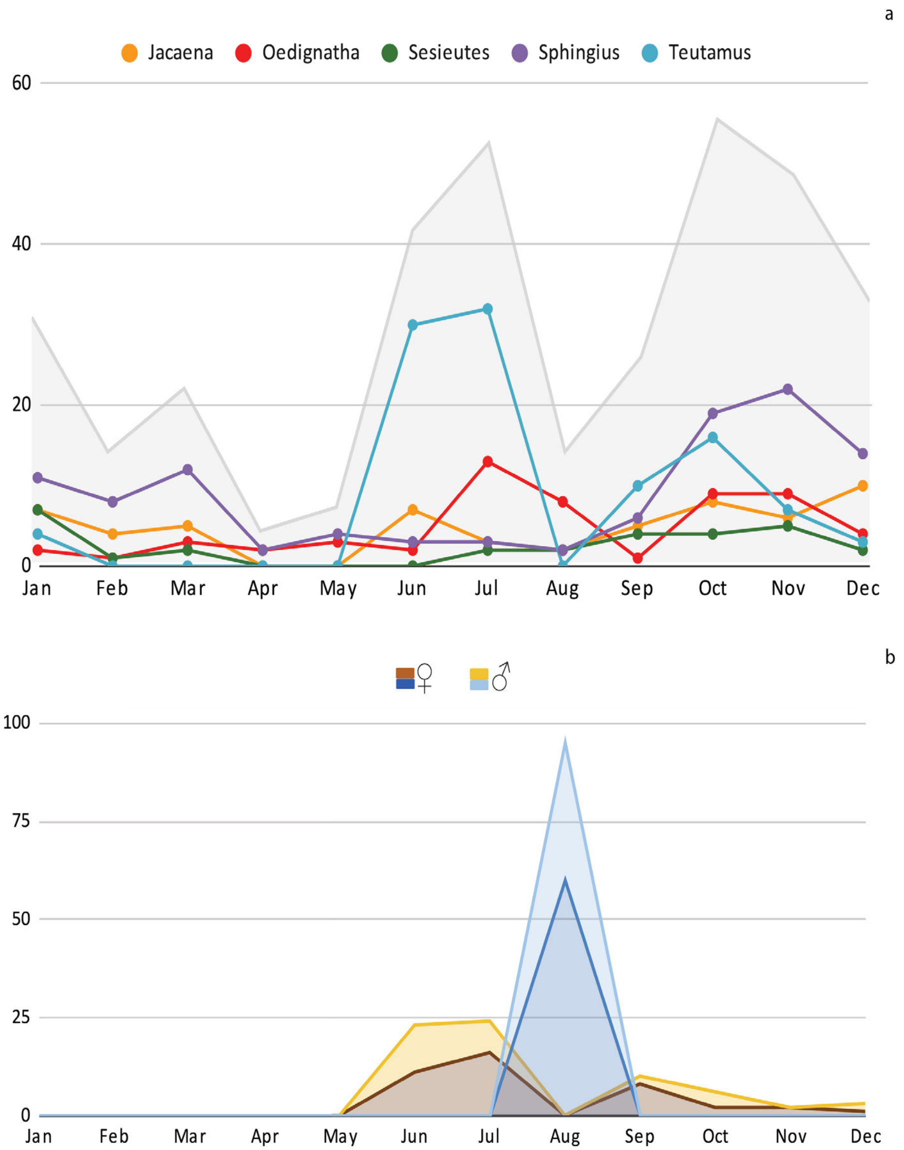

Figure 2.3.–Seasonal distribution of adult specimens of the *Teutamus* group in Thailand. Based on data extracted from 2 studies [19,21] using Plazi's retrospective workflow. a) Grey area indicates total number of specimens; lines detail richness per genus in literature. b) Relative abundances of males and females of *Teutamus politus*. Brown shades indicate specimens in literature; blue shades indicate specimens in our study.

identified specimens of the Liocranidae. We found four species of the TG in Chiang Mai: *Jacaena* lunulata, *Oedignatha* barbata, O. jocquei, and *Sphingius* cf. vivax; three species in Phuket: O. spadix, *Sesieutes* cf. minuatus, and *Teutamus politus*; and two species in Krabi: O. sp. and *T. politus*. Most of them were represented by males and females with the exception of J. lunulata and S. cf. vivax, where only males were found. These two, along with O. barbata and O. sp., were the rarest species having three or fewer individuals in our sample. The most abundant species were O. spadix and *T. politus* with 21 and 95 adults respectively.

Discussion

Literature data analysis– Detecting and understanding biodiversity patterns require large amounts of high quality data. In recent years global databased like GBIF and Plazi have set standards for collection, curation and dissemination of these biological data. GBIF, the largest biodiversity data repository, has aggregated digitized specimen records from many of the world's most important biodiversity collections institutions. In addition, records from observation networks such as iNaturalist are aggregated on GBIF. However, legacy taxonomic literature as a source of biodiversity data has remained relatively unexplored until recent years. Taxonomic literature holds a vast amount of high-quality biodiversity data [12,85,86]. Like data from institutional collections and unlike data from observations networks, these data typically point to specimen objects archived in a natural history institution. Such records have the potential to be re-evaluated in a way that records from observation networks cannot be. It is worth noting that many specimens cited in the taxonomic literature, although archived in a natural history collection, are not necessarily among the institutional collections data shared with GBIF.

Table 1.–Records of Teutaumus group (TG) species from three Thai provinces. Total records from taxonomic literature (Spp. in literature) vs. Literature records from June-August (Spp. July-August) vs. our field samples (Spp. in our study). * indicates new geographic distribution for the species.

Province	Species	Spp. in literature			Spp. in lit. (July- August)			Spp. in our Study		
		♂	♀	Total	♂	♀	**Total**	♂	♀	**Total**
Chiang Mai	*Jacaena angoonae*	-	4	**4**	-	-	-	-	-	-
	Jacaena lunulata	8	5	**13**	-	-	-	3	-	**3**
	Jacaena mihun	3	3	**6**	-	-	-	-	-	-
	Jacaena schwendingeri	3	9	**12**	-	3	**3**	-	-	-
	Oedignatha barbata	6	5	**11**	2	2	**4**	1	1	**2**
	Oedignatha jocquei	8	15	**23**	6	9	**15**	1	6	**7**
	Sesieutes zhui	5	4	**9**	-	-	-	-	-	-
	Sphingius gothicus	16	6	**22**	-	-	-	-	-	-
	Sphingius penicillus	17	3	**20**	-	-	-	-	-	-

	*Sphingius vivax**	-	-	-	-	-	-	1	-	**1**
Krabi	*Oedignatha* sp.*	-	-	-	-	-	-	1	1	**2**
	Sesieutes aberrans	2	-	**2**	2	-	**2**	-	-	-
	Sphingius punctatus	-	1	**1**	-	-	-	-	-	-
	Teutamus politus	20	19	**39**	1	-	**1**	5	14	**19**
	Teutamus rama	4	3	**7**	-	-	-	-	-	-
Phuket	*Oedignatha spadix**	-	-	-	-	-	-	6	15	**21**
	Sesieutes cf. *minuatus**	-	-	-	-	-	-	2	1	**3**
	Teutamus politus	8	19	**27**	7	16	**23**	30	46	**76**
Total specimens		100	96	**196**	18	30	**48**	50	84	**134**

Data extraction from taxonomic literature can proceed along two major pathways: 1) prospective, where data is mobilized and shared with GBIF as part of the routine publication process, as has been implemented some journals like EJT [13] and ZooKeys [2,8] and some revisionary studies [87]; and 2) retrospective, where data is mined from legacy taxonomic data [11,12]. This retrospective approach was tested in our study by semantically enhancing records from more than 50 legacy taxonomic documents. From these sources, ca. 3000 specimens of the family Liocranidae were structured and mobilized, including more than 1300 records from about 100 treatments of TG taxa (Supplementary Table 1). These data included relevant biodiversity information, such as geographical distribution, date of collection, sex, and number of specimens.

Although the data contained in taxonomical treatments has been curated by specialists and is highly dependable, it is not free from error and methodological bias. Meyer, Weigelt, and Kreft [88], in their study of land plant data available on GBIF, documented data biases in two major groups: coverage (geographical and temporal documentation gaps) and uncertainty (accuracy or credibility). Another bias observed in GBIF, as well as biodiversity studies and funding in general, is related to the taxonomic coverage and over representation of some groups like birds and plants and under representation of megadiverse groups like insects and arachnids [89–92] (Supplementary Figure 2.2; see also Data Aggregation, below).

In our analysis we did not find clear cases of uncertainty bias with the exception of the absence of geographical coordinates that made some of the occurrences spatially ambiguous. However, geographical and temporal coverage bias was observed. Scientists do not sample randomly or evenly from the whole world; therefore, it should be expected that some areas and times are studied more than others. This makes it difficult to distinguish seasonal changes in abundance from uneven sampling effort at different times of the year. Nevertheless, existing records at least indicate the time of year when specimens have been found in the past, and might therefore be found again. Overall, records of TG taxa were not evenly spread throughout the year. For example, zero specimens of *T. politus* are recorded for the month of August, suggesting that this might not be best time of year to search for this species in Thailand (Figs. 2.2-2.3). Although we had planned our sampling during the highest abundance peak (June-July; Fig. 2.3b), logistic constrains forced us to carry our sampling one month later. Nevertheless, we found a total of 188 specimens of this species during our collection, of which 95 were adults. Our results give evidence of the presence of these taxa during this time of the year, suggesting that the variation observed in legacy records is most probably due to temporal coverage bias and must be interpreted with care.

Another temporal coverage bias was observed when assessing specimen contributions per collector (Fig. 2.2b). We found P.J. Schwendinger to be the collector with most specimens contributed to the TG [19–23]; between 1983 and 2009 he collected 231 TG specimens in Thailand. However, most of his specimens, presumably, due to logistics, were reported around June and July, and December. Therefore, temporal distribution patterns, as observed in literature-extracted data (Figs. 2.2 and 2.3), could be an artifact of sampling bias and not necessarily reflect real seasonal variation of the taxa. Even taking into account these methodological biases, we consider specimen records in taxonomic literature to be among the best curated evidence of presence and, to some extent, relative abundances; and for many understudied and megadiverse taxa, this is the only source of specimen records available. Identifying and understanding data biases can help to identify temporal and spatial gaps were further sampling effort is needed.

Fieldwork– Data extracted from taxonomic literature on the family Liocranidae were used to create detailed profiles for the TG. These helped us to plan a collection that specifically targeted the re-collection of these taxa. Our analysis showed that within Southeast Asia, three provinces in Thailand, Chiang Mai, Phuket and Krabi were the best choice for targeting *T. politus* and its relatives.

This selection of times and places, in combination with specific methods for collecting ground spiders showed a high efficiency for sampling the TG. Our one-month expedition captured 134 adult spiders of the TG (Table 1) representing all TG genera previously reported for Thailand and six out of seven liocranid genera reported for this country (only missing Paratus Simon, 1898). In total, 351 adults of the TG had been reported from Thailand [19–23,73]; from these, ca. 200 had been reported in the same provinces we sampled (Chiang Mai, Krabi and Phuket) (Table 1). When comparing only the collections reported for the same months where we sample, we can observe that our approach was much more efficient, collecting 134 adults vs. 48 in literature. We collected a total of nine TG species vs. 14 reported from the same provinces and six reported from the same provinces and times. From these, *Teutamus politus* was the most abundant species in both literature and our study with 66 and 95 adults respectively (Fig. 2.3b).We collect more specimens of this species (188) than all the previous records in literature combined (102 specimens) [19,21]. *Oedignatha* spadix was the second most abundant in our study with 21 adult specimens; *Oedignatha* spadix is previously known only from Indonesia [19].

Data aggregation– The interoperable network of Plazi allows the extracted data to be automatically shared with other biodiversity databases like GBIF. This allows taxonomic literature data to be analyzed together with data from Natural History collections and observation networks. Many studies have explored the limits and capabilities of GBIF data for setting conservation priorities [93–96], modeling [93,97,98], aggregation of different kinds of data and its biases [88,92,95,96,99,100], among others. The major GBIF data domains (institutional collections databases, observation networks, taxonomic literature, and, in some cases, DNA sequence databases), each have their particular biases, but taken together are complementary enough to serve as a basis for building more complete biodiversity knowledge. In the case of the *Teutamus* group, virtually all records in GBIF were originated from digitized collection data with only five records contributed through human observation and one through iBOL [101]. Even in groups where other sources of data are not available, digitized collection data can give important insights on aspects like the group taxonomy and distributions. Two studies in the Amazonia highlight the importance of collection-based data, by aggregating museum specimen data of several unrelated taxa collected in Amazonia comparing their richness, distribution and endemism [102,103]. This approach allowed them to identify undersampling bias taxonomically and spatially, and map priority areas for conservation based on biodiversity data. They also observed that even when individual datasets might be imperfect, the aggregation of different approaches and sources can help to better assess and allocate conservation efforts.

In our study, the addition of records from the taxonomic literature, aggregated with complementary data from other sources available on GBIF, improved the taxonomic, geographic, and seasonal coverage of TG taxa (Table 2), giving us an improved picture of their overall biodiversity pattern. Semantic enhancement of taxonomic literature cannot compete in volume against the millions of records sourced from natural history collections databases and especially observation networks. But records from taxonomic literature may be the only source of data available for the vast portion of biodiversity about which we know very little. In other words, observation network records tend to be copious but dominated by few species, while specimen records from natural history collections and especially taxonomic literature tend to be fewer in number, but are often the only source of data on rare species. The Plazi approach gives free and persistent access to high quality data curated by taxonomic experts that might potentially help to identify and close knowledge gaps for some underrepresented groups.

Observation networks are some of the largest contributors to GBIF in terms of total records, but these tend to be quite limited in taxonomic focus and rarely include any but the most conspicuous and recognizable representatives of small bodied, high diversity groups like spiders. Here we emphasize the usefulness of the Plazi retrospective approach to close those gaps. Comparing a list of the currently valid species of the TG from the world spider catalog [27], the Plazi approach contributed with records on 89 out of 137 species. By contrast, only 41 species of the TG were present in GBIF before our study. Our contributions to the knowledge of these spiders can be also observed in the number of occurrences in GBIF. Literature extracted data on the TG currently represents 470 occurrences in GBIF versus the 180 occurrences that were available from collection-based data, observation and iBOL combined. Our marked-up documents account for 72% of the occurrences of the TG and the genus *Teutamus*, and 85% of records of our target species, *Teutamus politus* (Fig. 2.4). This gives evidence of the complementarity of these data sources and the importance of mobilizing and making publicly available all the specimen data contained in taxonomic literature.

It is worth noting that this complementarity can also mean that some records from literature and digitized collection data could be overlapping. However, ruling out these cases demands unambiguous collection numbers or specimen identifiers; or, in case this number is absent, comparing probable matches by collection date, locality, specimen count, and other data. For the *Teutamus* group, some records available in GBIF do have a unique collection number (e.g. *Teutamus politus* RMNH.ARA.15194). However, these identifiers are not always available (either in GBIF, on literature or on both) making difficult to reconcile data from different sources. Therefore setting unique identifiers and strengthening publication standards must be a top priority for the future [12,105–108]. This will help to generate usable and reliable datasets that can help to observe, study, and ultimately preserve biodiversity.

Structured, digitized specimen data extracted from taxonomic literature remains a small portion of the overall biodiversity data sphere, but it complements more mainstream data sources in important ways and has the potential to grow into a major source of data in its own right. Our study shows the importance of taxonomic literature records that, in combination with data from other sources, contributes to the most complete available assessment of spatial and temporal biodiversity pattern. Using this data for field work planning is but one possible application, but conservation risk assessment and species distribution modeling could be important in this context as well. The Plazi approach makes these data permanently available for others to re-use and add to in ways that we may or may not be able to currently imagine. Despite decades of ambitious and largely successful digitization efforts, much of the knowledge that biologists have accumulated about global biodiversity remains undigitized and unstructured, unqueryable, and difficult to access. The challenges presented by the global biodiversity crisis are daunting, and our best hope for addressing it begins with building a data infrastructure that faithfully represents the knowledge that generations of scientists have accumulated; specimen records from taxonomic literature are a key element in such an infrastructure.

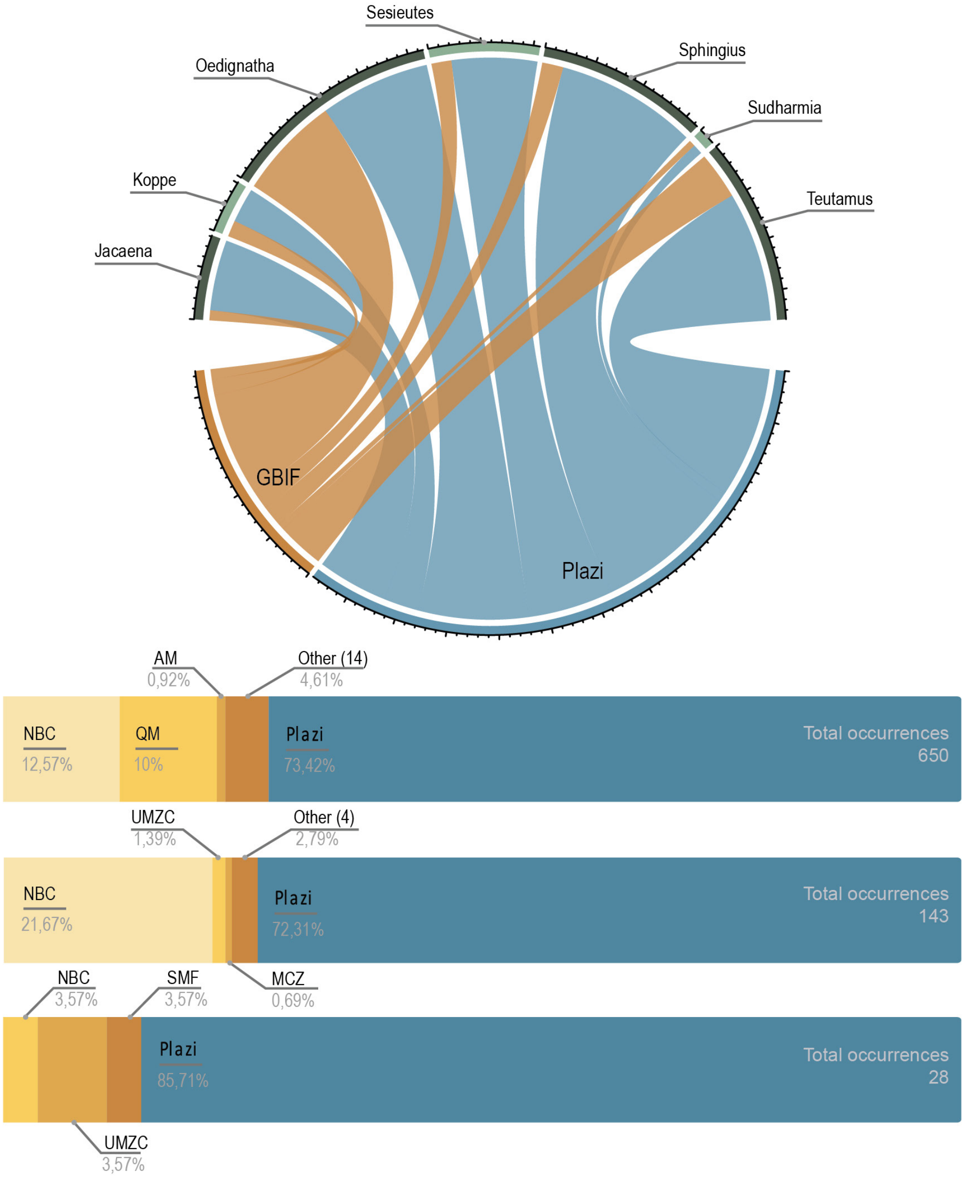

Figure 2.4.–Proportion of occurrences of the *Teutamus* group in GBIF [101]. Color indicates data source: digitized collection data (brown shaded) and taxonomic literature mined data (blue). Circle: Proportion per data source for the whole *Teutamus* group and each TG genera. Generated in RStudio[82,104]. Bars: detail of proportions and total occurrences TG (top), genus *Teutamus* (middle), and *Teutamus politus* (middle). Note the high proportion of data contributed through our mark-up and integration using Plazi's retrospective workflow). Collection abbreviations explained in Table 2.

Table 2.–*Teutamus* group in GBIF per collection/database comparing number of occurrences, total of specimens, geographical distribution and taxonomic coverage. Blue shaded squares indicate presence of each genus. J= *Jacaena*; K= *Koppe*; O= *Oedignatha*; Se= *Sesieutes*; Sp= *Sphingius*; Su= *Sudharmia*; T= *Teutamus*. Collection names: AM– Australian Museum, Australia; CAS– California academy of Sciences, USA; MACN– Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Argentina; MCZ– Museum of Comparative Zoology, Harvard, USA; MNHN–P–Muséum national d'Histoire naturelle-Paris, France; NBC– Naturalis Biodiversity Center (formerly RMNH), The Netherlands; NMNS– National Museum of Nature and Science, Japan; QM– Queensland Museum, Australia; SMF– Senckenberg Museum Frankfurt, Germany; SMNK– Staatliches Museum für Naturkunde Karlsruhe, Germany; UMZC– The University Museum of Zoology, Cambridge, UK; WAM– West Australia Museum, Australia; ZMUC– Zoological Museum, Natural History Museum, Denmark.

Source	Occurrences	Total Specimens	Geographical distribution	Genera representation						
				J	K	O	Se	Sp	Su	T
Plazi (23 documents in GBIF)	467	1035	China, India, Indonesia, Laos, Malaysia, Myanmar, Philippines, Seychelles, Singapore, Taiwan, Thailand, Vietnam	■	■	■	■	■	■	■
NBC	79	166	Indonesia, Malaysia, Sri Lanka, Netherlands, Thailand	■	■	■	■	■	■	■
QM	65	135	Australia, Malaysia, New Caledonia			■				
AM	6	ND	Australia, Papua New Guinea			■				
MCZ	5	5	Indonesia, Malaysia, Thailand	■		■				■
SMF	5	ND	Cambodia, India, Indonesia, Laos	■		■				■
MACN	2	ND	Thailand	■		■				
MNHN-P	2	4	Singapore, Sri Lanka		■		■			
UMZC	2	ND	Malaysia							■
WAM	2	3	Christmas Island			■				
NMNS	1	ND	Vietnam							■
CAS	1	1	Thailand					■		
SMNK	1	ND	Malaysia				■			
ZMUC	1	ND	Thailand							■
TOTAL	639	1349								

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Data accessibility

Extracted data is available from Plazi [14] tb.plazi.org/GgServer/srsStats (refining search as needed) and GBIF [101,109,110]. A list of all the Plazi document UUID used in this study can be found in the Supplementary Table 1.

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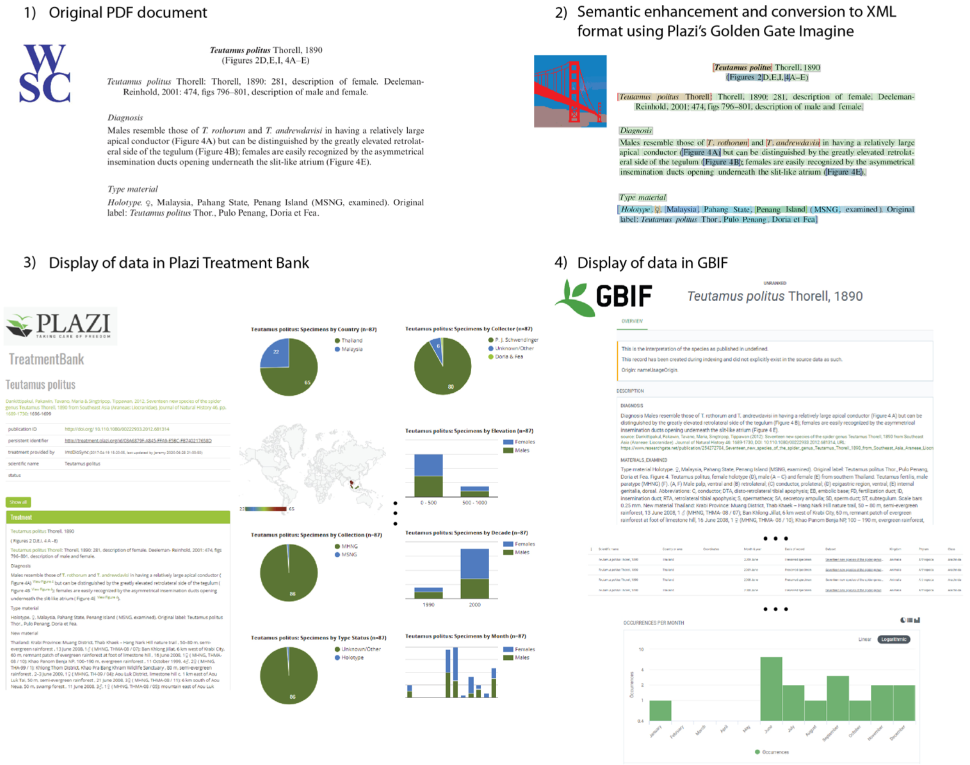
Supplementary Figure 2.1.–Visual summary of the data extraction process for *Teutamus politus* treatment. From Dankittipakul, Tavano, and Singtripop (2012). 1- Taxonomic document in PDF format downloaded from the World Spider Catalog https://wsc.nmbe.ch/species/7486. 2- Conversion to XML format using Golden Gate Imagine. Each color in the figure text represents a semantic tag in the extraction process (Sautter, Böhm, and Agosti 2007). 3- Extracted treatment as displayed in Plazi http://tb.plazi.org/GgServer/html/03A6879FA845FFA9E5BCFB740217658D. To the left, whole treatment text, illustrations and link to the original source, to the right charts and maps based on the specimen data. 4- Specimen data and taxonomic treatment text displayed in GBIF https://www.gbif.org/species/130509488.



1) Original PDF document

Teutamus politus Thorell, 1890
(Figures 2D,E,I, 4A–E)

Teutamus politus Thorell: Thorell, 1890: 281, description of female. Deeleman-Reinhold, 2001: 474, figs 796–801, description of male and female.

Diagnosis

Males resemble those of *T. rothorum* and *T. andrewdavisi* in having a relatively large apical conductor (Figure 4A) but can be distinguished by the greatly elevated retrolateral side of the tegulum (Figure 4B); females are easily recognized by the asymmetrical insemination ducts opening underneath the slit-like atrium (Figure 4E).

Type material

Holotype. ♀, Malaysia, Pahang State, Penang Island (MSNG, examined). Original label: *Teutamus politus* Thor., Pulo Penang, Doria et Fea.

2) Semantic enhancement and conversion to XML format using Plazi's Golden Gate Imagine

Teutamus politus Thorell, 1890
(Figures 2D,E,I, 4A–E)

Teutamus politus Thorell: Thorell, 1890: 281, description of female. Deeleman-Reinhold, 2001: 474, figs 796–801, description of male and female.

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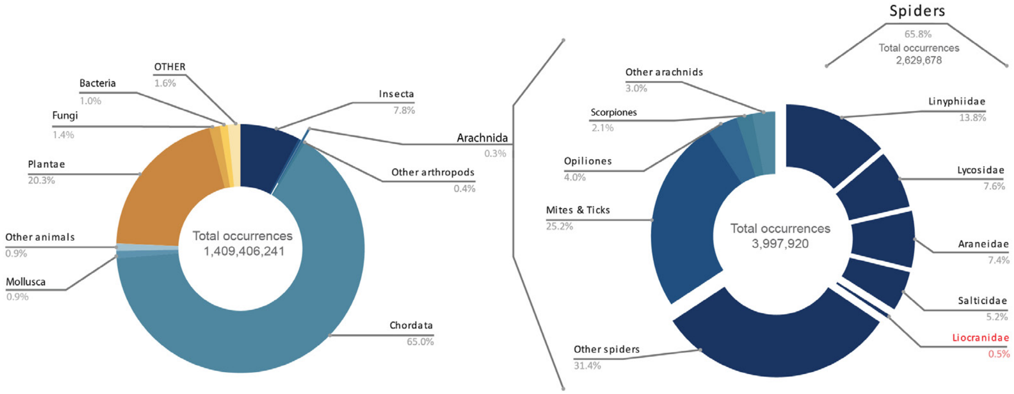
3) Display of data in Plazi Treatment Bank

4) Display of data in GBIF

Supplementary Figure 2.2.–Proportion of GBIF records per taxonomic group. Left circle represent the whole GBIF database. Right circle represent spiders and other arachnids detailing the proportion of the best represented spider families and the Liocranidae (in red).



Supplementary Table 1.–Complete list of processed publications of the family Liocranidae. Plazi UUID code (a unique persistent identifier given to documents), number of liocranid genera and species treated, and specimens listed per study. The UUID can be added to the prefix this prefix “http://tb.plazi.org/GgServer/summary/” to access the index of linked treatments for that source. A list of the references used in this table can be found at the end of this supplementary document.

Study	Article UUID	Non- TG			TG			Total Specimens
		Genera	Species	Specimens	Genera	Species	Specimens	
Barrion and Litsinger 1995	D6116953413AA950FFBB4926252AFFE1	-	-	-	3	3	8	8
Bastawade 2006	E370626DFFC6FFDCFFB4FFFFFFC3E666	-	-	-	1	1	12	12
Bastawade 2002	FFCC8B0CFFA3FF80A43F537CFFC6F93F	-	-	-	1	1	3	3
Bennett, Copley, and Copley 2013	8F7EFFBB8872FFB96C32B8600B71A62C	1	1	114	-	-	-	114
Biswas and Raychaudhuri 2000	FFEEFFB22C17FFEEFF9E8F29FFCBFFD8	-	-	-	1	1	14	14
Biswas and Roy 2008	FFF2D503FFD8154B7903FF201A02FF92	1	1	6	-	-	-	6
Biswas and Biswas 1992	06598512FFD6FFEFFF80FFD3F611FFF8	-	-	-	2	2	9	9
Biswas and Majumder 1995	8F48FF87FF9A9839B635FFD6FF85DD64	-	-	-	1	1	1	1
Bosmans and van Keer 2012	DE294F64CD29FFFCCA795F1BFF93FFAA	1	1	2	-	-	-	2
Bosmans 1999	FFAAFF87FFCDFFD9FF87461AFFA12804	4	7	339	-	-	-	339
Bosselaers 2009	FF805162FFD1F162FFF3F327FF8FD505	4	5	109	-	-	-	109
Bosselaers 2012	FF9CFFB9FB60691AFFEAFF9C85151220	1	1	1	-	-	-	1
Bosselaers et al. 2010	7F15FF9E4C7DFF817E28ED77920EEC4D	1	1	2	-	-	-	2
Candek et al. 2013	622F99677618FFF81D122429FFE4FF91	2	2	6	-	-	-	6
Chen and Huang 2009	FFEEFFF5C520246D3960E4674C4EE517	-	-	-	1	1	24	24
Crespo et al. 2018	FFA7F823FFB5FFF26305FFECFFC5B633	4	9	70	-	-	-	70
Danilov 1998	733D3830FFB3FFFFFFF4FFC8FFAFFF8F	1	3	21	-	-	-	21

Dankittipakul and Deeleman-Reinhold 2012	FA379502FFBEFFB0FFF8FFC06931FFFB	-	-	-	1	1	9	9
Dankittipakul and Deeleman-Reinhold 2013	FFF7FFD5FFEF5F40FF83FFDAFFBE5660	-	-	-	1	9	54	54
Dankittipakul, Tavano, and Singtripop 2011	566E5A667339121BA634FFDC3833FF8F	-	-	-	1	13	79	79
Dankittipakul, Tavano, and Singtripop 2012	FF9FFFE7A842FFA3E422FFF90169654C	-	-	-	1	18	205	205
Dankittipakul, Tavano, and Singtripop 2013	10136F22FFB5FF85AE6DD059FFD39809	-	-	-	1	9	43	43
Deeleman-Reinhold 2001	FF8A860AC93EFFE765528965DD50FFD6; FF8FE1734262FFA4FF8A4255DE29FF94; FFC9FFC63A1BFFDDFFD5FFC6DB37324B	1	1	6	7	38	564	570
Deltshev et al. 2013	FFD9FFE35975FFA9FFF1FFC1FFCDFF82	2	2	2	-	-	-	2
Deltshev and Wang 2016	FFB8FFF5C324E437FFF7FF910D10FC7A	1	1	4	-	-	-	4
Elverici, Özkütük, and Kunt 2013	FFA7C048B65EFFF5AE774A45FF9FF24A	2	2	25	-	-	-	25
Esyunin and Kazantsev 2007	5141AC78642A420DDF7CFFDFFFFB3311	1	1	4	-	-	-	4
Felton, Judd, and Merrett 2004	FFCCFF95D602FF82FF84FF82FFF1E808	1	1	6	-	-	-	6
Fu, Zhang, and Zhu 2009	4C1488709C14A741FF9CFFE8FF98FFB9	1	1	17	-	-	-	17
Hayashi 1992	FF9896040E0FFF90512CFFD1FFE9FFDC	1	2	4	-	-	-	4
Jäger 2007	2E4DFFC7FF823B04FF8434627515FFF5	-	-	-	1	1	1	1
Bosselaers and Jocqué 2013	9641FFF01026FF93FFC1CF4E123CD00A	1	7	227	-	-	-	227
Jonsson 2005	FFB49220FFB2FF91FFD2FFA9C165DB4A	1	1	7	-	-	-	7
Marusik and Koponen 2000	BE33BE057603DB13FFDE024BCB37C220	1	1	1	-	-	-	1
Marusik, Omelko, and Koponen 2016	3F632B49FFD3296A0D5CA5685D01FFB9	1	2	3	-	-	-	3
Marusik, Zheng, and Li 2008	F43D9C12004C613DA434AC5324270C62	1	2	16	-	-	-	16
Namkung 1989	FFBDFFD5063A094CFF937B5D13709146	1	1	3	-	-	-	3
Ono 2009	3513FF9301781725463FFF92FFA2FF89	-	-	-	1	1	1	1
Platnick and Di Franco 1992	4C59FFB0FFE19E6FFFB7FFD0FFEDFF8B	1	6	19	-	-	-	19

Reboleira et al. 2012	D40BFFF1FF9DFFC7FFB3FFD2FFE3B822	2	4	40	-	-	-	40
Reddy and Patel 1993	AF35FFE0FF850E68FFA5145FFFE6FFEB	-	-	-	1	1	3	3
Ribera and de Mas 2015	6864FFE79E0A1704FFFCFF8EFFBAF854	1	9	19	-	-	-	19
Bosmans 2011	FFE1FF97FFF5FFBFFFA5FFC935544357	2	2	11	-	-	-	11
Saaristo 2002	FF809205FFF21921FFC75A4699324D69	-	-	-	1	2	236	236
Sankaran et al. 2017	8618FF9AFF914734FFD0FFCF3A19FF86	1	1	17	-	-	-	17
Seo 2011	FF8BB520FFA9743EFFF97A74FFBAF37E	2	3	9	-	-	-	9
Seyyar et al. 2016	FFA1FFC04B7C6416FFAEE21F200EFFDC	1	1	3	-	-	-	3
Tso et al. 2005	BD41FFEDE627A13FB559E159D628093C	-	-	-	2	2	2	2
Ubick and Platnick 1991	FFB7AC78821CFFC2FFC2FFD7B54DAD40	1	1	41	-	-	-	41
Ubick and Vetter 2005	F117A4286D45FFE7233FFFA2FF906E1E	1	1	109	-	-	-	109
Vetter 2001	FF9BFFDBFFB54A58AC05FFCDFFD2FFAB	1	2	320	-	-	-	320
Warui and Jocqué 2002	FFDE4808FFABFFDCFFEDFFAFF36A181D	1	2	28	-	-	-	28
Wunderlich 2011	FFC1DD35277DCD7FBE6DFE7EFFCAFFE0	2	3	13	-	-	-	13
Zapata and Ramírez 2010	5924FF98422FFFB9FF823E088233FFC8	1	1	1	-	-	-	1
Zhang and Fu 2010	FF806164FFE5FFDF2F48FF90FFF6D054	-	-	-	1	1	15	15
Zhang, Fu, and Zhu 2009	FF8A9941EF368C07FFC8FFC4FFB1A240	-	-	-	1	4	23	23
Zhao and Peng 2013	8163FFFD6B76FFA8FFA5FFEE764AFFB6	1	1	3	2	2	3	6
Zonstein, Marusik, and Omelko 2015	FFA288738C19FFC1FFA7FFD054431A6B	1	1	8	-	-	-	8
		55	**94**	**1636**	**32**	**112**	**1309**	**2945**

Supplementary Table 2.–Detail of our sampling sites in Thailand.

Province	Site details	Geographic Coordinates and elevation	Date
Chiang Mai	Pha Daeng NP. Riparian tropical forest.	19º37.768'N 98º57.257'E, 560m.	16-19 July 2018.
	Pha Daeng NP. Bamboo forest.	19º37.668'N 98º57.131'E, 573m.	16-19 July 2018.
	Pha Daeng NP. Mixed Teak forest.	19º34.320'N 98º57.340'E, 474m.	16-19 July 2018.
	Pha Daeng NP. Dipterocarpus forest.	19º36.132'N 98º56.980'E, 571m.	17-19 July 2018.
	Doi Inthanon NP. Cloud forest.	18º35.268'N 98º29.240'E, 2572m.	21-24 July 2018.
	Doi Inthanon NP. Montane evergreen forest.	18º30.454'N 98º30.584'E, 1605m.	21-24 July 2018.
	Doi Inthanon NP. Mixed pine forest.	18º32.606'N 98º34.479'E, 995m.	21-24 July 2018.
	Doi Inthanon NP. Mixed oak-pine tropical forest.	18º32.436'N 98º31.858'E, 1279m.	21-24 July 2018.
	Doi Suthep NP. Montane evergreen forest with pine.	18º48.502'N 98º53.528'E, 1409m.	24-28 July 2018.
	Doi Suthep NP. Mixed oak tropical forest.	18º48.164'N 98º54.081'E, 1300m.	24-28 July 2018.
	Doi Suthep NP. Mixed bamboo tropical forest.	18º49.045'N 98º55.296'E, 802m.	25-28 July 2018.
	Doi Suthep NP. Dipterocarpus forest.	18º48.780'N 98º55.928'E, 643m.	25-28 July 2018.
Phuket	Ton Sai Waterfall. Mixed bamboo tropical forest.	8º1.673'N 98º22.019'E, 144m.	29 July - 2 August 2018.
	Ton Sai Waterfall. Mixed Kerriodoxa elegans tropical forest.	8º1.816'N 98º22.375'E, 215m.	29 July - 2 August 2018.
	Bang Pae Waterfall. Mixed bamboo tropical forest.	8º2.310'N 98º23.407'E, 135m.	30 July - 3 August 2018.
	Bang Pae Waterfall. Mixed tropical forest.	8º2.353'N 98º23.365'E, 173m.	31 July - 4 August 2018.
	Siray Island. Mixed tropical forest.	7º53.355'N 98º26.083'E, 132m.	2-6 August 2018.
	Siray Island. Rubber plantation.	7º53.384'N 98º26.102'E, 104m.	2-6 August 2018.
	Siray Island. Mixed tropical forest near banana plantation.	7º53.169'N 98º26.108'E, 88m.	3-6 August 2018.
	Siray Island. Mixed tropical forest near rubber plantation.	7º53.409'N 98º26.067'E, 117m.	4 August 2018.
Krabi	Community Forest near Than Bok Khorani NP. Mixed tropical forest.	8º29.536'N 98º44.353'E, 93m.	7-12 August 2018.
	Community Forest near Than Bok Khorani NP. Mixed bamboo tropical forest.	8º29.572'N 98º44.367'E, 85m.	8-12 August 2018.
	Community Forest near Than Bok Khorani NP. Mixed young tropical forest.	8º29.655'N 98º44.001'E, 60m.	9-12 August 2018.
	Community Forest near Than Bok Khorani NP. Oil palm plantation.	8º29.592'N 98º43.907'E, 56m.	9 August 2018.

Chapter 3

Imperfect and askew: A review of asymmetric genitalia in araneomorph spiders (Araneae: Araneomorphae)

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Abstract

Bilateral asymmetry in the genitalia is a rare but widely dispersed phenomenon in the animal tree of life. In arthropods, occurrences vary greatly from one group to another and there seems to be no common explanation for all the independent origins. In spiders, genital asymmetry appears to be especially rare. Most known species show almost perfectly symmetrical genitals with the right and left sides being mirror images of each other. However, some examples of asymmetric genitalia have been studied and many other reports are scattered in the taxonomic literature. Based on a broad literature survey, we found several species in thirteen families with evidence of genital asymmetry, mostly expressed only in females. Our review suggests that spider genital asymmetries, although rare, are more common than previously thought and taxonomic descriptions and illustrations are a useful but not entirely reliable tool for studying them. Here we also document thoroughly the case of the liocranid spider *Teutamus politus*. We collected live specimens of this species to document its genital morphology and to attempt observations of male-female interactions. We consider *T. politus* to be the first known case of directional asymmetry and the first report of morphologically asymmetric male genitals in Entelegynae spiders. Generalities, evolution and categorization of asymmetry in spiders are further discussed.

Keywords: Chirality, sexual selection, antisymmetry, Araneae, Synspermiata, Entelegynae, RTA, Liocranidae.

Introduction

Genital asymmetry is a trait that has evolved independently several times in many animal groups. Invertebrates show a wide range of genital asymmetries with probably thousands of independent origins. Many, sometimes not mutually exclusive, explanations have been proposed, namely: i) morphological compensation for selected changes in mating position; ii) sexually antagonistic co-evolution; iii) cryptic female choice for asymmetric male genitalia; iv) different functions for the left and right side; v) one-sided reduction to save space and resources; vi) functional constraints: to function properly, the separate parts of the genitalia need to connect in an asymmetric fashion [1–4].

Asymmetries are often classified as fluctuating (FA), antisymmetry (AS) or directional (DA) [3,5,6]. This categorization is based on the degree and relative frequencies of the different chiral forms found in a population. FA describes slight asymmetric variation around a symmetrical mean; the appearance of this type of asymmetry is usually related to developmental instability [5,7]. AS, also referred as 'random asymmetry' [8] describes cases where two mirror image forms, dextral and sinistral, are identifiable and within a population, occurring usually in equal or similar proportions [3]. Finally, DA refers to cases where only one asymmetric form is virtually always present [3]; this might be associated with mechanical, behavioral, or functional differentiation and selection of one asymmetrical form of the structures or organs [3,9].

Genital asymmetry, although rare as a whole, is a recurring phenomenon in a few groups of arthropods like mites, crustaceans, opiliones, and several insect orders. However, in spiders (Fig. 3.1a), sexual asymmetries seem to be an exception [1–4,10,11]. In insects, copulatory mechanics and the presence of a single male genital structure located at the posterior end of the abdomen might explain the great incidence of genital asymmetry in this group [1,3,12]. In contrast, spiders have two male copulatory organs derived from a modified pair of leg-like appendages, here called pedipalps, that usually are matched to paired copulatory openings on the female genitalia, here called epigynum (Fig. 3.1b) [13]. Pedialps are normally both used sequentially for sperm transfer during copulation and, in some cases, flexibility on the use of right and left sides has been observed [14,15]. The paired nature of spider genital structures has been hypothesized to act as an "evolutionary buffer" to the development of genital asymmetry, especially on male genitals [1,3,11].

Asymmetries can be catalogued as genetic (larval) and environmental (post-larval) depending on the developmental stage where they are originated [6,8]. In spiders, genital development is only apparent after the last molt. Therefore, the exact moment and mechanism by which asymmetry develops is difficult to interpret. Most cases of asymmetry in spiders have not been studied in detail or even discussed, with the notable exception of pholcids and theridiids [1,3]. Nevertheless, taxonomic illustrations and descriptions reveal asymmetrical genitalia in other families. Genital asymmetry has been documented in male pedipalps (with variation in shape, size or even presence of

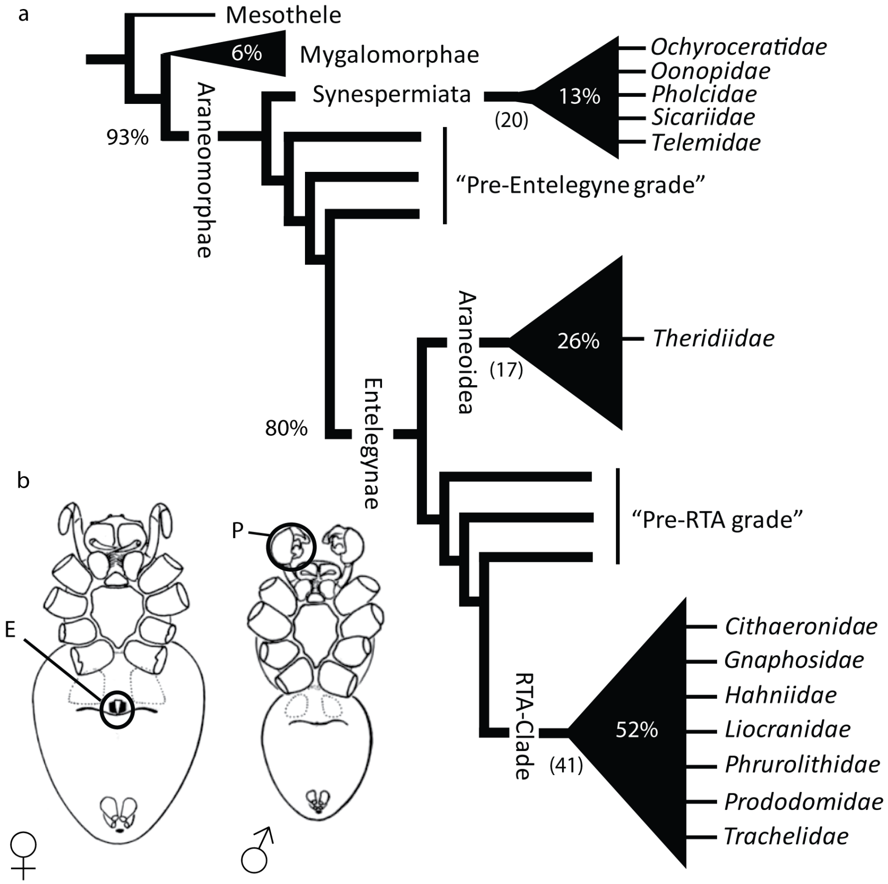

Figure 3.1.–Spider relations and spider genitalia. a) Schematic tree based on a comprehensive spider phylogeny by Wheeler *et al.* [16]. Family names indicate where genital asymmetries have been observed. b) Ventral view of spider copulatory organs: ♀ Female genitalia or Epigynum (E) and ♂ Male Pedipalp (P); modified from Foelix [13]. Number of families per clade are indicated between parentheses. Proportion of species per clade in relation to the Order Araneae is also given.

the copulatory organs) and female epigyna. Female genital asymmetry can be further divided into external (position of copulatory openings) and internal (position and shape of sperm conducting and storing structures).

Several independent origins of genital asymmetry have been found in the spider tree of life. However, all known cases have been reported in two major clades: Synspermiata and Entelegynae that include about 13% and 80% of known spider diversity, respectively (Fig. 3.1a). Morphologically, Synspermiata spiders tend to have structurally simpler genitalia than entelegyne spiders in both sexes. Asymmetries in Synspermiata have been documented in two families: Pholcidae (Fig. 3.2a, h) and Oonopidae (Fig. 3.2e, g). Additionally, taxonomic descriptions and illustrations of some Ochyroceratidae (Fig. 3.2b, d), Telemidae (Fig. 3.2f) and Sicariidae (Fig. 3.2c) depict female genital asymmetry too. In Entelegynae, examples appear more scattered and have been documented in two clades: Araneoidea and the RTA (*sensu* Wheeler et al. [16]). In the former, all known cases are found in the family Theridiidae (Fig. 3.3a–c) and in the latter, several examples have been illustrated in at least six families (Fig. 3.3b, d–h). Explanations for genital asymmetry in spiders are diverse and could include individual variation, natural selection, or sexual selection [1,3,11,15,17].

Spider genital asymmetry can be classified as follows: Fluctuating asymmetry (FA) is probably the most common type and has been documented in some Lycosidae [31–37], Pholcidae [38], and Oxyopidae [11,39]. Other examples of seemingly asymmetric structures like the pedipalps of the one known male specimen of *Pimoa petita* [40] or the numerous documented anomalies and deformities [41–44] might easily be explained by developmental malformations (Fig. 3.4).

Antisymmetry (AS) is the second most common form of asymmetry in spiders and has been documented in three genera of the Theridiidae (Asygyna, *Echinotheridion*, and *Tidarren*) (Fig. 3.3a, c) [24,45,46]; one genus of Pholcidae (Metagonia) (Fig. 3.2a, h) [23]; one genus of Phrurolithidae (Scotinella) (Fig. 3.3b) [47] and scattered cases such as in Trachelidae (Fig. 3.3f) [29,48,49], Cithaeronidae (Fig. 3.3h) [50] and other RTA families. Directional asymmetry (DA) is the rarest type and, until now, it had only been reported in the pholcid *Metagonia mariguitarensis* (Fig. 3.2h) [9]; DA has also been implied in some descriptions within the Oonopidae (Fig. 3.2e) [21,51], and in the liocranid *Teutamus politus* female genitalia [52]. All of these, other isolated reports, and scattered descriptions and illustrations, suggest that genital asymmetries in spiders have originated independently several times and their study might give better insights into how and when this phenomenon has evolved and the selective mechanisms behind it.

A particularly interesting example is the Liocranidae where two different types of asymmetry are present [52–54]. For example, *Jacaena mihun* (Fig. 3.3g) shows no external chirality, but internally the asymmetric copulation ducts are highly variable among individuals. Another example, *Teutamus politus* (Fig. 3.5–3.7), shows external asymmetry in the female genitalia with both copulatory openings fused together in one atrium placed on the left side of the epigyne (see Deeleman-Reinhold [52]: fig 800, 801). Deeleman-Reinhold [52] mentioned female asymmetry as a diagnostic character for this species and noted that in all six of the specimens available for examination, the atrium is located in the left side. A revision of the genus *Teutamus* [53] also included external asymmetry in the female genitalia as a diagnostic character for *T. politus*, and expanded the sample of specimens examined; asymmetry in male pedipalp was not reported in either of these studies.

Here we present a general review of genital asymmetries in the spider literature, grouping them in previously described categories of genital asymmetry and discussing the existence of a new category of female genital asymmetry (here called Chaotic Asymmetry). We also analyzed the specific case of the species *Teutamus politus* by collecting new specimens in Thailand and documenting male and female genitalia using

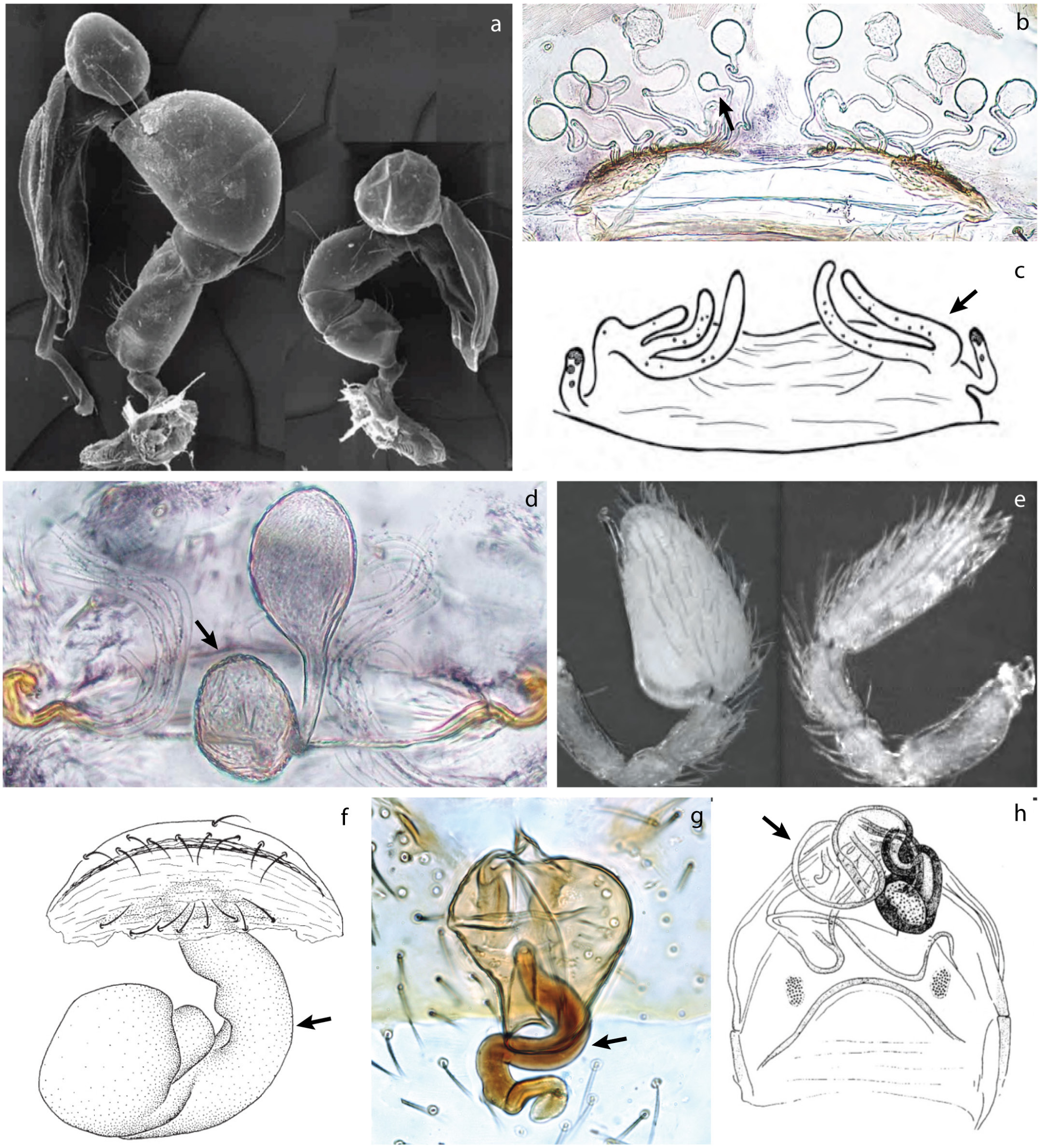

Figure 3.2.–Examples of genital asymmetry in Synspermiata. a, e) male pedipalps, lateral view. b–d, f–h) vulva, dorsal view. a) Pholcidae: *Metagonia mariquitarensis*; modified from Huber [9]. b) Ochyroceratidae: *Althepus naphongensis*; modified from Li *et al.* [18]. c) Sicariidae: *Hexophthalma albospinosa*; modified from Magalhaes and Brescovit [19]. d) Ochyroceratidae: *Speocera cattien*; modified from Tong, *et al.* [20]. e) Oonopidae: *Paradysderina righty*; modified from Platnick and Dupérré [21]. f) Telemidae: *Telema exiloculata*; modified from Lin and Li [22]. g) Oonopidae: *Triaeris stenaspis*. h) Pholcidae: *Metagonia delicata*; modified from Huber [23]. Arrows indicate the asymmetric structure: b, c: number and development of spermathecae. d: size of spermathecae. f–g: direction of seminal receptacle.

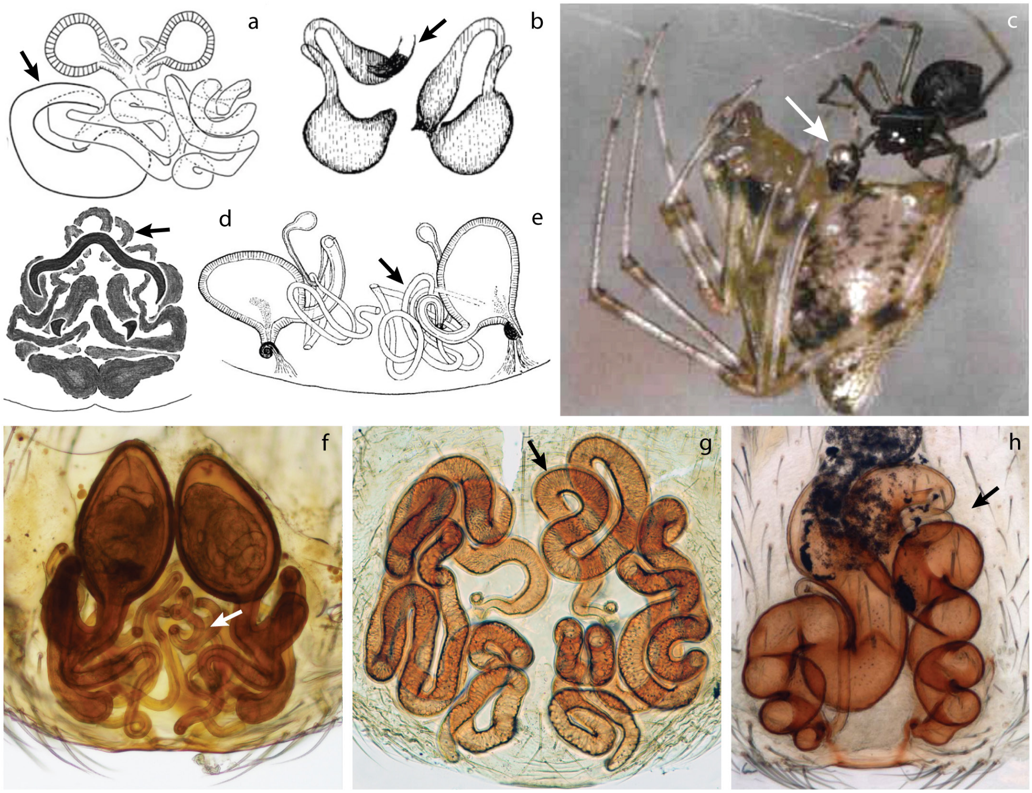

Figure 3.3.–Examples of genital asymmetry in Entelegynae. a, b, d–h) vulva, dorsal view. c) male and female during copulation. a) Theridiidae: *Asygyna coddingtoni*; modified from Agnarsson [24]. b) Phrurolithidae: *Scotinella fratella*; modified from Dondale and Redner [25]. c) Theridiidae: *Tidarren sisyphoides*. [26]. d) Gnaphosidae: *Apopyllus gandarella*; modified from Azevedo *et al.* [27]. e) Hahniidae: *Iberina difficilis* ; modified from Harm, 1966 [28]. f) Trachelidae: *Trachelas ductonuda*; modified from Rivera-Quiroz and Alvarez-Padilla [29]. g) Liocranidae: *Jacaena mihun*. h) Cithaeronidae: *Cithaeron praedonius*; modified from Ruiz and Bonaldo [30]. Arrows indicate the asymmetric structure: a, d–h: copulatory ducts. b: copulatory openings. c: male pedipalp.

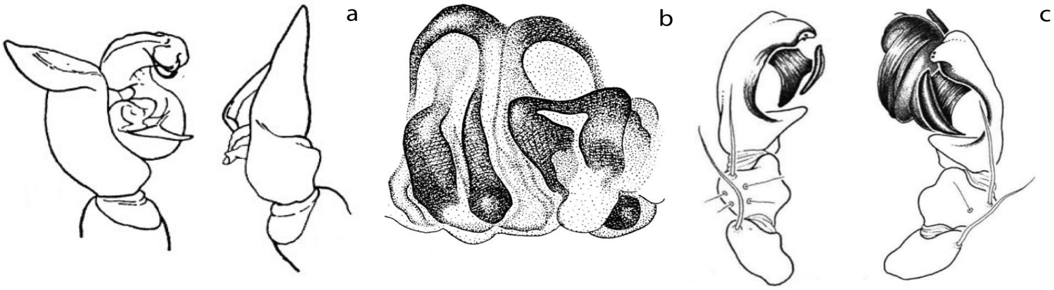

Figure 3.4.–Examples of genital malformation in spiders. a, c) male pedipalps, posterior-lateral view. b) vulva, ventral view. a) *Lycosa ammophila*; modified from Kaston [42]. b) *Pardosa sagei*; modified from Kaston [42]. c) *Pimoa petita*; modified from Hormiga [40].

diverse morphological methods. This gives evidence of the first cases of both directional asymmetry in males and females, and developmental male genital asymmetry in Entelegynae spiders.

Material and Methods

Literature review— We performed an informal search in taxonomic literature of several Synspermiata and Entelegyne families. Selection of publications was initially based on reported cases in literature [1,3,9,11,12] and then expanded depending on the occurrences found within each family. We did not contemplate cases of FA but this type of asymmetry is included in our discussion. We considered *T. politus* as a good model for testing basic hypotheses on genital asymmetry because of the clear external and internal morphology of female genitalia and Deeleman-Reinhold's [52] note suggesting this could be a case of DA. Furthermore, we hypothesized that morphological or behavioral compensation for female genital asymmetry could be found in the male.

We considered male asymmetry as those cases that result in clear morphological differences between right and left pedipalp regardless of having a developmental or behavioral origin. Based on this, we also considered the pedipalp amputation that males of *Echinoitheridion* and *Tidarren* perform on themselves in our review; especially since the asymmetry has clear adaptive and evolutionary implications [15,46,55–57].

Fieldwork— We selected study sites and collecting dates based on the relative numbers of collected adult specimens of *T. politus* mentioned in the literature [52,53]. Fieldwork was carried out in Thailand between July 29th and August 12th 2018; here we sampled 12 sites in total: eight in Phuket Island (8º1.673'N 98º22.019'E, 144m; 8º1.816'N 98º22.375'E, 215m; 8º2.310'N 98º23.407'E, 135m; 8º2.353'N 98º23.365'E, 173m; 7º53.355'N 98º26.083'E, 132m; 7º53.384'N 98º26.102'E, 104m; 7º53.169'N 98º26.108'E, 88m; 7º53.409'N 98º26.067'E, 117m) and four more in Krabi Province (8º29.536'N 98º44.353'E, 93m; 8º29.572'N 98º44.367'E, 85m; 8º29.655'N 98º44.001'E, 60m; 8º29.592'N 98º43.907'E, 56m). We attempted to cover a variety of vegetation types ranging from relatively well preserved mixed forests to rubber and oil palm plantations. In each site we processed leaf litter using Winkler extractors and direct collecting on ground, among leaf litter and under rocks and logs. Hand collected specimens were kept alive in individual tubes. Winkler specimens were collected in a mixture of propylene glycol and 96% ethanol. All *Teutamus* specimens used in this study were collected under permit 5830802 emitted by the Department of National Parks, Wildlife and Plant Conservation, Thailand. Specimens were deposited in the collection of the Naturalis Biodiversity Center, Leiden, The Netherlands (RMNH.5084632–RMNH.5084651), and the Natural History Museum of the National Science Museum, Thailand (THNHM-I-12251–12252).

Behavioral observations— Live specimens were kept individually in clean 15ml Falcon tubes and fed with termites every two days. Seventeen males and 19 females were selected and assigned unique numbers. Couples were formed preferably with specimens from the same locality. Spiders were placed in a Petri dish (diameter 5 cm, height 1 cm); each dish was divided by a paper wall with a small opening so spiders could roam freely but flee in case of aggression. Each couple was kept in the dish under constant observation for a period of about three hours. Travel logistics and specimen sensitivity (especially of males) to environmental changes, did not allow to further test different times and conditions. After observations, all specimens were sacrificed and stored in 96% ethanol (see Appendix 1 p.92 for more information on the mating trials).

Morphological methods— Somatic characters and male sexual structures were photographed using a Leica MI6SC Stereomicroscope equipped with a Nikon DS-Ri2 camera. Female genitalia were dissected, digested using a pancreatine solution [58], cleared with methyl salicylate. Observations were made using semi-permanent slide preparations [59] in a Leica DM 2500 microscope with the same camera as above. Male genitals were expanded using 10% KOH and distilled water in three 3 min. cycles leaving the pedipalps in distilled water overnight to stabilize them for photography (modified from Shear [60]). Female epigyna and male pedipalps were prepared for SEM and mounted following Alvarez-Padilla and Hormiga [58] SEM images were obtained using a JEOL JSM-6480LV electron microscope.

The following abbreviations are used in the text and figures: **Female genitalia**: A, atrium; Cd, copulatory ducts; Co, copulatory openings; Fd, fertilization ducts; Sa, secretory ampullae (*sensu* Dankittipakul, *et al*. [53]); S, spermatheca. **Male genitalia**: B, male pedipalp bulb; Cy, cymbium; C, pedipalp conductor; E, embolus; Fe, femur; H, basal hematodocha; Pa, patella; RTA, tibia retro lateral apophysis; Sd, sperm duct; sT, sub tegulum; T, tegulum; Ti, tibia.

Results

Literature review—We reviewed publications that directly focus on genital asymmetry as well as taxonomic literature that allusively describe or illustrate asymmetrical morphology. We found more than 150 species across thirteen spider families with indications of asymmetric genitalia (Table 1) representing less than 0.3% of all spider species World Spider Catalog (WSC) [61]; and about 13.5% of all the currently valid species in the genera reviewed for this study. Synspermiata has at least five families (Ochyroceratidae, Oonopidae, Pholcidae, Sicariidae and Telemidae) where some kind of asymmetry has evolved accounting for ca. 90 species (Table 1). Asymmetry was found in both female and male genitalia; female asymmetry is more frequent, being found in at least five oonopid, three sicariid, two pholcid and two ochyroceratid genera. In addition, most genera in the Telemidae have evolved a single sac-like seminal receptacle; some species show seemingly asymmetric modifications of this sac, leaning and

Table 1.–Spider taxa with genital asymmetry reports in literature.

Family	Species	External/ Internal	Female / Male	Type of asymmetry	Distribution	Source
Synspermiata						
Oonopidae	*Aschnaoonops marta*	E	M	AS/DA?[1]	Neotropical	Platnick *et al.* [65]
	Aschnaoonops meta	I	F	AS?[1,3]	Neotropical	“
	Escaphiella (8 spp)	E	M	AS/DA?[1]	Neotropical	Platnick and Dupérré [51]*
	Lionneta (2 spp)	I	F	AS/FA?[2,3]	Seychelles	Saaristo [66]
	Ischnothyreus (whole genus?)	I	F	AS/CA/FA?[3]	Tropical Asia	Edward and Harvey [67]; Tong *et al.* [68]; Brescovit *et al.* [69]
	Paradysderina (10 spp)	E	M, F	AS/DA?[1]	Neotropical	Platnick and Dupérré [21]*
	Reductoonops (2 spp)	I	F	AS?[1]	Neotropical	Platnick and Berniker [70]
	Triaeris (5 spp)	I	F	DA?[3]	Pantropical	Platnick *et al.* [71]
Ochyroceratidae	*Althepus* (5 spp)	I	F	AS/FA?[1,2,3]	South-East Asia	Deeleman-Reinhold [72]; Li *et al.* [18]
	Speocera (8spp)	I, E	M, F	AS?[3]	Pantropical	Lin, *et al.* [22];Tong and Li [64]; Tong *et al.* [20]
Pholcidae	*Mesabolivar yuruani*	I	F	DA?[1]	Venezuela	Huber [17]
	Metagonia (9 spp)	I	F	AS	South America	Huber [23]*;Ferreira *et al.* [73];Huber [74] Huber *et al.* [75]; Machado, Ferreira and Brescovit [76]; Perez-Gonzalez and Huber [77]
	Metagonia mariguitarensis	E male/ I female	M, F	DA	Brazil	Huber [9]*
	Panjange lanthana group (3 spp)	E	M	DA?[1]	Philippines	Huber [11]

Sicariidae	*Hexophthalma* (3spp)	I	F	FA?[3]	South America	Magalhaes, Brescovit, and Santos [19]
	Loxosceles (4spp)	I	F	FA	North to South America; Africa	Gertsch and Ennik [78]*; Lotz [79]
	Sicarius (14 spp)	I	F	FA	South America	Magalhaes, Brescovit, and Santos [19]*
Telemidae	*Cangoderses christae*	I	F	AS/FA?[1,3]	Côte d'Ivoire	Wang and Li [80]
	Kinku turumanya	I	F	AS/FA?[3]	Ecuador	Dupérré and Tapia [62]
	Telema (14 spp)	I	F	AS/FA?[1,3]	East and South-East Asia	Wang and Li [63]; Wang and Li [81]; Lin and Li [82]; Lin, Pham and Li [22]
Entelegynae						
Araneoidea						
Theridiidae	*Asygyna* (2 spp)	E, I	F	AS	Madagascar	Agnarsson [24] *
	Echinotheridion (whole genus)	E	M	AS	Neotropical	Knoflach [46]*
	Tidarren (whole genus)	E	M	AS	America, Tropical Africa	Knoflach and van Harten [15]*
RTA						
Cithaeronidae	*Cithaeron* (2 spp)	I	F	CA	South America, South-East Asia, North Africa	Platnick [83]*; Platnick and Gajbe [50]; Ruiz and Bonaldo [30]
Hahniidae	*Neoantistea* (2 spp)	I	F	AS/CA/FA?[1,2,3]	Nearctic	Opell and Beatty [84]
	Hahnia (3 spp)	I	F	AS/CA/FA?[3]	Palearctic	Harm [28]
	Iberina mazarredoi	I	F	AS/CA/FA?[3]	Spain	Ledoux [85]
	Mastigusa (2 spp)	I	F	CA?[1,3]	Northern Europe	Almquist [86] Azarikina and Trilikauskas [87]
Gnaphosidae	*Apopyllus* (9 spp)	I	F	CA/FA?[3]	Neotropical	Azevedo, *et al.* [27]

Liocranidae	*Jacaena mihun*	I	F	CA	Thailand,	Deeleman-Reinhold [52]; Dankittipakul, *et al.* [54]
	Teutamus politus	E, I	M[a], F	DA	Thailand, Malaysia	Deeleman-Reinhold, [52]*; Dankittipakul, *et al.* [53] and this study*
	Teutamus (4 spp)	E, I	F	AS/FA? [2]	Sumatra	Deeleman-Reinhold [52]*; Dankittipakul, *et al.* [53]
Phrurolithidae	*Scotinella* (2 spp)	E, I	F	AS	USA	Penniman [47]
Prodidiomidae	*Moreno ramirezi*	I	F	CA? [3]	Argentina	Platnick, Shadab, and Sorkin [88]
Trachelidae	*Trachelas* (7 spp)	I	F	CA? [1,2,3]	North and Central America	Platnick and Shadab [49]; Platnick and Shadab [48]; Rivera-Quiroz and Alvarez-Padilla [29]
Lycosidae	*Several cases of FA, here we list a few examples:*					
	Delirosa karadagensis	E	F	FA	Ukraine	Kovblyuk [36]
	Geolycosa latyfrons	E	F	FA	USA	Wallace [35]
	Tsamanicosa subrufa	E	F	FA	Australia	Framenau and Baher [37]

Summary of cases and types of spider genital asymmetry, mostly from taxonomic literature. (AS, antisymmetry; CA, chaotic asymmetry; DA, directional asymmetry; FA, fluctuating asymmetry). Tenuous asymmetry categorizationsindicated by: ?[1] small sample sizes, ?[2] imprecise illustrations, ?[3] information ambiguous or incomplete. * indicates where intraspecific variation is reported. [a] described in the present work.

sometimes spiraling to one side (Fig. 3.2; fig. 5 [62]; fig. 7a,b [63]). However, intraspecific variation has not been documented. Male asymmetry is less common, being found in three oonopid (*Aschnaoonops*, *Escaphiella*, and *Paradysderyna*) and two pholcid (*Metagonia* and *Panjange*) genera, and ambiguously suggested for two ochyroceratid species [20,64]. Nevertheless, it is prevalent in *Escaphiella* and *Paradysderina*, where about 20 species show apparent directional asymmetry in male pedipalps (Fig. 3.2e).

In Entelegynae, more than 60 species in eight families show genital asymmetry. Almost half of the cases were found in the Theridiidae with ca. 35 species in three genera (*Asygyna*, *Echinotheridion*, and *Tidarren*). The rest are scattered among seven families in the RTA clade (Cithaeronidae, Hahniidae, Gnaphosidae, Liocranidae, Phrurolithidae, Prodidiomidae, and Trachelidae) (Table 1). Most genital asymmetry reports in Entelegynae include only female genitalia. From these, internal asymmetry was the most common, showing a wide range of variation on spermathecae and copulatory ducts (Fig. 3.3d–h). In comparison, external asymmetry was not as usual being found only in *Asygyna* (Fig. 3.3a), *Scotinella* (Fig. 3.3b) and *Teutamus* (Fig. 3.7a, d). Male genital asymmetry in Entelegynae had only been reported in the theridiids *Echinotheridion* and *Tidarren* (Fig. 3.3c); these two genera exemplify a unique behavior that results in genital mutilation. Developmental asymmetry, rather than behaviorally induced, had never been described in Entelegynae literature before this work.

Remarks on *Teutamus politus* Thorell, 1890

(Figures 3.5–3.7, S1–S2)

A total of 60 female and 35 male specimens were collected in Thailand. The whole series of specimens were used for external female genitalia and male pedipalps observation and comparison. All the specimens showed the same direction in the genital asymmetry. Five females and five males had their genitals dissected and prepared for detailed examination. Additional images documenting external intra-specific genital variation using standard views of the genitalia can be found in the supporting information files (S1 and S2).

Male genital morphology— All pedipalp segments with the exception of the bulb (B) seem to be completely symmetrical. Bulbs show at least three clear asymmetries between the right and left sides: i) the left conductor (C) is conical and straight (Fig. 3.5f, 3.6b), slightly pointing towards the cymbium (Cy) in lateral view (Fig. 3.5d, f); while the right C is flattened, hook-shaped (Fig. 3.6c) and pointing away from the Cy in lateral view (Fig. 3.5a, c); ii) the left side has a flatter and wider tegulum (T) (Fig. 3.5f) projected anteriorly in retrolateral view (Fig. 3.5e); and iii) the left B is slightly wider than the right one (Fig. 3.5b, e; c, f; 3.6a). There is no apparent difference in the length and shape of the emboli (E) or the spermatic ducts (Sd).

Female genital morphology— Externally, the epigynal plate is flattened and fused to the ventral scutum (Fig. 3.7a). Copulatory openings (Co) are placed close together, forming an atrium facing the left side of the venter and located anteriorly to the bean-shaped spermatheca (Fig. 3.7a–c). Left spermatheca is slightly shorter than right one (Fig. 3.7c). Copulatory ducts (Cd) are equally long. Right Cd anterior to the right spermatheca, left Cd located in between both spermathecae (Fig. 3.7c, e). Asymmetric attachment of Cd to spermathecae with the right being anterior to that of the left one (Fig. 3.7b, c). Both Cd have secretory ampullae (Sa) close to their middle portion (Fig. 3.7b, c). Fertilization ducts (Fd) are short and simple, originating from the posterior end of the spermatheca and pointing in the same direction (Fig. 3.7e).

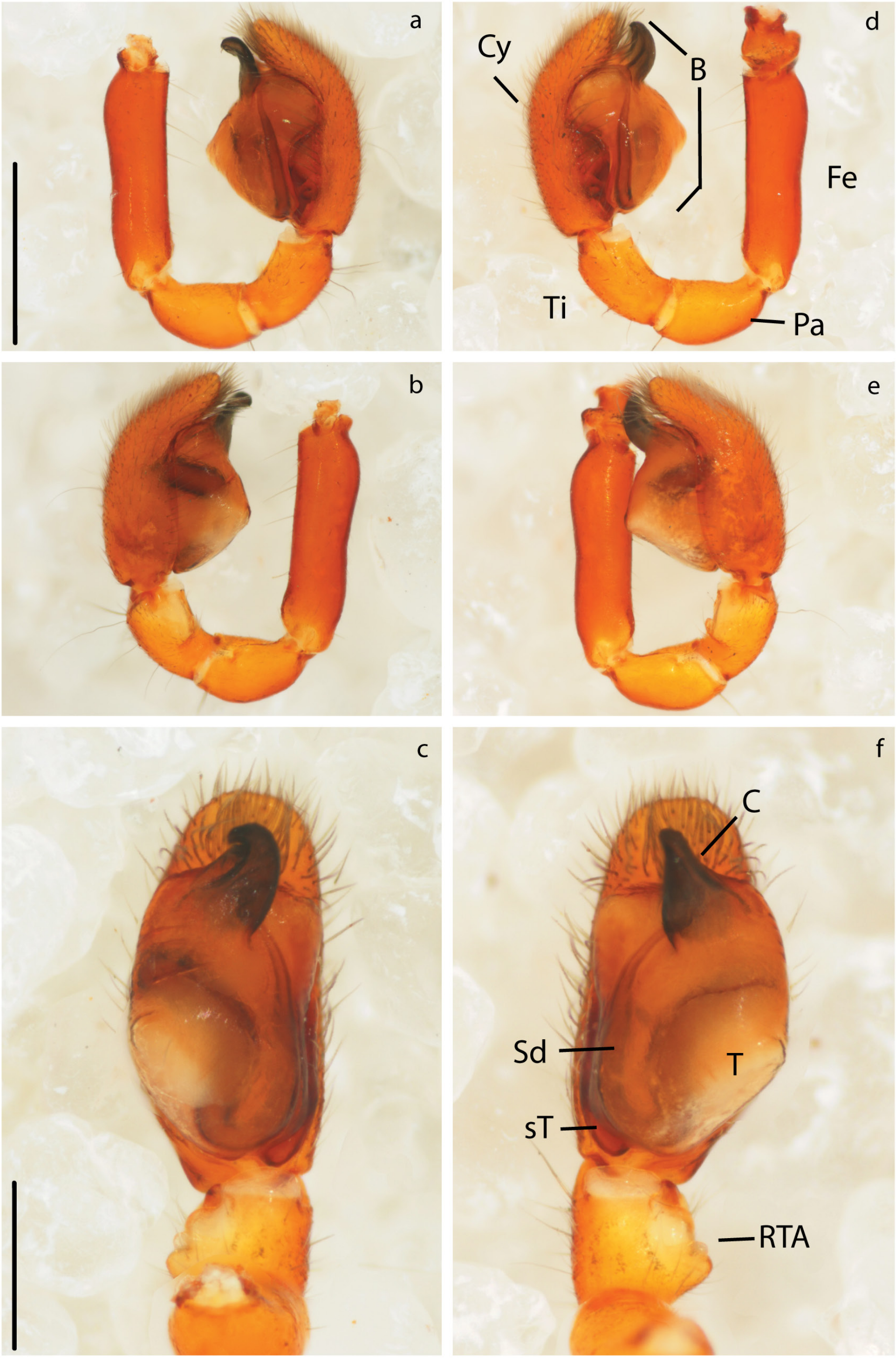

Figure 3.5.–Male genitalia of *Teutamus politus* (unexpanded). Right pedipalp: a) prolateral view. b) retrolateral view. c) ventral view. Left pedipalp: d) prolateral view. e) retrolateral view. f) ventral view. Scale bars: **a**, **b**, **d**, **e** = 0.5 mm. **c**, **f** = 0.25 mm. B – Bulb; C – Conductor; Cy – Cymbium; Fe – Femur; Pa – Patella; RTA – Retrolateral tibial apophysis; Sd – Spermatic duct; sT – Subtegulum; T – Tegulum; Ti – Tibia.

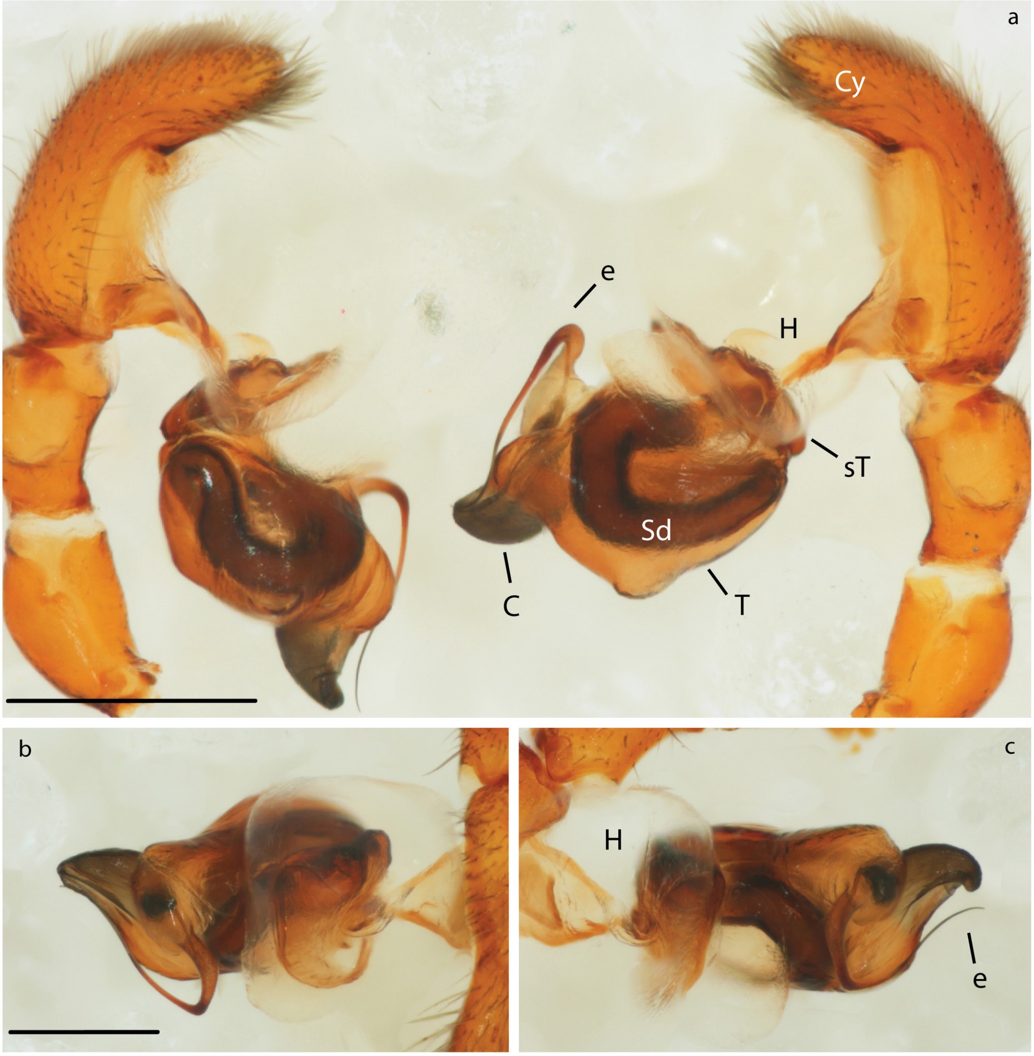

Figure 3.6.–Male genitalia of *Teutamus politus* (expanded). a) comparative retrolateral view. b) left pedipalp prolateral view. c) right pedipalp prolateral view. Scale bars: a = 0.5 mm. b, c = 0.25 mm. C – Conductor; Cy – Cymbium; e – Embolus; Fe – Femur; H – Hematodocha; Sd – Spermatic duct; sT – Subtegulum; T – Tegulum.

Behavioral observations— A total of 25 different couples were tested. Initially couples were formed with males and females from the same collection site. Males were more difficult to keep alive than females with most males dying within three days of collection. Due to this, males and females from different sites were also coupled. There were no successful observations of either courtship or mating. Spiders preferred to explore the dish or stand still and, whenever they got too close, they usually avoided each other. In general, interactions between females and males were brief and non-aggressive. Four females laid egg sacs in the Falcon tubes.

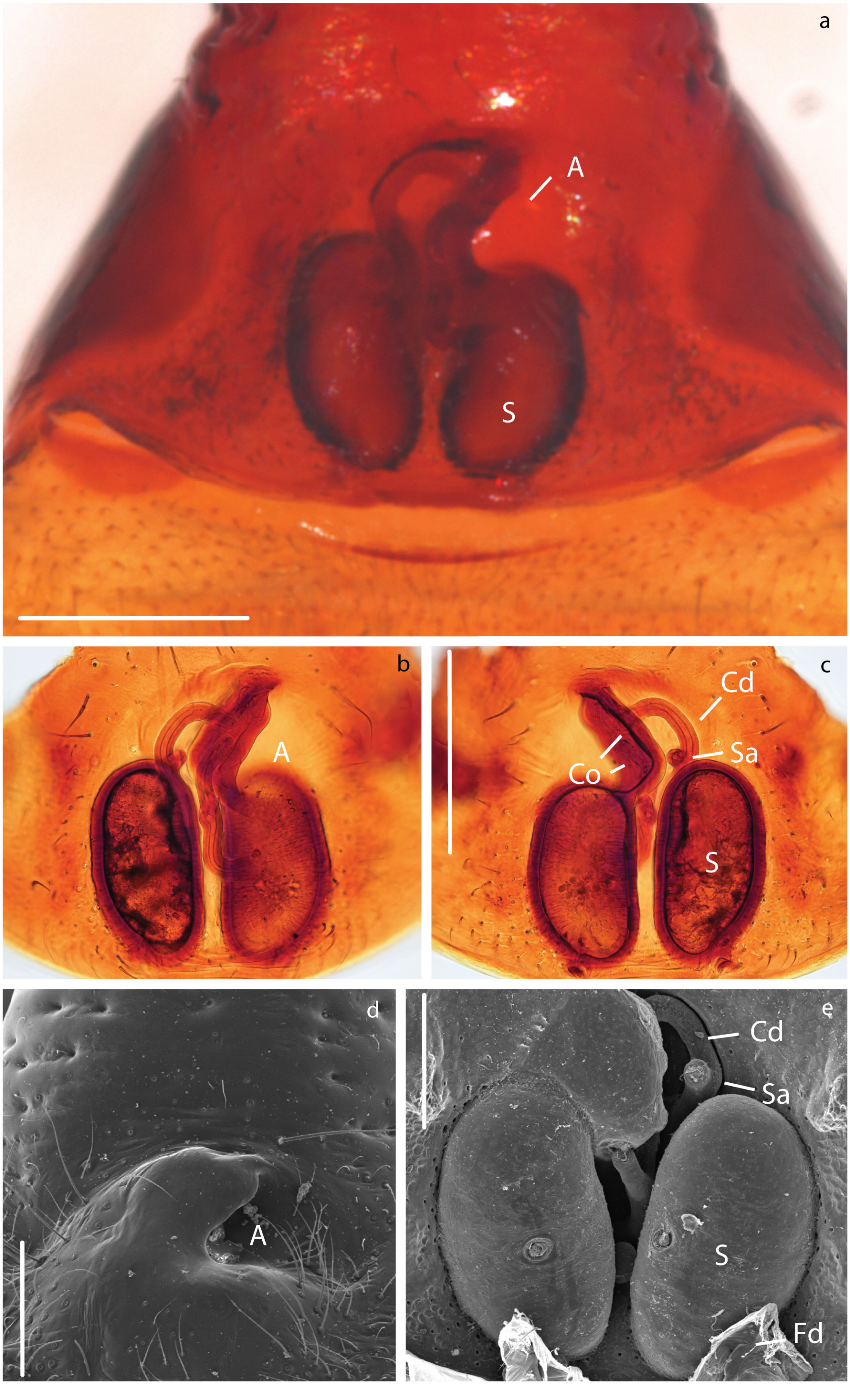

Figure 3.7.–Female genitalia of *Teutamus politus*. a) epigynum ventral view. b) dissected and cleared vulva ventral view. c) same, dorsal view. d) vulva, ventral view, SEM. e) same, dorsal view. Scale bars: **a**, **b**, **c** = 0.25 mm. **d** = 150 um. **e** = 100 um. A – Epigynal atrium; Cd – Copulatory duct; Co – Copulatory opening; Fd – Fertilization duct; S – Spermatheca; Sa – Sececretory ampullae.

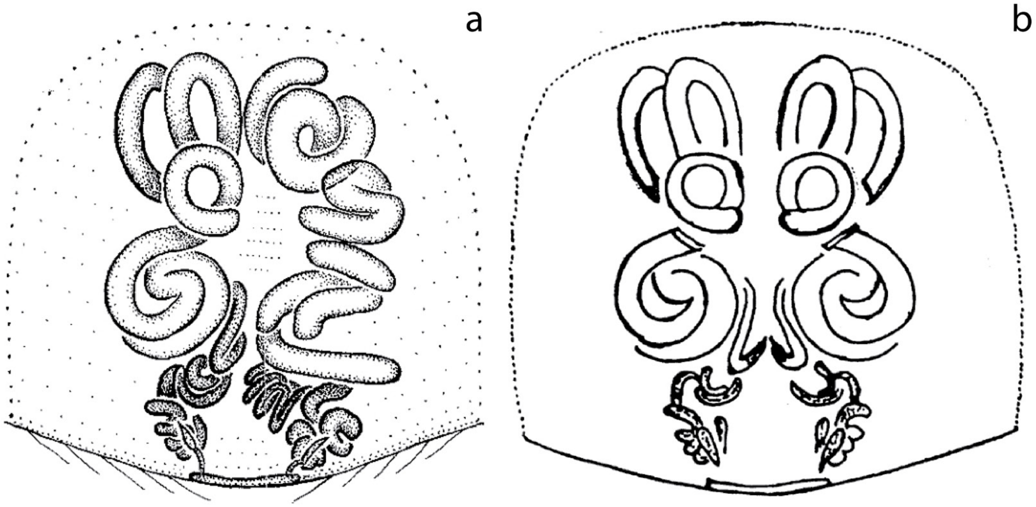

Figure 3.8.–Example of illustration bias. Vulva, ventral view. a) *Cithaeron indicus*; modified from Platnick and Gajbe [50]. b) Same; modified from Gajbe [89].

Discussion

Literature review—The taxonomic literature is the biggest repository of primary descriptive data on the world's biodiversity. However, illustrations and description are difficult to interpret and might be influenced by the number of studied specimens, state of preservation, preparation artifacts and even illustration techniques. As an example, the species *Cithaeron indicus* shows clear asymmetric female genitalia in its original description [50] but appears symmetrical in a later publication [89] (Fig. 3.8). Illustrators sometimes avoid introducing variation by drawing one half of a given structure and then tracing the other side based on it. This might simplify understanding and drawing some structures but could also lead to overlooking important information in the illustration process. Similar biases have been observed in some species of *Trachelas* [48,49] and could be present elsewhere. As pointed out by Huber and Nuñeza [11], preparation artifacts might also play a role in the identification and interpretation of asymmetric structures. Weakly sclerotized internal genitalia (as that typically found in non-Entelegynae spiders) are often prone to create artifacts during specimen preparation and an interpretation without sufficient knowledge of intraspecific variation might be misleading. Entelegyne spiders tend to have more heavily sclerotized bodies being less prone to artifacts during the preparation process and allowing a more robust interpretation of their genital morphology.

Descriptions of male spider genitalia are also subject to preparation artifacts or methodological biases. Male genitalia preparation and examination is usually done by dissecting, studying and illustrating only one pedipalp. Although this is a very efficient approach and does not represent a problem on most occasions, some cases of asymmetric

Table 2.–Number of specimens examined per species in literature.

Family	Species	Females	Males	Type of asymmetry	Source
Synspermiata					
Oonopidae					
	Escaphiella gertschi	446	285●	DA	Platnick and Dupérré [51]
	E. itys	529	220●	DA	“
	E. tayrona	27	28●	DA	“
	E. betin	17	18●	DA	“
	E. acapulco	3	1●	?	“
	E. catemaco	7	4●	DA?	“
	E. chiapa	52	32●	DA	“
	E. Colima	-	2●	DA?	“
	Paradysderina asymmetrica	4	7●	DA	Platnick and Dupérré [21]
	P. boyaca	1	2●	DA?	“
	P. carrizal	0	11●	DA	“
	P. chinacota	0	1●	DA?	“
	P. fusiscuta	2	1●	DA?	“
	P. lefty	2●	1●	DA?	“
	P. monstrosa	2	6●	DA	“
	P. righty	12	6●	DA	“
	P. schizo	0	1●	DA?	“
	P. tambopata	1	1●	DA?	“
Pholcidae	*Mesabolivar yuruani*	4●	1	DA?	Huber [17]
	Metagonia delicate	55 [6/7]●	34	AS	Huber [23]
	M. uvita	55 [22/32]●	32	AS	“
	M. talamanca	16 [5/9]●	7	AS	“
	M. beni	7●	3	?	Huber [74]
	M. globulosa	5●	2	AS	Ferreira *et al.* [73]
	M. furcata	1●	1	?	“
	M. potiguar	1●	1	?	“
	M. diamantina	1●	1	?	Machado, *et al.* [76]
	M. mariguitarensis	12●	4●	DA	Huber [9]
	Panjange casaroro	5	3●	DA?	Huber [11]
	P. malagos	4	1●	DA?	“
	P. camiguin	56	24●	DA	“
Sicariidae	*Sicarius thomisoides*	5●	5	FA	Magalhaes, Brescovit, and Santos [19]
	S. fumosus	5●	5	FA	“
	S. crustosus	5●	3	FA	“

	S. lanuginosus	3●	5	FA	“
	S. yurensis	5●	3	FA	“
	S. peuensis	5●	5	FA	“
	S. gracilis	5●	5	FA	“
	S. boliviensis	5●	5	FA	“
	S. rupestris	5●	7	FA	“
	S. mapuche	5●	6	FA	“
	S. levii	5●	6	FA	“
	S. saci	5●	5	FA	“
	S. jequitinonha	5●	2	FA	“
	S. rugosus	3●	3	FA	“
Entelegynae					
Araneoidea					
Theridiidae	*Asygyna coddingtonii*	15 [4/11] ●	5	AS	Agnarsson [24]
	Asygyna huberi	10 [2/8] ●	3	AS	“
RTA					
Cithaeronidae	*Cithaeron praedonius*	8●	4	CA	Platnick [83]
Liocranidae	*Jacaena mihun*	4●	6	CA	Deeleman-Reinhold [52]; Dankittipakul, *et al.* [54]
	Teutamus politus	113 (60)*●	67 (35)*●	DA	Deeleman-Reinhold [52]; Dankittipakul, *et al.* [53], and this study
Phrurolithidae	*Scotinella britcheri*	7 [4/3]●	N.S.	AS	Penniman [47]
	S. fratellus	24 [15/9]●	N.S.	AS	“

Details of the number of specimens examined per species and study. Species where asymmetry has been observed but number of specimens or variation are not mentioned in the original work are not noted here. (AS, antisymmetry; CA, chaotic asymmetry; DA, directional asymmetry; FA, fluctuating asymmetry). Individual variation indicated between brackets [right/left]. ● Indicates the asymmetric sex.* Specimens examined in this study. N.S. not specified in the original study. *Echinotheridion* and *Tidarren* are not detailed here since all the valid species show the same type of asymmetry.

genitalia might go unnoticed. This has resulted in a more difficult assessment of male asymmetry; as an example, *Metagonia mariguitarensis* was considerd to be the only species with male genital asymmetry [9]. However, DA in males of *T. politus* had not been reported before, apparently because the right male pedipalp had been overlooked in previous descriptions. Similarly two *Speocera* species have their male pedipalps ambiguously described as “asymmetric” but no more details were given [20,64].

In contrast, recent revisionary studies on the oonopid genera *Aschnaoonops*, *Escaphiella*, *Paradysderyna* and *Reductonoops* [21,51,65,70] took special care in comparing the right and left male pedipalps revealing many more cases of genital asymmetry. In all of these, male pedipalps show consistent differences in bulb development and embolus shape between right and left (Fig. 3.2e).In fact, for some species, enough specimens have been examined to confirm that asymmetry is directional [51].

Knowing intraspecific variation and having a big-enough specimen sample are crucial for confidently categorizing the types of asymmetry, and understanding the underlying evolutionary processes. Unfortunately, this kind of fine-grained data is rare (Table 2). A few studies have described intraspecific variation in detail. *Asygyna* [24], two species of *Scotinella* [47], *Tidarren cuneolatum* [15] several species of *Sicarius* [19] and the Pholcidae [9,23] are some examples that report individual variation and (or) proportion of forms within the studied population. Other publications, like *Escaphiella* [51], *Paradysderina* [21] and *T. politus* [52] imply in the species descriptions that *all* the examined specimens show the same asymmetric morphology. Similarly, other studies deal with internal morphology variation by explicitly citing it in text as in *Cithaeron* [83]or showing it in comparative pictures as in *Jacaena* (fig. 8A-D: Dankittipakul, *et al.* [54]) and *Loxoceles* (fig. 72-86:Getrsch [78]). Nevertheless, many other studies included in our revision show illustrations or photographs where asymmetric morphology is evident; but, no information about the variation within the species or proportion of forms is given.

Patterns of genital asymmetry— We found evidence of more than 150 cases of asymmetry in spider genitals in thirteen different families. Previous broad-scoped reviews noted only some examples in Pholcidae and Theridiidae [1–3,11]. We identified multiple independent origins of asymmetry, some even occurring within the same family (as seen in Oonopidae, Pholcidae, Theridiidae Hahniidae and Liocranidae). Reports on insects suggest that genital asymmetry rarely appears isolated and is usually a shared trait between closely related species [3,4,90]. Here, we found some similar patterns with several species within a genus showing at least one type of genital asymmetry. Some cases like Jacaena mihun and Teutamus politus were seemingly isolated (until more cases are confirmed). However, we found some conspicuous examples of asymmetry shared between closely related species. These are the cases of male asymmetry in Escaphiella and Paradysderina, female asymmetry in Asygyna, Metagonia and Trachelas, and the emasculatory behavior in all the species of *Tidarren* and *Echinotheridion* (arguably closely related groups [46,57,91,92]).

This pattern is more common in the Synspermiata, but was also observed in Entelegynae (Table 1). Although the known number of cases and families with asymmetrical genitalia has increased significantly, this still represents less than 0.3% of all known spider species. The low incidence of genital asymmetry in spiders has been mainly explained by the presence of two sperm transfer structures in the male [1,3]. Huber, et al.[1] remarks that in comparison to insects, most spider asymmetry originates in females instead of males. Many examples support this hypothesis, which also fits a cryptic female choice hypothesis [10]. Nevertheless, we found numerous “new” examples of male asymmetry hidden in the taxonomic literature (Table 1), highlighting the many cases in the Oonopidae where male asymmetry has apparently not coincided with modified female genitalia (further discussed in DA). Huber, et al.[1] also observed that most insect asymmetry evolves first as DA, while most (or all) spider asymmetry appears firstly as AS. Here we found that DA might not be as rare as previously thought. Examples of DA are included in Table 2 and discussed below. Many spider asymmetries seem to fit in the AS category, although only a handful have been evaluated for the appearance of right or left-sided asymmetries within a sample as in Phrurolithidae and Theridiidae [24]. Also, we found some cases in which female copulatory ducts are long, coiled and entangled in a way that does not fit any of the three known types of asymmetry. We called this chaotic asymmetry (CA) because the great variation between individuals of the same species does not allow distinguishing either a dextral or a sinistral form.

Other cases difficult to assess are: the reduction of spermathecae to a single receptacle, as seen in some oonopids [67,69,71,93] , pholcids [73,76,77], and telemids [22,62,63,80–82] (Fig. 3.2f, g); and the presence of odd numbered spermathecae in some sicariids [19,78,79,94], ochyroceratids [18,72,95] (Fig. 3.2b, c) and probably some mecymaucheniids [96]. Both phenomena can sometimes generate a seemingly asymmetric morphology. Although good illustrations and photographs of these are available in literature (e.g. figs. 20: Magalhaes, Brescovit, and Santos [19]; figs 14 and 19: Li et al. [18]; fig. 8: Lin, Pham and Li [22]; fig. 7: Wang and Li [63]) only some cases in the Sicariidae [19,78] have reported intraspecific variation.

As mentioned earlier, a correct interpretation of the type of asymmetry based only on the available literature is complicated. Intraspecific variation and proportion of forms are key pieces of information to distinguish the type of asymmetry and the evolutionary mechanisms behind it; however, these details are often overlooked. Here we include examples that, to the best of our knowledge, fit the definition of each type of genital asymmetry and give hypotheses that could explain their origin.

Fluctuating Asymmetry (FA)— This kind of asymmetry is defined by van Valen [5] as "the inability of organisms to develop in precisely determined paths". In other words, FA refers to small random morphological fluctuations around a symmetric mean [3,5,38,97,98]. FA incidence, relation to environmental factors, and its influence within populations has been studied on some Lycosidae and Pholcidae [17–21]. Here we found that some cases, like the hahniid *Neoanthistea*, some more lycosid, oonopid, and telemid genera (mentioned as FA? in Table 1), and other "malformed" specimens in literature might be cases of FA.

Similarly, the great intraspecific variation observed in the female genitalia of some sicariids [19,78], range from asymmetries in number, size and shape of spermathecae to almost symmetric structures. This suggests that asymmetries in this family and similar cases in the ochyroceratid *Althepus* [18,72] might be fluctuating. A few species that show AS (*Scotinella britcheri* and *S. fratella*) and all species with CA (*Cithaeron prae-*

donius, *Jacaena mihun*, among others) also have a range of morphological variation in female internal genitalia within the population. However, these variations are clearly bimodal (as seen in our examples of AS) or larger than the usual 1-2% observed in FA [7]. Thus we do not consider them to be fluctuating; these and other examples are discussed in the following sections.

Antisymmetry (AS)— This kind of asymmetry describes cases where two mirror image forms, dextral and sinistral, are identifiable and within a population, usually occurring in similar proportions. Evidence from snails [99], crustaceans [6], and insects [6,90,100] suggest AS to be an evolutionarily unstable or transitional state between symmetry and DA [3,6,101]. In spiders, the co-ocurrence of these two kinds of asymmetry within the same genus has only been found in the pholcid genus *Metagonia* [9,74].

Besides *Metagonia***,** AS has been reported in at least two entelegyne families: Theridiidae and Phrurolithidae. Although the evolutionary scenario is different in each of the cases, it is interesting to observe the sex biased incidence of AS. In *Asygyna* and *Scotinella,* asymmetry has only been reported in females; while the theridiids *Echinotheridion* and *Tidarren* only show asymmetry of male pedipalps. Sex biased incidence of AS has also been observed some insect groups like Odonata, Orthopthera, Mantodea, and others [1,2,90].

Antisymmetry in female genitalia has been confirmed in three genera: *Asygyna* [24], *Metagonia* [23,73,74], and *Scotinella* [47]. All of these show both, dextral and sinistral forms within the studied samples. In *Scotinella*, two basic forms with some range of variation in-between were described. Nevertheless, no significant predominance of either within the studied populations was found [47]. The only known example of male AS is the one induced by an uncommon genital automutilation behavior. Notably, all known species in the theridiid genera *Echinotheridion* and *Tidarren* share this trait. In these genera, male spiders show no preference for either left or right pedipalp self-emasculation; furthermore, females show completely symmetric genitalia suggesting that no selection of right or left male forms is done by females. Experiments and observation on some species of *Tidarren* have shown that males can display two mating positions being able to inseminate any of the female spermathecae [1,15]. This particular phenomenon has been related to other evolutionary oddities in these genera like mandatory mate consumption from females and extreme sex dimorphism in size [15,46,55–57,91,92,102].

With the exception of the studies on male emasculation [15,46,55,56,102]**,** the mechanical, behavioral or functional implications of AS have not been reported. Huber [17] suggest that AS in Pholcidae might respond to the exaggerated development of internal genital structures forcing a reduction in one of the sides, becoming asymmetrical. Agnarsson [24] explains the AS in *Asygyna* by sexual selection by female choice, either by reducing copulation times (leaving less chance for potential predators) or by discriminating males according to their abilities to introduce sperm. A similar scenario could also explain the case of *Scotinella*.

Chaotic asymmetry (CA)— This new category of asymmetry does not fit the definition of any of the three traditional types. In species of this type, females usually develop long and convoluted copulation ducts where the great variation between specimens does not allow a clear distinction between a dextral and sinistral form. All known examples of this type of asymmetry are found in the Entelegynae clade. Platnick [83] mentioned for *Cithaeron praedonius* (Cithaeronidae): “No two females show identical patterns of epigynal duct coiling; for that matter, no individual specimen shows identical coiling of the ducts of the right and left sides”. Similar morphological variation (Fig. 3.3d–h) has been observed in *Jacaena,* (Liocranidae) [54]. Apparent CA has also been observed in *Apopyllus* (Gnaphosidae) [27], *Neoantistea* and *Mastigusa* (Hahniidae) [84,86,87], *Moreno* (Prodidiomidae) [88], and *Trachelas* (Trachelidae) [29,48,49]. However, the variation within each species is not known, therefore, their categorization as CA is highly tenuous.

The origin of these internal genital modifications has not been investigated and its relation to a functional differentiation between sides or packing of other internal organs cannot be ruled out. We hypothesize that the development of this kind of asymmetry is related to complexity in internal female genitalia and this could explain the absence of examples in the genitally simple Synspermiata. The absence of a clear right/left pattern and great variation between individuals suggest that copulatory duct shape is not under a strong selection. This might be related to a simplification in pedipalp sclerite complexity and embolus length (as seen in *Trachelas*, *Jacaena* and *Moreno*). In contrast, some *Apopyllus* and *Mastigusa* males have fairly complex male genitals with an extremely long embolus that usually coils around the bulb. In the case of *Apopyllus*, female ducts show slight asymmetries between right and left sides and authors mention internal variation between conspecific females. This genus also shows intraspecific variation in the RTA and external genitalia and it is hypothesized to be an instance of male-female coevolution [27] which could be further explained by mechanical fit of genitalia during copulation in a female choice context *sensu* Eberhard [103] (see also the discussion on DA in *T. politus*).The cases of *Cithaeron indicus* [50]*, Moreno ramirezi* [88] and both *Neoantistea* [84] species are doubtful; in the former, the male is not known, and in *Moreno* and *Neoantistea*, species were described based on just one female or variation was not documented. Therefore, the observed asymmetry could be fluctuating, antisymmetric, a developmental abnormality or even an artifact of preparation.

If pedipalp bulb sclerite reduction is related to the appearance of CA, the question would be why is it so rare? Within Entelegynae, several groups have reduced male pedipalp complexity; however, CA has not evolved nearly as many times. Long and convoluted ducts are hypothesized to be a way of avoiding premature fertilization and discriminating between different males sperm [104]. Although in many species male embolus deposits the sperm directly in the spermatheca (i. e. *Anyphaena accentuata*

[105]), several cases have been found where female ducts are much longer than the male embolus (i. e. *Clubiona pallidula* [105]). In these cases, sperm transport by the female is necessary and pre- or copulatory stimulation may be related to it [104]. The "lengthening-hypothesis" in *Sparassidae* showed a correlation between emboli and copulatory duct length and complexity [106]. Also, this study show that in this family, evolution tended to be towards elongating instead of shortening. We could speculate that CA appears when long ducts are a preexisting condition, and its shape is not under selective pressure by male intromittent structures. Then, the shape of the ducts could vary randomly without compromising copulation but still keeping the sperm screening advantages predicted by the cryptic female choice model. More research on the physiological means of sperm transport and copulation mechanics of these species could shed some light on the evolution of CA.

Directional asymmetry (DA)— In insects, DA is the most common type of asymmetry [1,2]; however, in spiders, DA seems to be quite rare. In Synspermiata literature, only the pholcid *Metagonia mariguitarensis* had been confirmed as DA, [9]. However, after our survey, we identified several reports of consistent one-sided asymmetries in other members of this clade. Some species of *Escaphiella* and *Paradysderina* show an extreme underdevelopment of the right palp in comparison to the left one [21,51]. From these, *E. gertschi* and *P. carrizal*, among others (Table 2) had enough specimens checked to confirm directionality (more than 200 specimens reported for *E. gertschi* and *E. itys*!). Other cases like *E. acapulco* or *P. boyaca*, had only a few specimens reported and were considered to be inconclusive (marked with "?" in Table 2). Other seemingly consistent male genital asymmetries have been described for three *Panjange* species of the *lanthana* group [11], *Aschnaoonops marta* [65], and at least six species of *Paradysderina* [21]. Likewise, female internal genitalia of *Mesabolivar yuruani* [17]; and some species of *Ischnothryeus* [67,68,93], *Paradysderina* [21], *Reductoonops* [70] and *Triaeris* [71] show asymmetries that seem to be consistent within their species. Nevertheless, either the number of specimens examined is low or variation within the species is not explicitly described making it difficult to confirm directionality.

The story seems to be different for Entelegynae spiders where more complex development of genitals might inhibit the evolution of directional asymmetry. Although implicit in the description of *Teutamus politus* female genitalia by Deeleman-Reinhold [52], the present study is the first report of DA in the entelegyne clade. Teutamus politus is also the first example of developmental male genital asymmetry in the Entelegynae. Previously, male asymmetry in this clade was only known from teratogenic specimens and the unique AS phenotype created by self-emasculation in *Tidarren* and *Echinotheridion*.

Putative cases of male DA in *Escaphiella* and other oonopids have only been observed in males and may not be related to modifications in female genitalia [51,65]. In all these cases, underdevelopment of one pedipalp might indicate a functional specialization of one side over the other. Observations in other oonopids have shown that during copulation both palps are inserted simultaneously [107,108]. In the cases we found, pedipalps asymmetry could potentially lead to a reduction in copulation times, a more efficient transfer of sperm or a better stimulation of the female genitalia. Similarly, female genital asymmetry in other oonopids like *Triaeris*, has not been linked to male pedipalp modifications. Oonopid internal female genitalia has proven to be one of the most complex in Synspermiata [93,107–111]. More studies on it and on male-female interactions might lead to interesting discoveries like the sperm control mechanisms on [108,109,111] or the potential parthenogenesis in some *Triaeris* species parthenogenetic [71].

In contrast, to the cases before, directional genital asymmetries in *M. mariguitarensis* and *T. politus* have been found in both sexes, which might indicate that selection by female choice is the underlying cause. In *T. politus*, most asymmetries appear to be external, affecting the atrium (A) and Co in females (Fig. 3.7, S1), and C and T in males (Figs. 3.5, 3.6 and S2). Genital parts involved in storage (Sd, S and Sa and glands), transport (Cd, Fd) and transfer of sperm (e) do not seem to be as modified. This scenario could be explained via mechanical fit and/or selective cooperation of the female [103,104]. Here, the female genitalia grooves, for instance, the atrium (Fig. 3.7a, d) anchor and control the coupling of the male palps conductor (Fig. 3.5c, f). Directional asymmetry observed in pholcidae and oonopidae appear to be more related to the size and shape of sperm transfer and storage structures suggesting a functional specialization of one side over the other.

Besides the simple mechanical fit of genitalia, stimulatory cues may also be a driving factor in the evolution of DA. Spider genitalia were thought to be numb mechanical structures without nervous input. However, recent studies have found neurons in spider genitalia [112,113] that might provide sensory input and stimulation during copulation. Similar asymmetries in shape and size have been found in males of some sepsid flies. Here, the asymmetric intromittent structures are rhythmically used to stimulate the female during copulation [114]. This hypothesis was not tested in the present work; however, the appearance of asymmetrycal sclerites (as seen in *T. politus*, *Metagonia* [17] and *Panjanje* [11]) and might be related to a differential stimulation of the female genitalia.

Changes in mating position have also been associated with many cases of DA in insect genitalia [1,4,12]. Unfortunately we were not able to test this in the case of *T. politus* using live specimens; nevertheless, observations in *Agroeca* [115] and other RTA spiders [13,116] suggest that copulation is achieved by the male climbing over the female and stretching over a side while the female slightly turns her abdomen; this process is alternated between right and left side. In *T. politus*, female genital opening location makes it virtually impossible to have successful mating attempt from a right-side position. Instead, a male must insert both pedipalps always from the left side in relation to the female body. Morphological modifications like the difference in conductor shape (Fig. 3.5c, f; 3.6b, c) and seemingly flatter tegulum of the left side (Fig. 3.5e, f) are consistent with this hypothesis. In addition, this evidence seems to back the hypothesis discussed by Schilthuizen [3] and Huber, et al. [1] stating that in spiders asymmetry is most likely female-initiated and male changes appear as an evolutionary response.

Conclusions

Genital evolution is a complex and interesting topic. The appearance of asymmetric morphologies is a puzzling phenomenon that has often been overlooked. Here we reported *T. politus* as the first case of directional asymmetry, and the first developmental asymmetry in male genitals, in Entelegynae spiders. We also searched for as many cases as possible in taxonomic literature; however, many more might be waiting to be (re) discovered. Our review revealed multiple origins of genital asymmetry in at least thirteen families, and in some cases (e.g. Oonopidae, Pholcidae, Theridiidae, Liocranidae) two or more within the same family. A correct assessment of genital asymmetry based on taxonomic legacy literature is difficult mainly due to the lack of data, description and illustration biases, and limited number of specimens and variation in descriptions.

As noted previously for genital asymmetry in insects and spiders, there is no single explanation for the evolution of this trait, but some generalizations can be made. In contrast to insects and other arthropod groups, the low number of genital asymmetric species in spiders might indicate that the appearance of these morphological modifications reduce subsequent speciation rates or even increase extinction rates; specialized lineages tend to have a reduced capacity to diversify and therefore might be considered evolutionary dead ends [117]. However, our observations indicate that cases of sexual asymmetry in spiders, although rare, are more common than was previously thought. Furthermore, they have evolved independently several times but rarely appear isolated and most of the times seem to be clustered within a genus or closely related genera, as in the cases of Oonopidae, Pholcidae, Theridiidae, and probably Liocranidae. The evolution of genital asymmetries in spiders might be a good candidate to be tested as a potential evolutionary dead end.

Several hypotheses for the appearance of asymmetry in spiders have been proposed and include natural selection [9,102], sexual selection [11,17] and antagonistic co-evolution [1,15,56] (not mutually exclusive). We considered *Echinotheridion* and *Tidarren* to be examples of antagonistic co-evolution where the male has evolved self-emasculation in response to the extreme sexual dimorphism in size and aggressive behavior in the female. No selection between left and right is apparent in these genera, thus no directionality is observed. DA cases like *T. politus* seem to support the hypothesis that correlates changes in mating position to genital asymmetry; however, other examples still need to be studied. DA in *T. politus* and some pholcid examples, AS in *Scotinella* and *Asygyna*, and CA cases in *Jacaena*, *Cithaeron* and *Trachelas* support the hypothesis of female-initiated asymmetry in spiders. However, male DA in Oonopidae and AS in some theridiids conflict with this explanation. Further and more detailed study on internal genitalia and comparative study of male right and left pedipalps may yield new and valuable information to explain the evolutionary pattern of genital asymmetry. We hope that this review will aid in the study, development and testing of hypotheses on sexual evolution. We specifically hope it sparks discussions on the complex interactions between males and females, and appearance of interesting phenomena like genital asymmetry.

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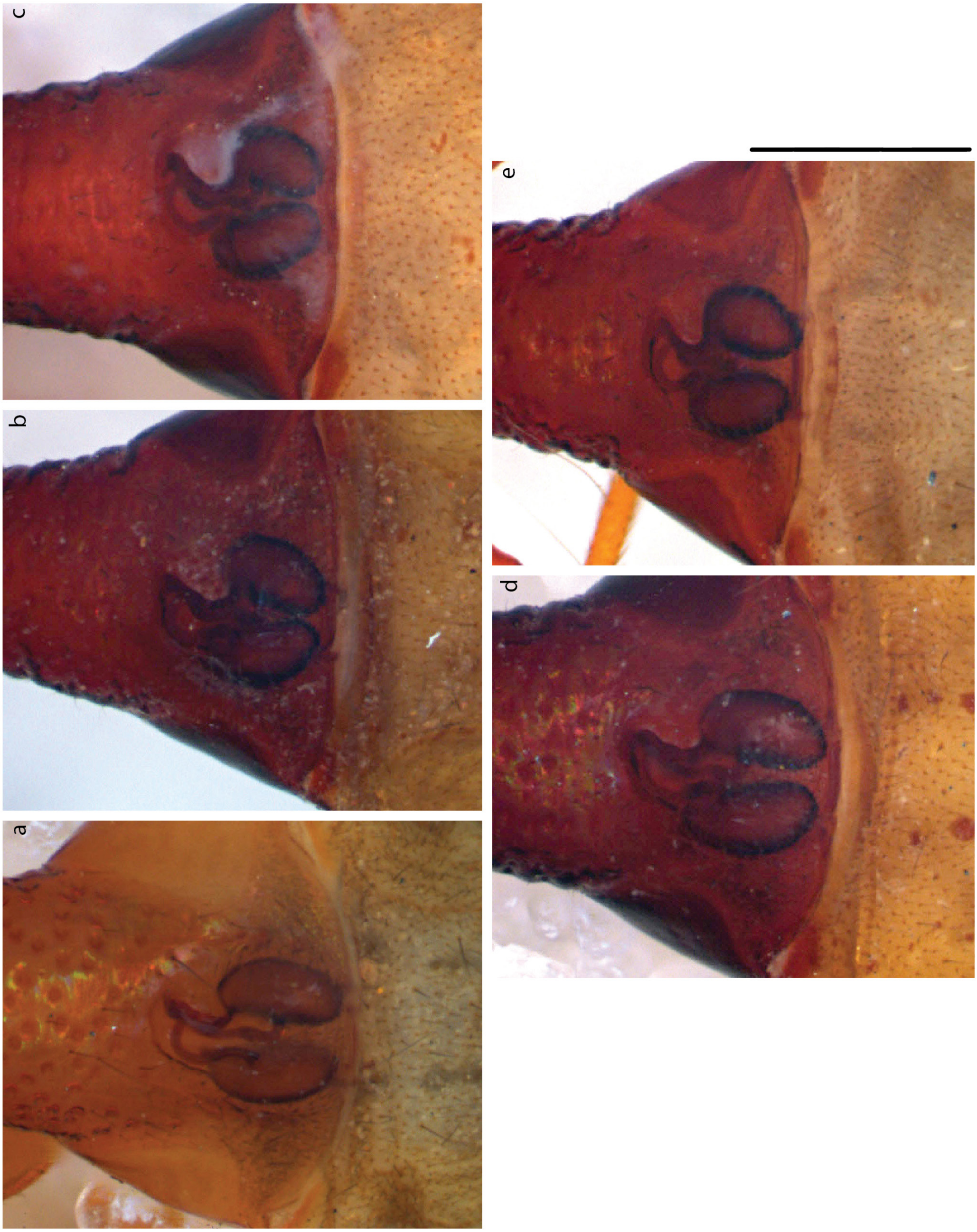
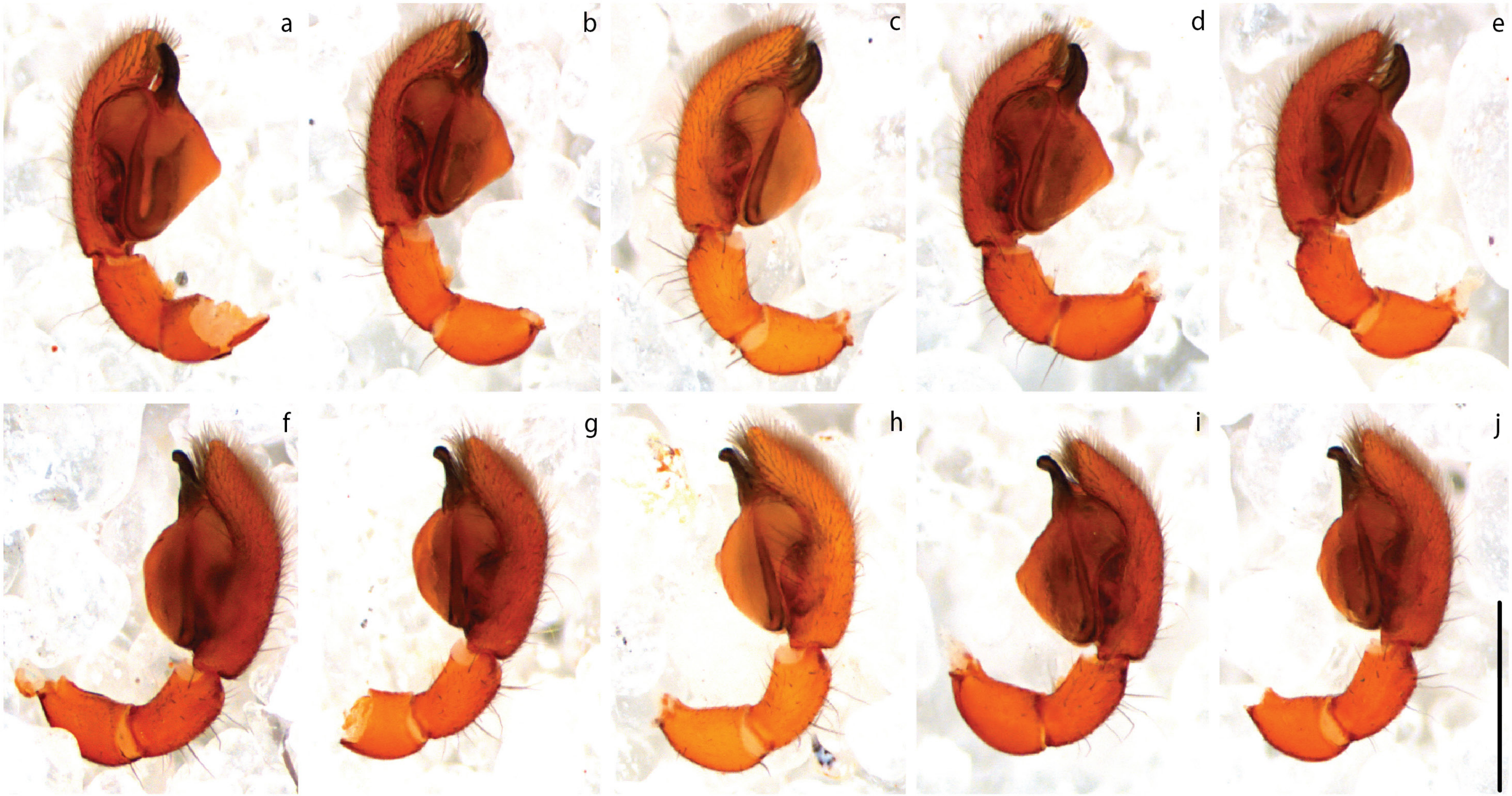
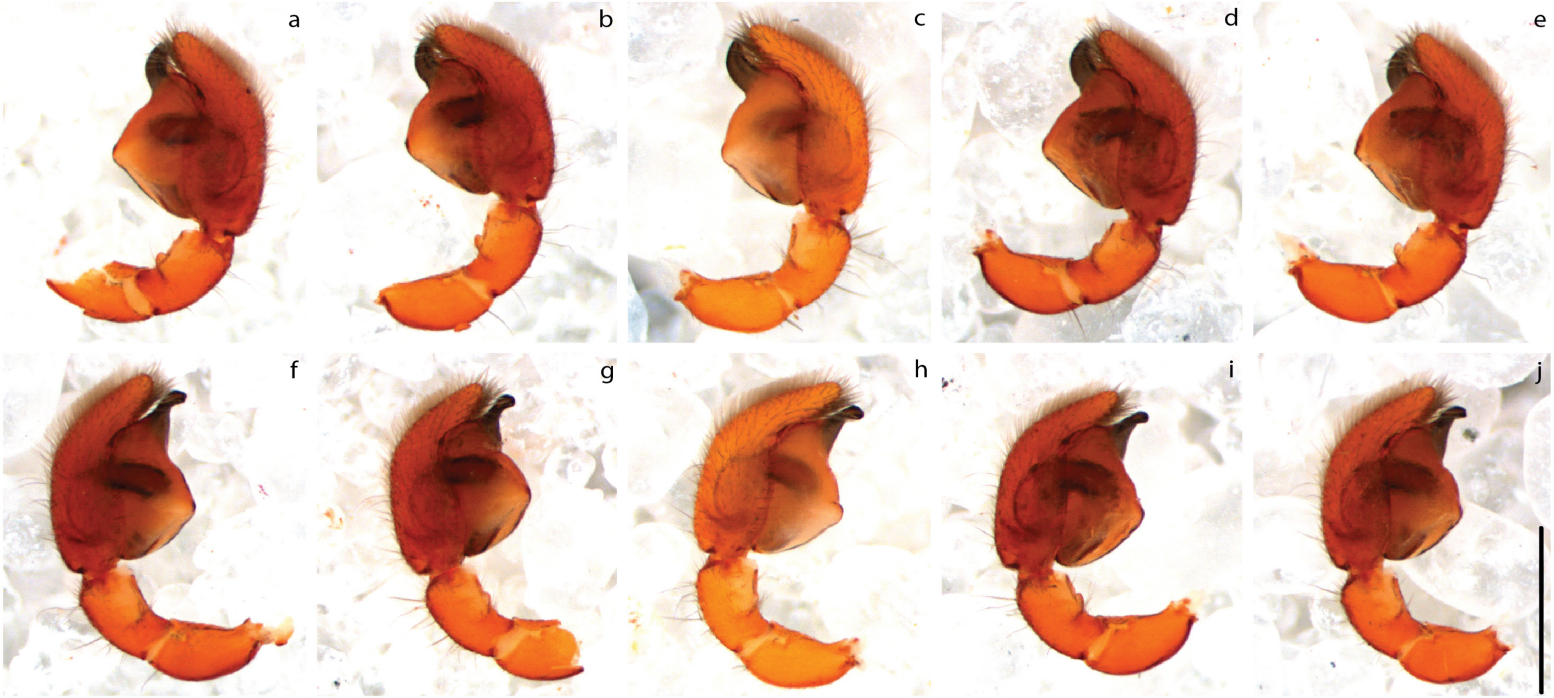
Supporting information



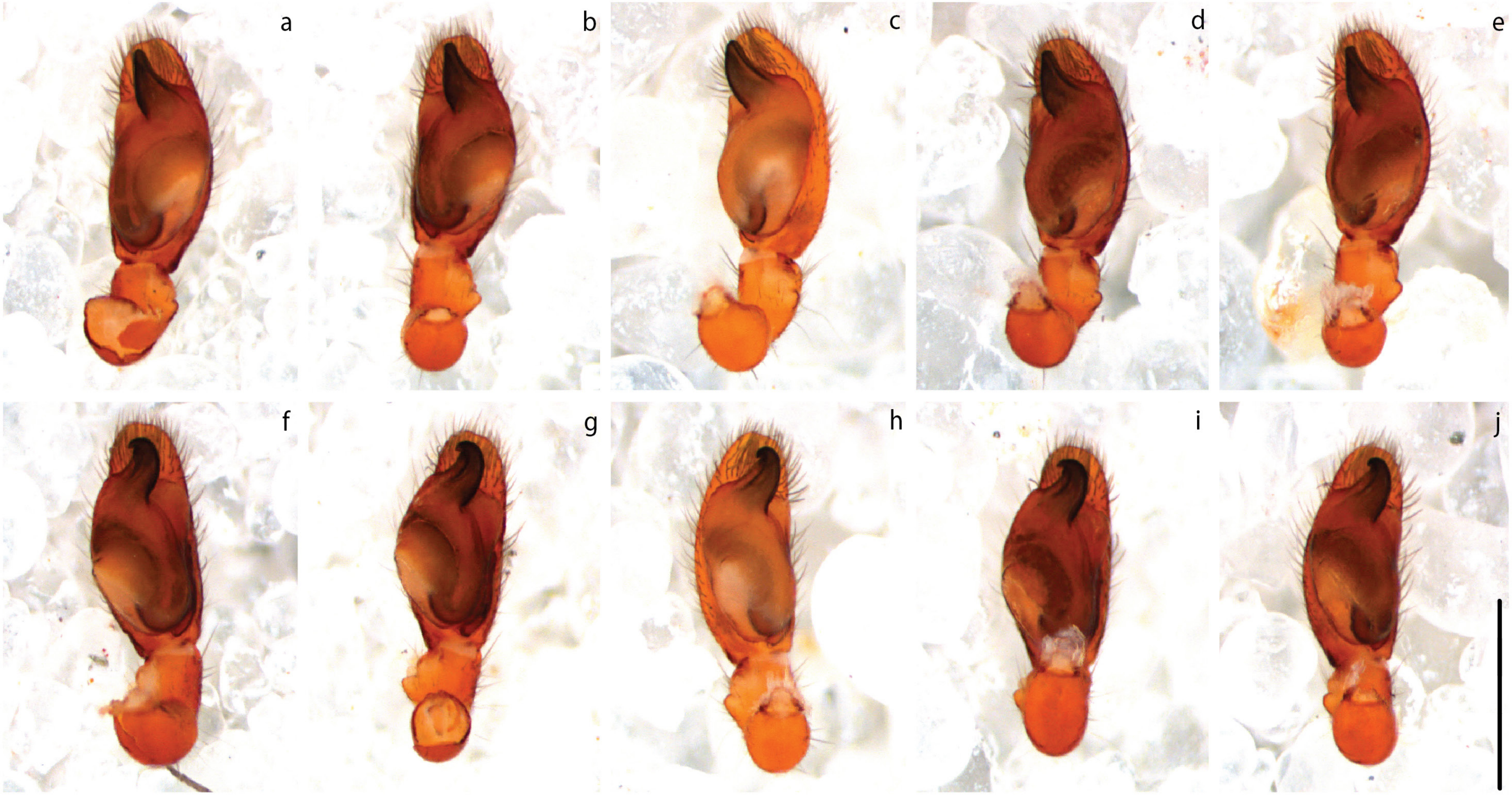
S1.–Intra-specific variation female external genitalia. Standard views of sexual structures used to aid in DA comparison. One comparative plates of the epigyna ventral view is given. Scalebars =0.5 mm. (Individual pictures of five female specimens can be found in https://doi.org/10.1371/journal.pone.0220354.s001)



S2.–Intra-specific variation of male genitalia. Standard views of sexual structures used to aid in DA comparison. Comparative plates of the pedipalp in prolateral view. Top row=left pedipalp; bottom row= right pedipal. Scalebars =0.5 mm. (Individual pictures of both palps from five specimens can be found in https://doi.org/10.1371/journal.pone.0220354.s002)



S2.–Intra-specific variation of male genitalia. Standard views of sexual structures used to aid in DA comparison. Comparative plates of the pedipalp in retroateral view. Top row=left pedipalp; bottom row= right pedipal. Scalebars =0.5 mm. (Individual pictures of both palps from five specimens can be found in https://doi.org/10.1371/journal.pone.0220354.s002)



S2.–Intra-specific variation of male genitalia. Standard views of sexual structures used to aid in DA comparison. Comparative plates of the pedipalp in ventral view. Top row=left pedipalp; bottom row= right pedipal. Scalebars =0.5 mm. (Individual pictures of both palps from five specimens can be found in https://doi.org/10.1371/journal.pone.0220354.s002)

Appendix 1 • Mating trials

The following information about the mating trials and experimental design for the study of mating behavior and courtship was not included in the original publication of this chapter. For this short report we used the "ARRIVE essential 10 protocol" for reporting animal research [1]. This guideline shows a list of ten steps for optimal transparency and reproducibility of in-vivo animal experiments. Although this list includes the reporting of outcomes, statistical analyses, among other results; our study did not have successful observations. Therefore, we cannot address those parts of the procedure. Nevertheless, here we will summarize our study design (including sampling methods and localities), sample size, inclusion and exclusion criteria, sample randomization, and experimental procedures.

Live specimens of *Teutamus politus* were collected during our fieldwork in Thailand in the southern provinces of Phuket and Krabi (see supplementary table 2 on p.53 for more information on the specific collecting sites). The specimens used for our behavioral observations were collected using an entomological aspirator —also known as pooter— (Fig. Ap1a, b). All specimens were found and captured among leaf litter and always close or on top of nests of *Odontomachus* sp. ants. All our collections were carried out during the day; therefore we do not have evidence of variations of their activity throughout the day.

Teutamus politus is easy to identify and tell apart from other ground spiders in the area. Therefore, all the specimens were immediately determined and placed individually in 15ml Falcon tubes. Specimens were brought back to our headquarters where adults and juvenile spiders were separated. Juvenile individuals were fixed and stored in 96% ethanol. Adult specimens were kept alive at room temperature (ca. 25ºC), fed with termites and other small insects every two days, and hydrated by placing a small piece of moist cotton wool inside their enclosures. A total of 17 males and 19 females were assigned unique codes for the mating trials.

In a first stage, we only paired males and females collected in the same site (Fig. Ap1c–e); this was done to avoid introducing the variable of possible differences between populations in our study. Nevertheless, life in captivity of our spiders (especially males) proved to be a limiting factor, with many specimens dying two or three days after being collected. This forced us to pair up spiders from different collecting sites in order to keep making our observations. Every couple was placed in a mating arena formed by a 5 cm petri dish (a broad variety of these setups are commonly used for sexual behavior studies in spiders e.g. [2–6]) divided by a paper wall with a small opening so spiders could roam freely but flee in case of aggression (Fig. Ap1f, g). Each pair was kept in the dish under constant observation for a period of about three hours. Each animal was used only in one mating trial per day to avoid inducing unnecessary stress to the animals. Twenty five different couples were tested in total with females being used in more tests due to their resilience and longer life in captivity in relation to males. Travel logistics and specimen sensitivity to environmental changes did not allow to further test under different times and conditions.

Ap1.–Sampling and mating trails of *Teutamus politus*. Collecting method, direct collection of specimens using an aspirator: a) sifted leaf litter on a white surface, this technique provides great contrast allowing seeing and collecting small specimens more easily. b) collection of specimens directly on the ground and among leaf litter, this technique avoids damaging bigger specimens in the sifting process. Mating trails: c–e) male and female specimens barely interacting during our behavioral observations. f–g) Photographs of our mating arenas: showing our setup and several parallel observations running simultaneously. Egg sacs: h) a handful of esgg sacs laid in the spider enclosures. i–j) detail of the egg sacs shape and size. Scale bars: 0.5 cm.

There were no successful observations of either courtship or mating during our trails. Spiders preferred to explore the dish or stand still and; whenever they got too close, they usually avoided each other. In general, interactions between females and males were brief and non-aggressive. No attempt of courtship —including vibrations, or tapping— was observed. Four females laid egg sacs in the Falcon tubes (Fig. Ap1h–j); these were round, flattened, disk-like sacs of about 5mm in diameter and were in all cases laid overnight. Although we tried to take care of the egg sacs, none of the eggs actually hatched. This, together with their relatively restricted distributions and close relation to *Odontomachus* ants suggests that this species might require very specific environmental conditions to survive and thrive.

On the one hand, the poor results obtained in these mating trials might imply that *T. politus* need very specific conditions of light, humidity and/or substrate to display their normal behavior. On the other hand, the egg sacs laid in the spiders' enclosures also suggest that at least some of the females we sampled had already mated and might therefore be non-receptive to other male advances. Besides, the short life in captivity of the males —in relation to females—, and the temporal variation patterns observed in Fig 2.3 (Chapter 2) might indicate that our sampling was carried out late in the phenology of this species. Even if our trials were not successful, we hope they can serve as a base for future studies on the behavior and ecology of these interesting species and their kin.

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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

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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	H3	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

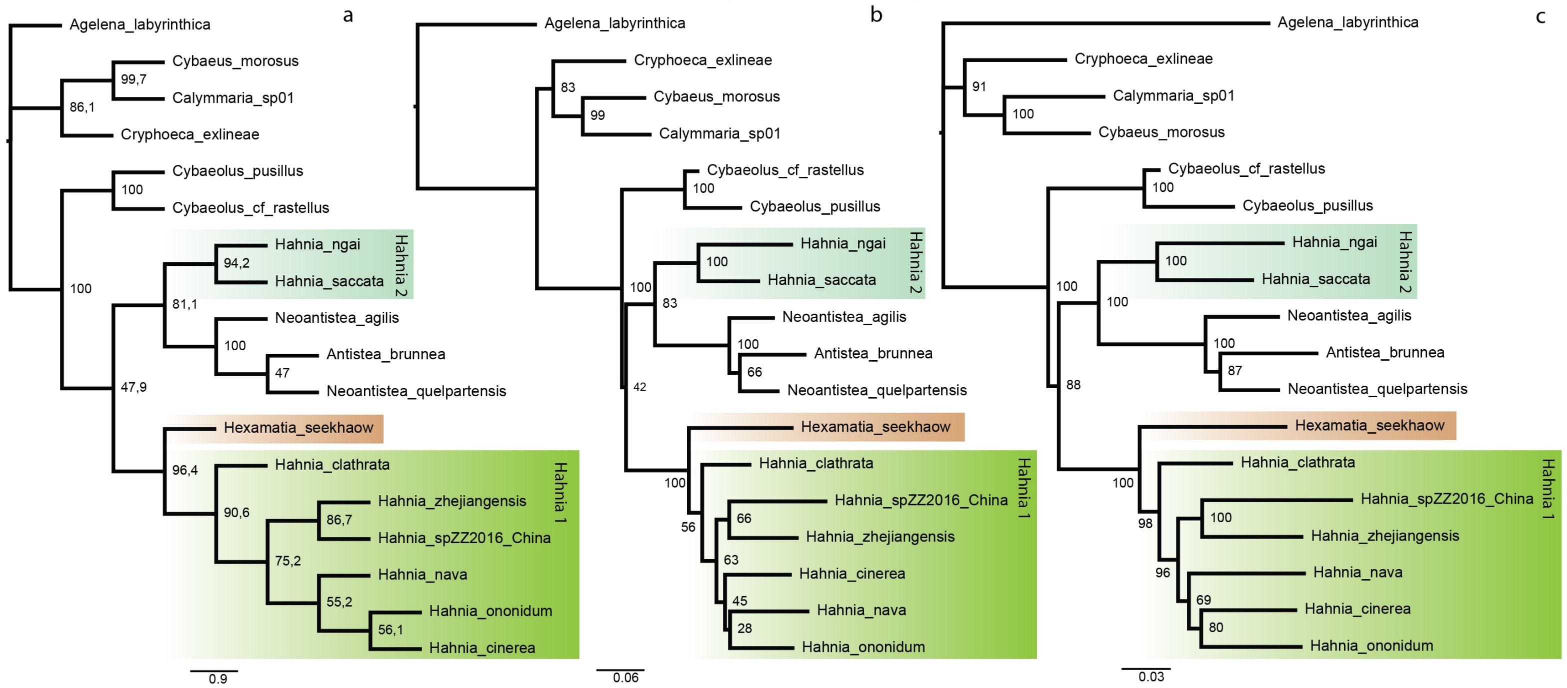

Figure 4.1.–a–c. Tree topologies obtained by different analyses. a– Maximum Parsimony most parsimonious tree, numbers at node indicate bootstrap support; b– Maximum Likelihood, numbers at node indicate bootstrap support; c–Bayesian Inference, numbers at node indicate posterior probabilities. The agelenid *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.

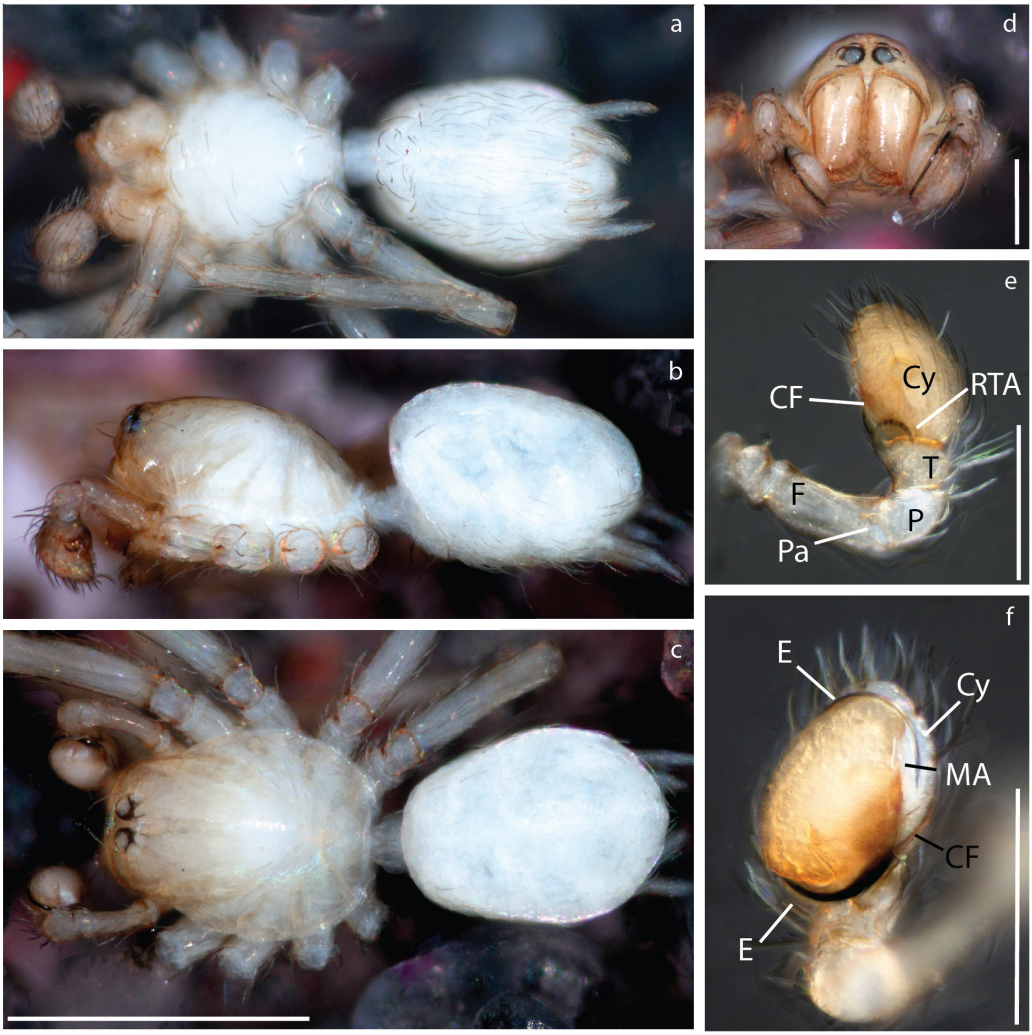

Figure 4.2.–a–d. *Hexamatia seekhaow* sp. nov. Male: Habitus: a– ventral view; b– lateral view; c– dorsal view. Prosoma: d– anterior view. Palp: e–retrolateral view; f– ventral view. Scale bars: a, b= 0. 5 mm; d– f= 0.15 mm. CF – Cymbial furrow; Cy – Cymbium; E – Embolus; F – Femur; MA – Median apophysis; 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; 18º48.502’N, 98º53.528’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; 18º48.502’N, 98º53.528’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; 18º35.268’N,

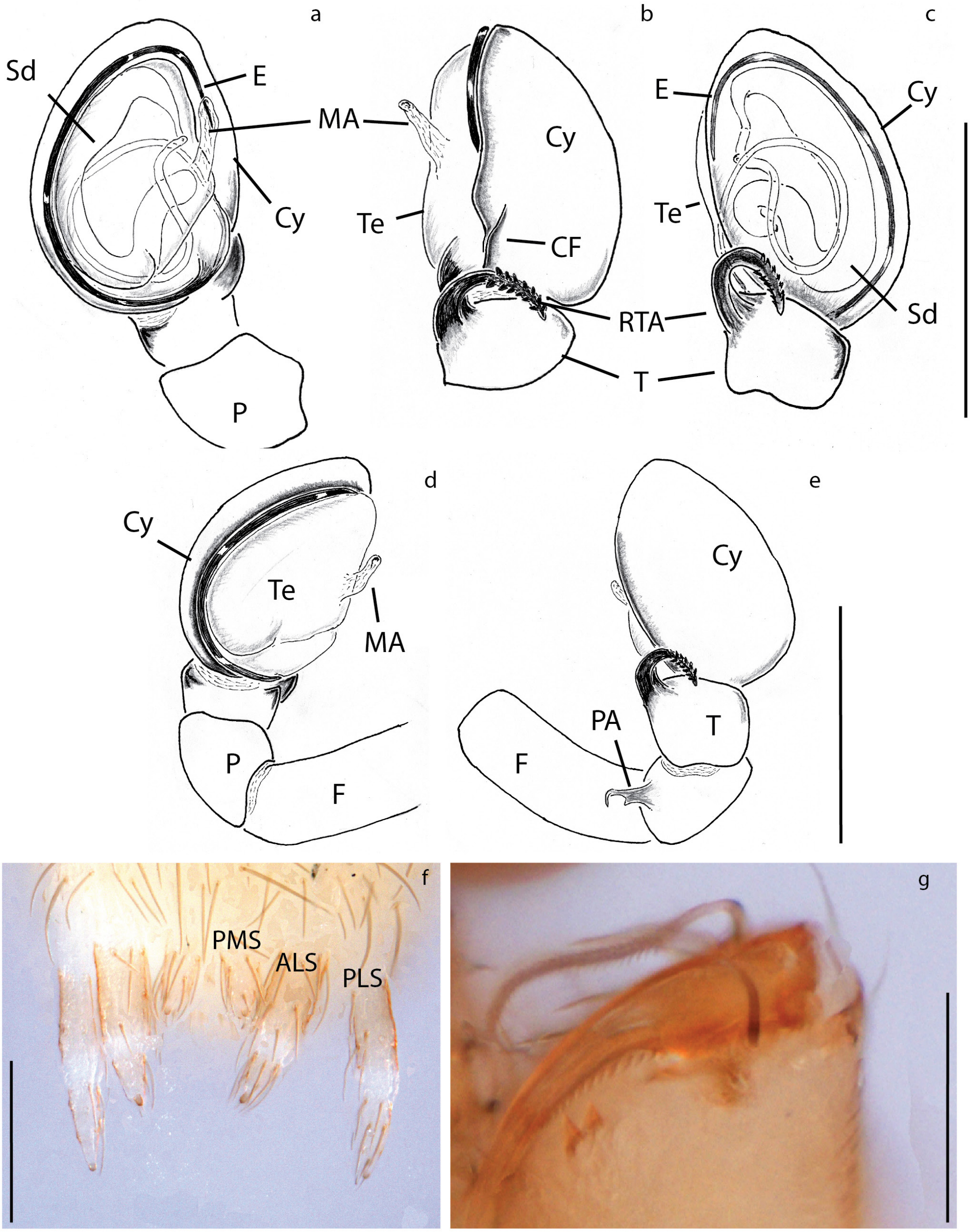

Figure 4.3.–a–d. *Hexamatia seekhaow* sp. nov. Male palp: a– ventral view, cleared; b– retrolateral view; c– dorso–retrolateral view, cleared; d– prolateral view; e– dorso–retrolateral view. Male spinnerets: f– ventral view. Scale bars: a–f = 0.15 mm; g = 05mm. ALS – Anterior lateral spinnerets; CF – 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.

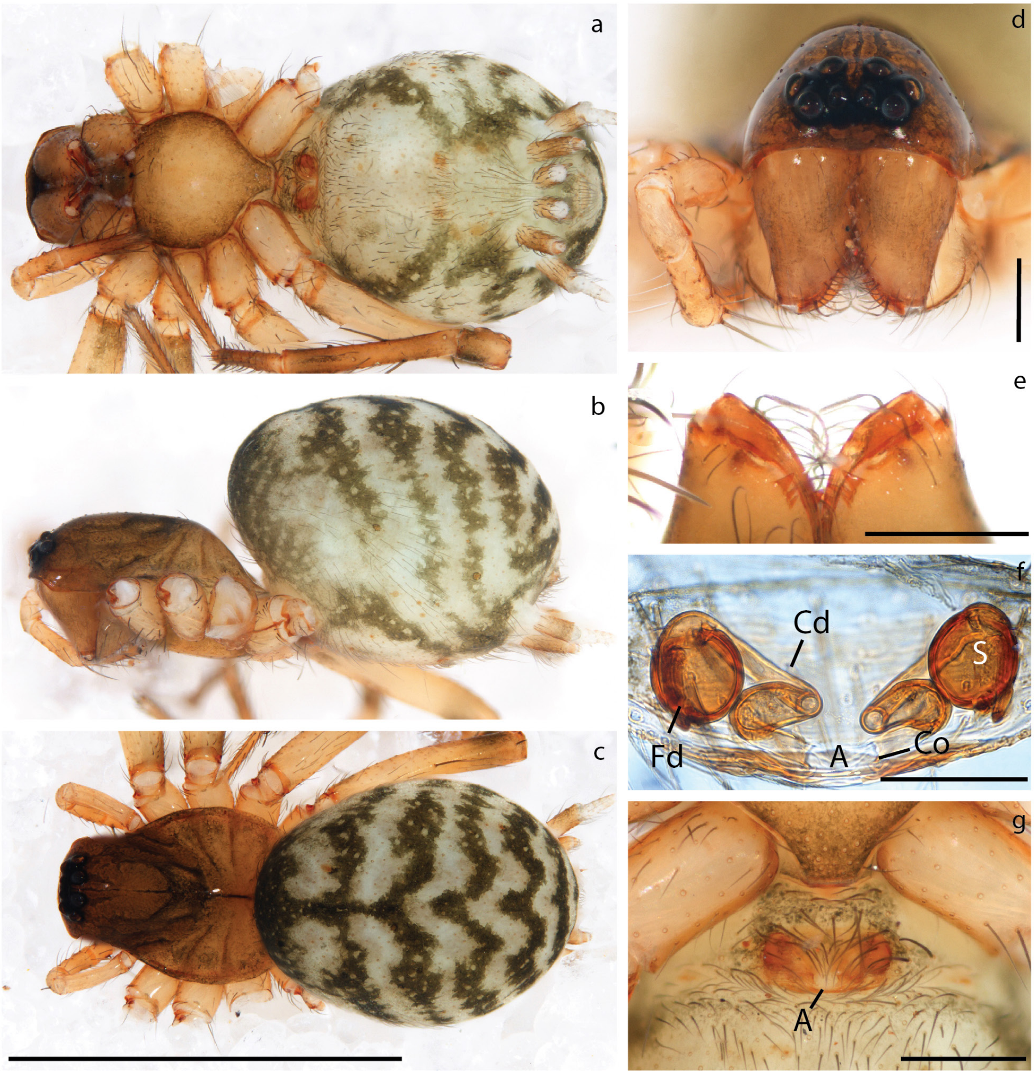

Figure 4.4.–a–c. *Hahnia ngai* sp. nov. Female: Habitus: a– ventral view; b– lateral view; c– dorsal view. Prosoma: d– anterior view. Chelicerae: e–posterior view. Epigynum: f– dorsal view, cleared; g– ventral view. Scale bars: a–c= 1.0 mm; d, e, g= 0.25 mm; f= 0.1 mm. A – Epigynal atrium; Cd – Copulatory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

98º29.240’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

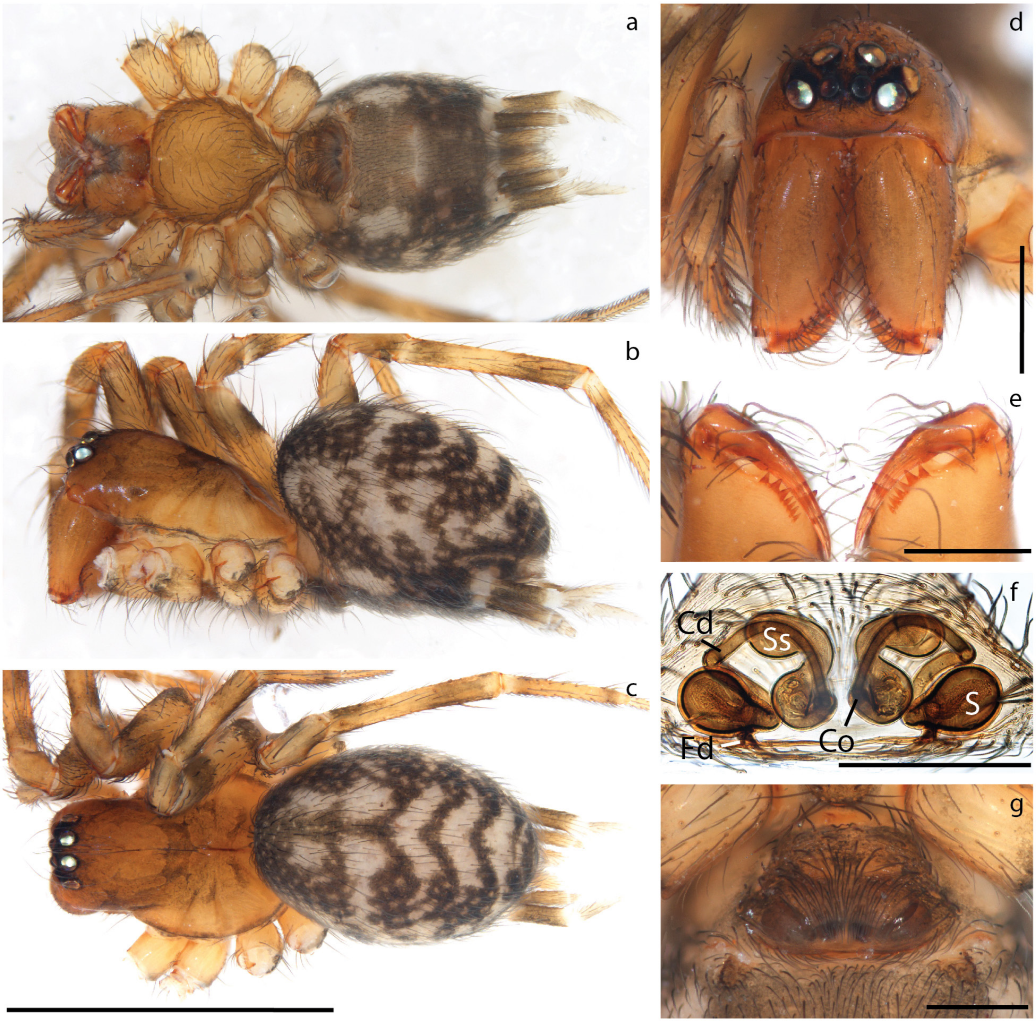

Figure 4.5.–a–d. *Hahnia saccata* Zhang, Li & Zheng, 2011. Female: Habitus: a– ventral view; b– lateral view; c– dorsal view. Prosoma: d– anterior view. Chelicerae: e–posterior view. Epigynum: f– dorsal view, cleared; g– ventral view. Scale bars: a–c= 1.0 mm; d= 0.50 mm; e–g = 0.25 mm. A – Epigynal atrium; 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

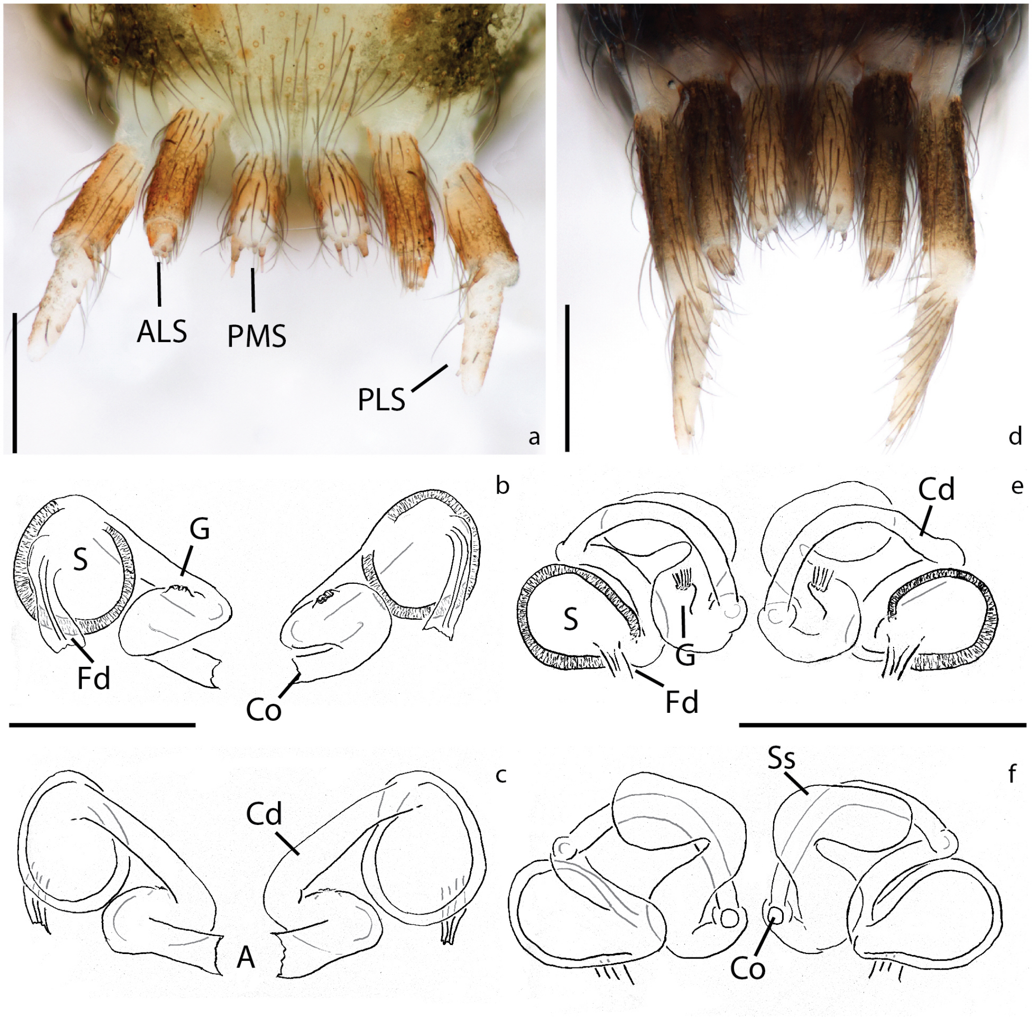

Figure 4.6.–a–f. Female spinnerets and genitals: *Hahnia ngai* sp. nov. Spinnerets. a– ventral view. Epigynum, cleared. b– dorsal view; c –ventral view. *Hahnia saccata* Zhang, Li & Zheng, 2011. Spinnerets. d– ventral view. Epigynum, cleared. e– dorsal view; f –ventral view. Scale bars: a, d– f=0.25 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

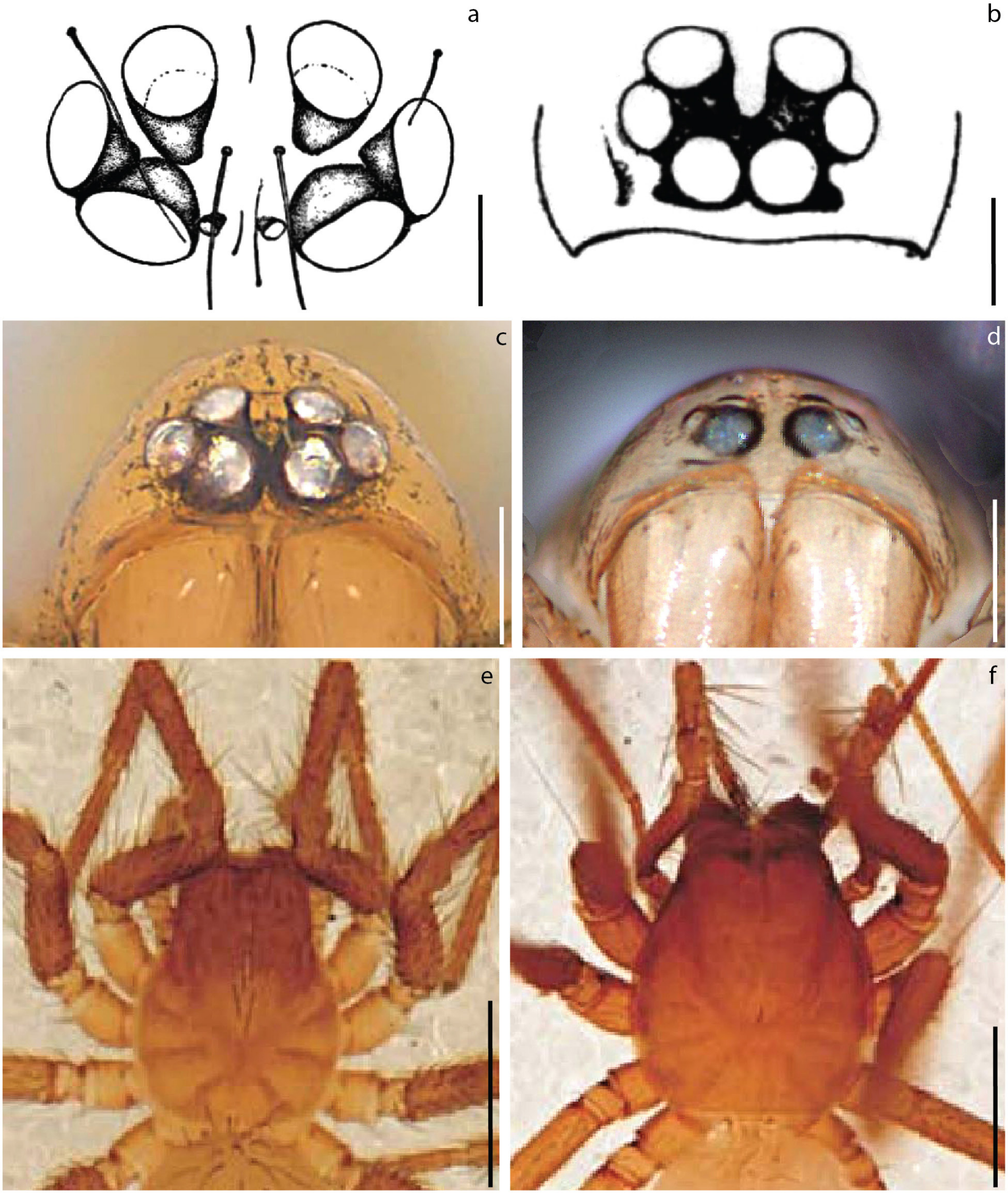

Figure 4.7.–Examples of eye reduction in the Hahniidae. Eight eyes with minute AME: a– *Alistra myops*; modified from Schiapelli and Gerschman de P. 1959. Six eyes: b– *Amaloxenops vianai*; modified from Schiapelli and Gerschman de P. 1958; c– *Scotospilus longus*; modified from Zhang, Li, and Pham 2013; d– *Hexamatia seekhaow* sp. nov. No eyes: e, f– I*berina 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; 18º48.780’N, 98º55.928’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)

Chapter 5

First records and three new species of the family Symphytognathidae (Arachnida: Araneae) from Thailand, and the circumscription of the genus *Crassignatha* Wunderlich, 1995

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Abstract

The family Symphytognathidae is reported from Thailand for the first time. Three new species: *Anapistula choojaiae* sp. nov., *Crassignatha seeliam* sp. nov., and *Crassignatha seedam* sp. nov. are described and illustrated. Distribution is expanded and additional morphological data are reported for *Patu shiluensis* Lin & Li, 2009. Specimens were collected in Thailand between July and August 2018. The newly described species were found in the north mountainous region of Chiang Mai, and *Patu shiluensis* was collected in the coastal region of Phuket. DNA sequences are provided for all the species here studied. The relations of these symphytognathid species were tested using previously published phylogenetic analyses on micro orb-weavers. Also, we used micro CT analysis to build 3D models of the male genitalia and somatic characters of two species of *Crassignatha* Wunderlich, 1995. The molecular phylogeny and 3D models were used to discuss the taxonomy and circumscription of the currently valid symphytognathid genera, with focus on *Crassignatha* and *Patu* Marples 1951. Based on this, three new combinations are suggested: *Crassignatha bicorniventris* (Lin & Li, 2009) comb. nov., *Crassignatha quadriventris* (Lin & Li, 2009) comb. nov., and *Crassignatha spinathoraxi* (Lin & Li, 2009) comb. nov. A new record of *Crassignatha danaugirangensis* Miller et al, 2014 is reported from Brunei.

Keywords: 3D reconstruction, *Anapistula*, Borneo, Computed tomography, micro-CT, *Patu*, Sabah, Symphytognathoids

Introduction

The family Symphytognathidae includes some of the tiniest spiders known. According to a recent "Spider World Record" study [1], this family holds the records for the smallest female, smallest male and smallest web. The Symphytognathidae has traditionally been put together with other small size araneoids (Anapidae, Mysmenidae and Theridiosomatidae, sometimes along with synaphrids and micropholcommatids) in a group informally called the symphytognathoids [2,3]. Although phylogenetic relationships among the Symphytognathidae have not been directly studied, some representatives have been used as part of other phylogenetic studies targeting the family Mysmenidae [4,5], as well as a broad scope analysis of the whole order Araneae [6,7]. Symphytognathids can be separated from other relatives by the following combination of characters: the loss of the posterior median eyes, reducing eye number to six (with the further loss of the anterior median eyes in the case of the four-eyed genus *Anapistula*), fusion of the chelicerae (but see below), extreme reduction or loss of female pedipalp, the labium being much wider than long, loss of the colulus, sternum broadly truncated posteriorly, the absence of book lungs, and the presence of one or two promarginal cheliceral teeth originating from a common base [3,4,8–10].

The family is widespread in the tropics and subtropical regions, with most species described from the southern hemisphere. At present 8 genera and 74 species are recorded worldwide. In Asia, six genera and 29 species have been recorded [11]. From these, 19 species have been recorded from China [10,12–15] and six from South East Asia (Indonesia, Malaysia and Vietnam) [16–19]. Here, the family Symphytognathidae is formally reported from Thailand for the first time, although Lopardo et al. [4] did include a Thai symphytognathid in their study, designated SYMP-004-THAI , which was later identified as *Crassignatha* (Lopardo, *pers. comm.*). We describe three new species of the genera *Anapistula* and *Crassignatha* and expand the known distribution of *Patu shiluensis.* We used a combination of newly generated sequences and sequences available in GeneBank to build a molecular phylogeny of the Symphytognathidae —and related micro orb-weaver families— in order to test the familial placement of our new species. Additionally, we discuss the taxonomy of the Symphytognathidae with emphasis on the genera *Crassignatha* and *Patu*.

Material and Methods

Fieldwork — The symphytognathid specimens reported here were collected in Chiang Mai and Phuket, Thailand, between July 16th and August 6th 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, and among sifted leaf litter.

Molecular data — To test the relationships and position of the novel species within the Symphytognathidae, we selected one specimen from each species we collected and used all four right legs to extracted genomic DNA and sequence six gene fragments: COI, H3, 12S, 16S, 18S and 28S (primers in SM1) following [6,20] protocols. Sequences were edited in Geneious Prime 2020.0.5 and deposited in GenBank; accession numbers are reported in Table 1. We used these sequences and a selection of taxa previously used to test the phylogeny of mysmenid spiders [4,5]. A total of 47 species of "symphytognathoids" from the families Anapidae, Mysmenidae, Symphytognathidae and Theridiosomatidae were used. Two more species of Tetragnathidae were used as an outgroup to the symphytognathoids. We used MAFFT v.7.450 online (https://mafft.cbrc.jp/alignment/server/) with default parameters to align the sequences. Matrix was built using in Sequence Matrix v.1.8 (http://www.ggvaidya.com/taxondna/); matrix available in SM1. Each locus was treated as a partition and examined with jModelTest2 [21] in CIPRES [22] to get the best model fit for each; GTR+I+G was selected in all cases. Our datasets were analyzed using MEGA X [23] for Maximum Parsimony (SPR, default values, bootstrap= 1000); RaXML [24] in CIPRES for Maximum Likelihood (GTR, bootstrap= 1000) and MrBayes v. 3.2.6 [25] in CIPRES for the Bayesian Inference (GTR+I+G, two independent runs with one cold and three heated chains, mcmc=50,000,000 gen, samplefreq=1000, burnin=2500; partitions are indicated in the NEXUS file). The program Tracer v. 1.7.1 [26] was used to analyze the performance of our BI analyses.

Table 1. GenBank accession numbers of DNA sequences generated for the present work.

Species	COI	H3	16s	12s	18s	28s
Anapistula choojaiae	MT712393	MT782018		MT711286	MT711238	MT711242
Crassignatha seedam	MT712396	MT782021			MT711241	
Crassignatha seeliam	MT712394	MT782019			MT711239	
Patu shiluensis	MT712395	MT782020	MT711285		MT711240	

Morphological data — Specimens were photographed with a Nikon DS-Ri2 camera attached to a Leica DM 2500 microscope. Specimens were observed in ethanol using semi-permanent slide preparations [27]. Female genitalia were dissected, digested using pancreatine solution [28], and cleared with methyl salicylate. For the 3D scans, whole male spiders were stained in 1% Iodine -70% et- OH for 24 hours. Specimens were fixed in a modified 10ul pipette tip and scanned using a Zeiss X-radia 520 versa. 3D model and subsequent segmentation of the internal ducts of male pedipalps were done in Avizo 9.5.0. All the specimens have been deposited in the collection of the Naturalis Biodiversity Center, Leiden, the Netherlands. Additionally, two males of *Crassignatha danaugirangensis* Miller et al., 2014, recently collected in Brunei, were analyzed using micro-CT scanning. 3D reconstructions were used to clarify some anatomical details of this species and the genus *Crassignatha*, including the internal and external structure of the male pedipalp, cheliceral armature, and carapace texture.

Nomenclature of the genital structures was based on Harvey [17] and Lin, Tao, and Li [14] for *Anapistula*, and Lin and Li [13] and Miller, Griswold, and Yin [10] for *Crassignatha* and *Patu.* Abbreviations in text and figures: A – Epigynal atrium; AME – Anterior median eyes; BI – Bayesian Inference; C – Conductor; C1 – Conductor, anterior projection; C2 – conductor, posterior projection; Cd – Copulatory duct; Ch – Chelicera; ChT– cheliceral tooth; Co – Copulatory opening; Ct – cymbial tooth; Cy – Cymbium; E – Embolus; Em– Embolic membrane; EMD – Epigynal median duct; F – Femur; Fd – Fertilization duct; Lb – lateral branch of the EMD; LE – lateral eyes; Mcl– male leg II mating clasper; ML – Maximum Likelihood; MP – Maximum Parsimony; Pa – Patella; Pc – Paracymbium; PME – Posterior median eyes; S – Spermatheca; Sa – Secretory ampulla; Sc – Epigynal scape; Sd – Spermatic duct; T – Tibia.

Results

Phylogenetic Analysis

Tree topologies inferred by the different phylogenetic analyses performed (Figs. 5.1–5.3) show some consistencies in several groupings; however, low support values are common, especially in the MP and ML trees. There is an inconsistent and problematic placement of the Symphytognathidae in relation to the Anapidae. All tree analyses recovered Mysmenidae as monophyletic and a sister group of Anapidae + Symphytognathidae. Theridiosomatidae is recovered as monophyletic in the MP and ML analyses with medium to high support (Figs. 5.1–5.2); nevertheless, in the BI the position of this family is not resolved (Fig. 5.3). Similarly, the position of Micropholcommatinae, currently considered part of the Anapidae, is not clear, being found as paraphyletic in the MP, unresolved in the BI, and a poorly supported monophyletic clade in the ML analysis (Fig. 5.1–5.3). The Anapidae is closely related to the Symphytognathidae in all our trees (with the notable exception of the two micropholcommatines in the ML and BI); however, it appears as a poorly supported monophyletic group in the ML (Fig. 5.2), and paraphyletic in the MP and BI (Fig. 5.1, 5.3). The Symphytognathidae appear monophyletic with moderate to high support in all the analyses (Figs. 5.1–5.2). In the BI analysis, this family is monophyletic and highly supported but found in an unresolved branch that includes the paraphyletic Anapidae (Fig. 5.3). The internal relations of the Symphytognathidae are similar in all our trees forming one clade that includes *Symphytognatha picta*, one species (SYMP_008_DR) identified as S*ymphytognatha*, one as *Patu* (*Patu*_SYMP_001_DR), and one more (SYMP_005_AUST) that remained unidentified. The other clade recovers the rest of the *Patu* species + *Crassignatha.* Here, two terminals (SYMP_002_MAD and SYMP_003_MAD) are closer to *Patu shiluensis* —and related to the three *Crassignatha* representatives—; and two other (SYMP_006_AUS and SYMP_007_AUS) are consistently found outside of the *Crassignatha* + *Patu* clade. SYMP-004-THAI consistently clusters with *Crassignatha seeliam* sp. nov., and unpublished morphological observations (Lopardo, pers. comm.) are consistent with the possibility that these are conspecific.

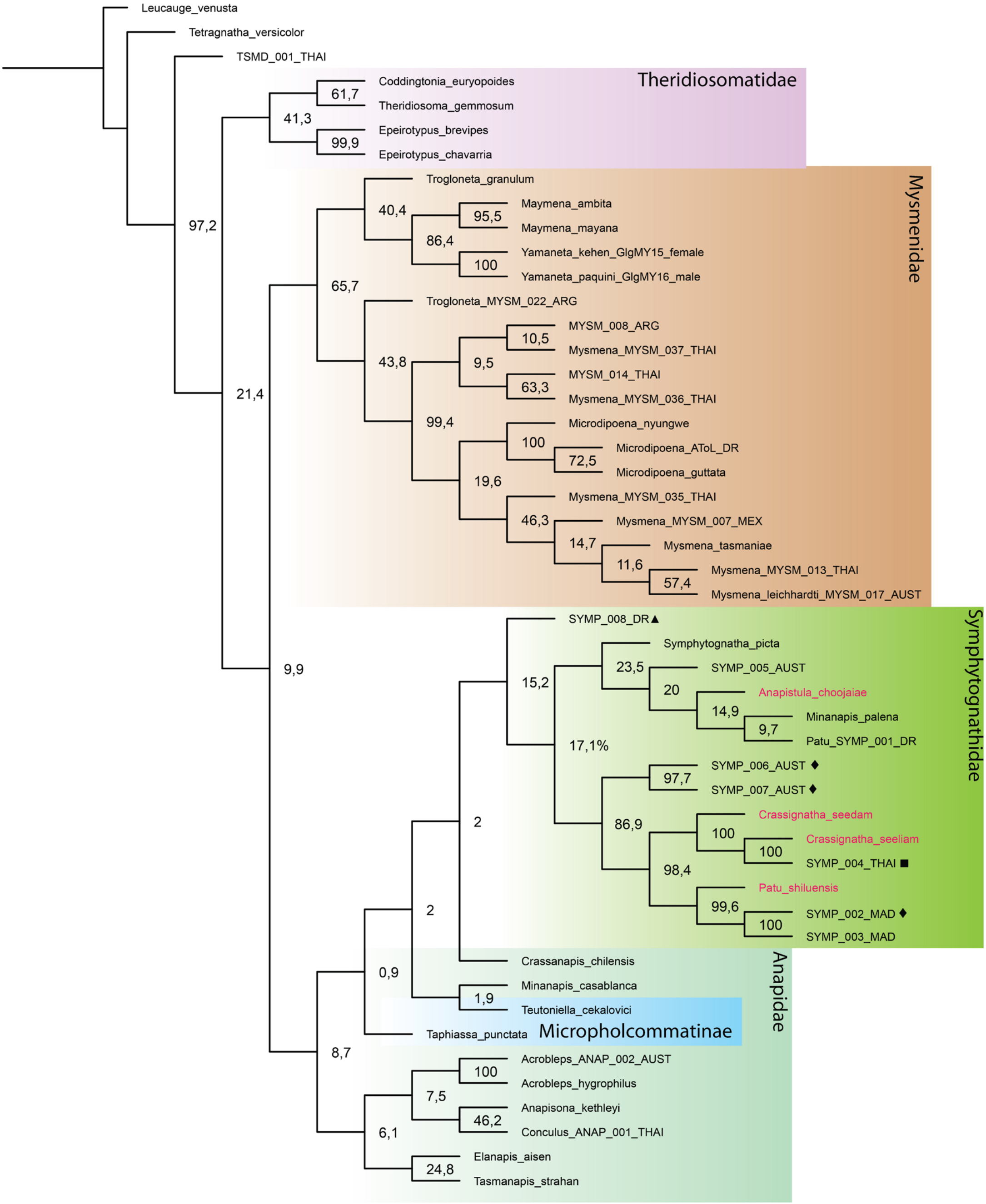

Figure 5.1.– Maximum Parsimony Tree. Obtained in MEGA-X using a modified version of Lopardo et al. [4] and Feng et al. [5] plus the four symphytognathid species from our study (in red). Numbers at nodes indicate bootstrap support. Note the paraphyly of Anapidae and the high support of *Crassignatha* and *Patu* in the Symphytognathidae. Molecular vouchers used for previous "symphytognathoid" studies [4,31] identified to genus level by L. Lopardo (pers. comm.) as follows: ■ *Crassignatha* (apparently conspecific with *C. seeliam*); ◆*Patu*; and ▲*Symphytognatha*.

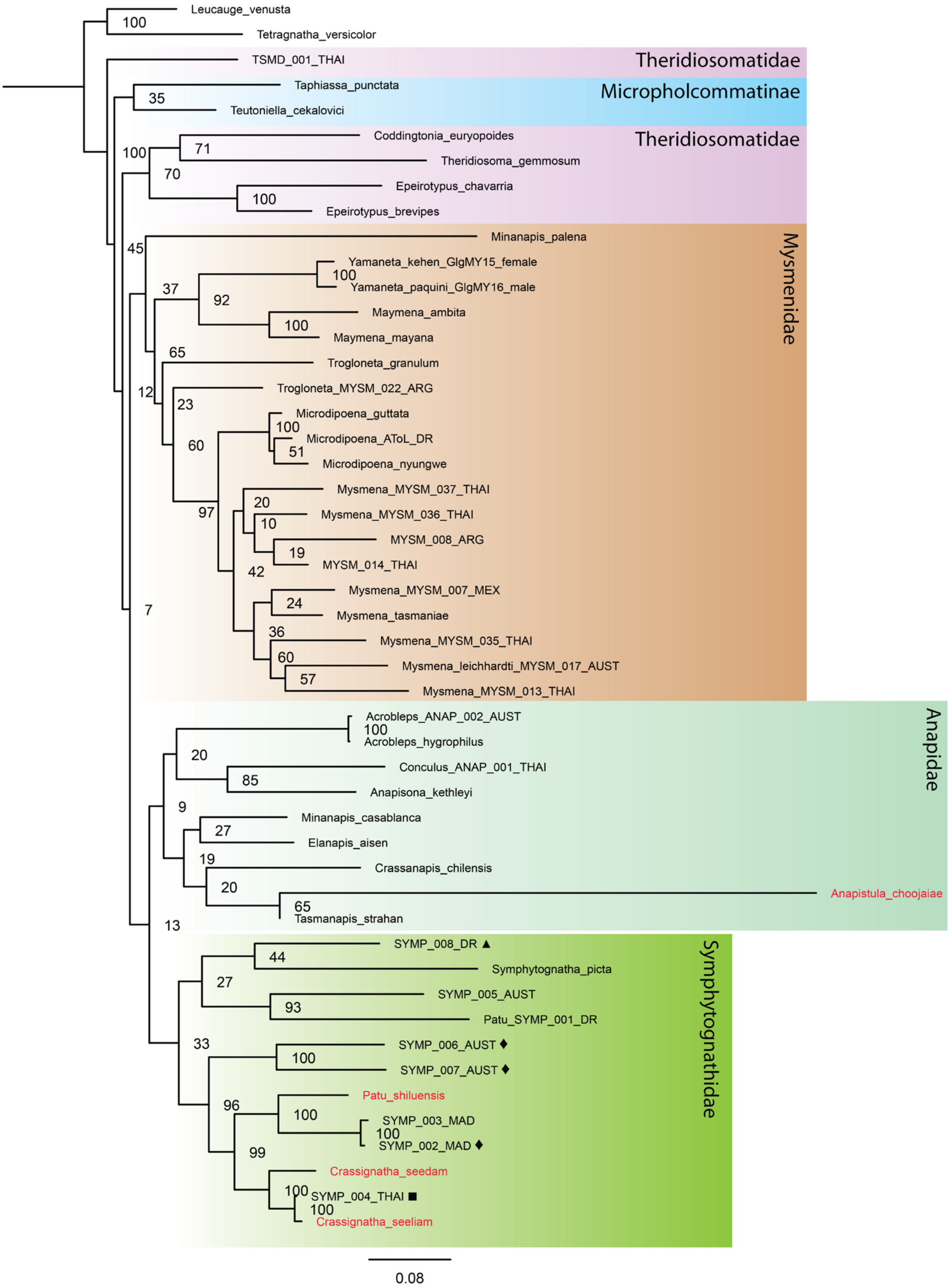

Figure 5.2.– Maximum Likelihood Tree. Obtained in RAxML using a modified version of Lopardo et al. [4] and Feng et al. [5] plus the four symphytognathid species from our study (in red). Numbers at nodes indicate bootstrap support. Note the long branch of *Anapistula* and its position within Anapidae; and the high support of *Crassignatha* and *Patu* in the Symphytognathidae. Molecular vouchers used for previous "symphytognathoid" studies [4,31] identified to genus level by L. Lopardo (pers. comm.) as follows: ■ *Crassignatha* (apparently conspecific with *C. seeliam*); ◆*Patu*; and ▲*Symphytognatha*.

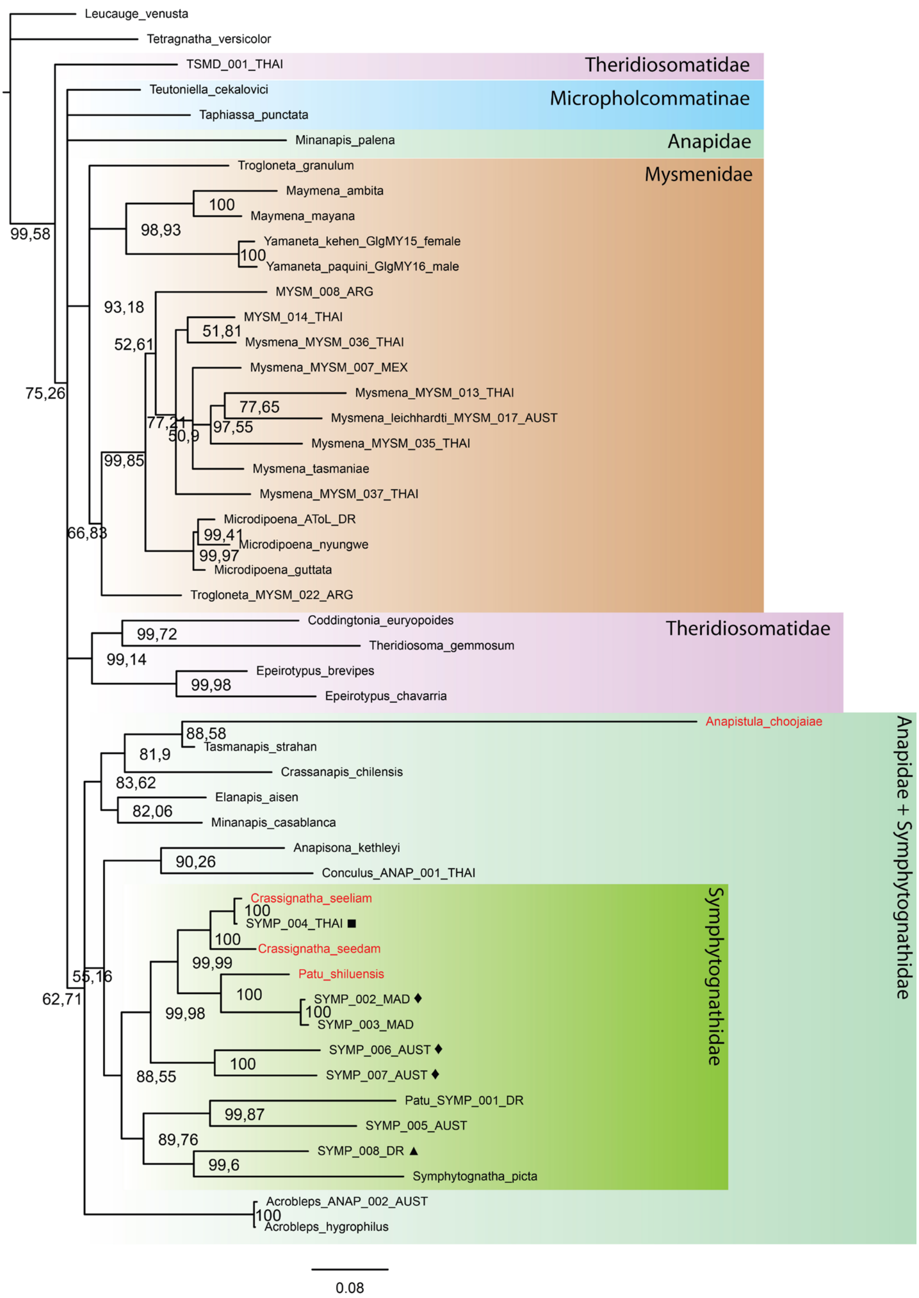

Figure 5.3.– Bayesian Inference Tree. Obtained in Mr. Bayes using a modified version of Lopardo et al. [4] and Feng et al. [5] plus the four symphytognathid species from our study (in red). Numbers at nodes indicate percent posterior probabilities. Note the unresolved relations of the Anapidae and the highly supported monophyly of Symphytognathidae. Molecular vouchers used for previous "symphytognathoid" studies [4,31] identified to genus level by L. Lopardo (pers. comm.) as follows: ■ *Crassignatha* (apparently conspecific with *C. seeliam*); ◆*Patu*; and ▲*Symphytognatha*.

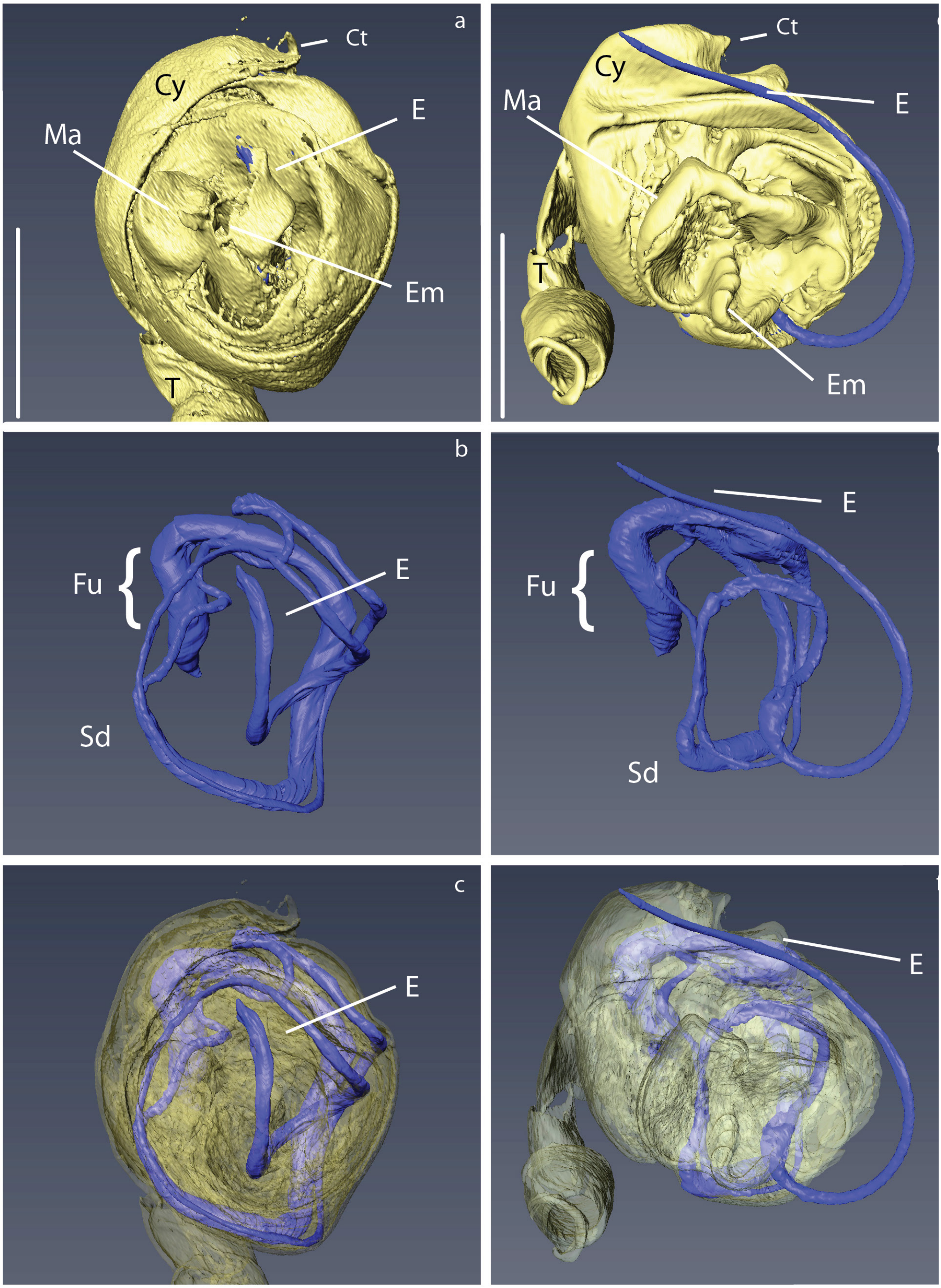

Figure 5.4.- 3D reconstruction of the male palp of *Crassignatha*. With detail in the spermatic ducts: a–c *C. seeliam* sp. n; d–f *C. danaugirangensis*. Scale bars: 0.1 mm. Ct – Cymbial tooth; Cy – Cymbium; E – Embolus; Em– Embolic membrane; Fu – Fundus; MA – Median apophysis; Pa – Patella; Sd – Spermatic duct; T – Tibia.

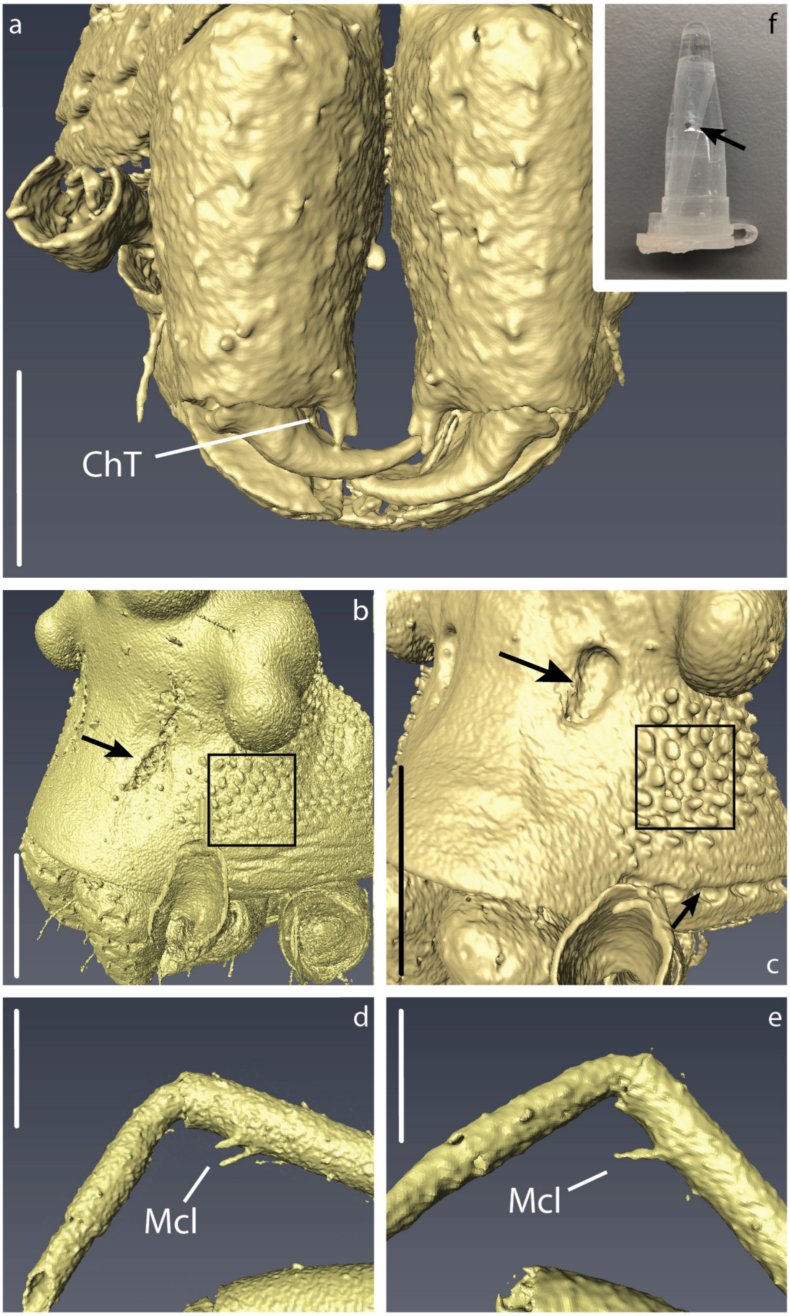

Figure 5.5.- 3D reconstruction of some diagnostic characters of *Crassignatha*. a, c, e *C. danaugirangensis*. b, d *C. seeliam* sp. n.; a chelicerae, arrow pointing at the bifurcated tooth; b, c detail of the carapace; cephalothorax tubercles (in the squares), and pore bearing sulcus (arrows); d, e Male leg II clasper; f whole male specimen of *C. danaugirangensis* prepared for micro-CT inside a modified 10ul pipette tip and a 0.5ml Eppendorf tube filled with 70% Et-OH. Scale bars: 0.06 mm (a); 0.1 mm (b–e). ChT– cheliceral tooth; Mcl– male leg II mating clasper.

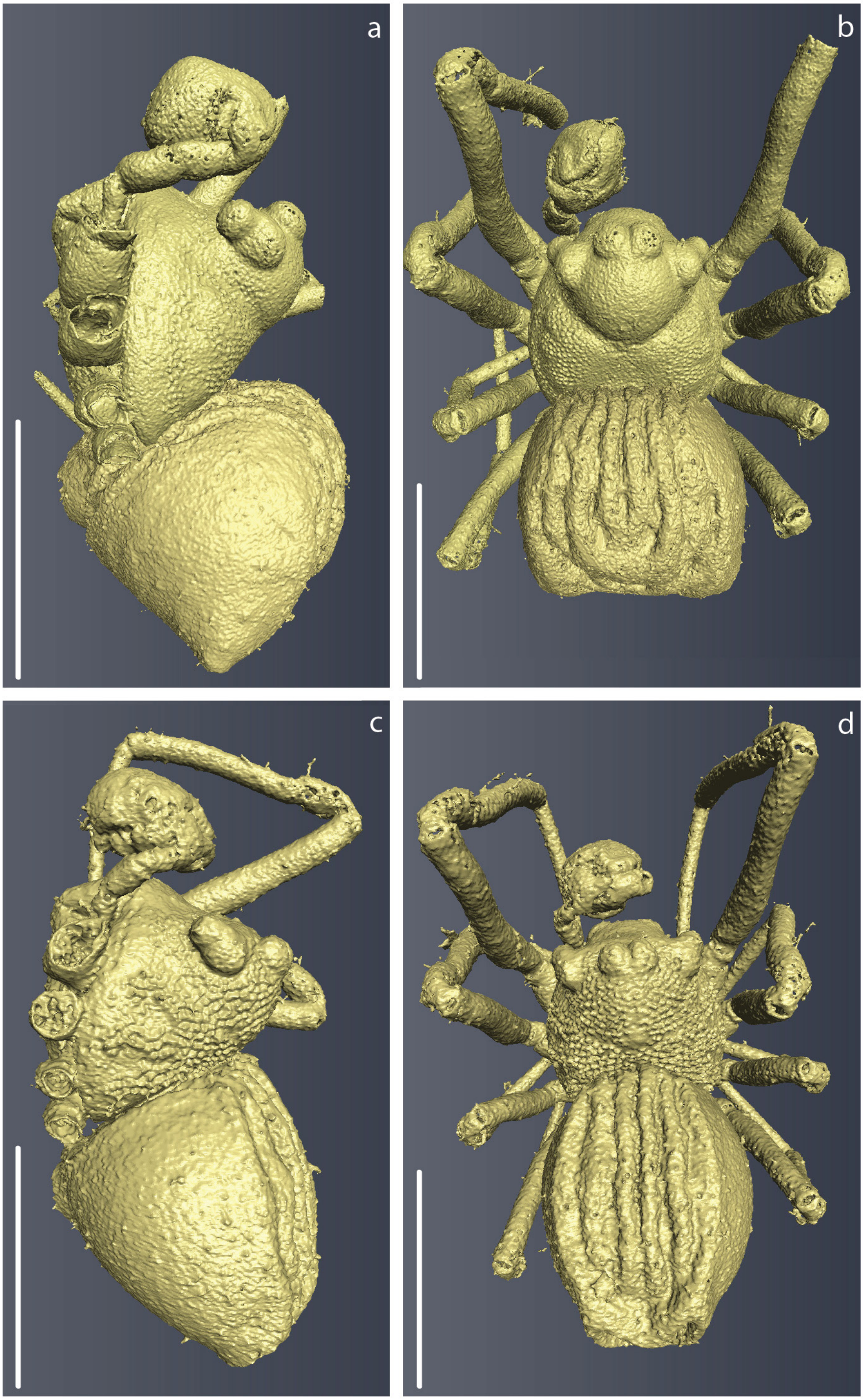

Figure 5.**6.- 3D reconstruction of the habitus of *Crassignatha.*** males: **a, b** *C. seeliam* sp. n; **c, d** *C. danaugirangensis*. Right pedipalp was dissected previous to the scanning. Scale bars: 0.3 mm.

Micro-CT and 3D modelling

The micro computed tomography scans allowed us to observe in detail small structures of the surface and internal ducts of the male genitalia (Figs. 5.4a–f). Structures like the cheliceral teeth (Fig. 5.5a), cephalothorax tubercles (Figs. 5.5b, c), and mating clasper on male tibia II (Figs. 5.5d, e) were also observed. We reconstructed 3D models of the whole body surface of *Crassignatha seeliam* (Figs. 5.6a–b) and *Crassignata danaugirangensis* (Figs. 5.6c–d). All of these images were important to examine, interpret and clarify the diagnostic characters of the genus *Crassignatha*. Additional views of the pedipalps, spermatic ducts and habitus can be found in the Supplementary Material (SM2, SM3)

Taxonomy

Family Symphytognathidae Hickman, 1931
Genus *Anapistula* Gertsch, 1941

Anapistula Gertsch, 1941: 2. Type species *Anapistula secreta* Gertsch, 1941.

***Anapistula choojaiae* sp. nov.**

Figs. 5.7–5.9

Holotype: THAILAND • ♂; Chiang Mai, Pha Daeng National Park. Riparian tropical forest; 19º37.768’N, 98º57.257’E. 560m; July 16-19, 2018; Booppa Petcharad, Jeremy Miller, F. Andres Rivera-Quiroz leg.; Winkler extractor; RMNH.ARA.18442. **Paratypes:** THAILAND • ♀ allotype; same data as holotype • 1♂ 1♀; same data as holotype; RMNH.5106639 • 2♀; Pha Daeng National Park. Bamboo forest; 19º37.668’N, 98º57.131’E. 573m, same dates and collectors as holotype; RMNH.ARA.18443.

Etymology: The species epithet is a Latinized matronym of the second authors’ daughter.

Diagnosis: Female genitalia in *Anapistula* show little morphological variation between congeneric species making it generally difficult to tell species apart. However, *A. choojaiae* sp. nov. can be distinguished from most *Anapistula* species by the presence of an epigynal atrium; *A. aquytabuera* Rheims & Brescovit, 2003, *A. pocaruguara* and *A. ybyquyra* Rheims & Brescovit, 2003 —from Brazil—, *A. panensis* Lin, Tao, and Li 2013 and *A. zhengi* Lin, Tao, and Li 2013 —from China—, and *A. seychellensis* Saaristo, 1996 —from the Seychelles— also share this character. *A. choojaiae* differs from all of these by the relative size and shape of the atrium, the width of the EMD and the bifurcation of the Lb (compare Fig. 5.8d and 5.9c to figs. 16, 18, 21: [29]; figs. 3, 4, 8, 9:[14]; fig. 3: [30]).

Male pedipalp of *A. choojaiae* similar to *A. panensis* in the overall shape of the palp and in having C1 and C2 roughly the same length, but differs on the width of C1 in respect to C2 and the length of the E in relation to C1 (compare Figs. 5.7c, 5.9a to fig. 1-2:[14]).

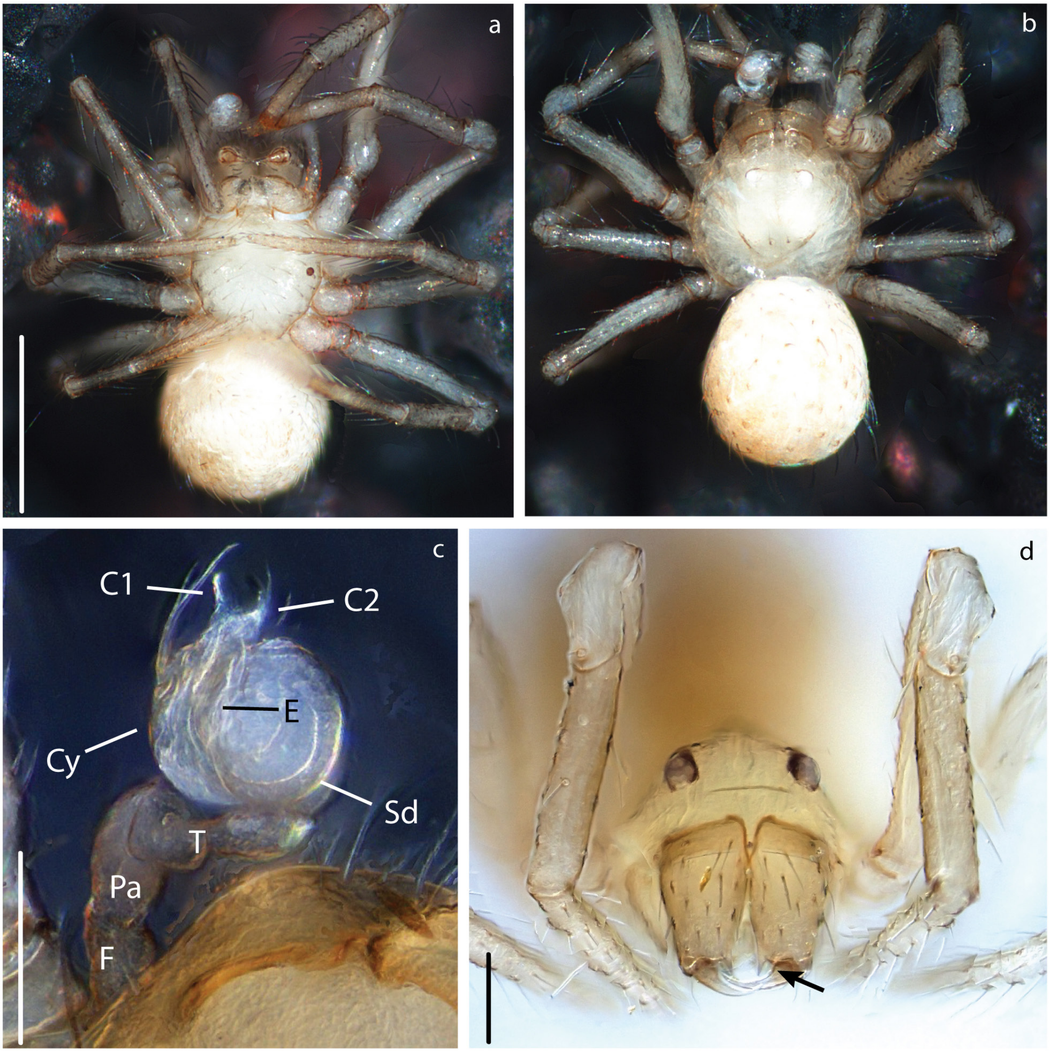

Figure 5.7.- ***Anapistula choojaiae*** sp. n. male: Habitus: **a** ventral view; b dorsal view. Palp: **c** ventral view. Female: Prosoma: **d** anterior view. Scale bars: 0.2 mm (**a, b**); 0.07 mm (**c**); 0.06 mm (**d**). Arrow pointing to the cheliceral teeth. C1 – Conductor, anterior projection; C2 – Conductor, posterior projection; Cy – Cymbium; E – Embolus; F – Femur; Pa – Patella; Sd – Spermatic duct; T – Tibia.

Description: Carapace ovoid, yellowish-white with smooth texture (Figs. 5.7a, b; **5.**8a, b). AME absent (Fig. 5.7d). Male LE without pigmentation (Figs. 5.7b; **5.**8b). Chelicerae with two promarginal teeth (Fig. 5.7d). Legs same color as carapace with slightly darker color on distal segments. Abdomen sub-spherical with small sparse sclerotized patches, some bearing long setae (Figs. 5.7b; 5.8b). Scuta absent in both sexes.

Male palp: Weakly sclerotized (Fig. 5.7c). Semicircular from ventral view (Figs. 5.7c; **5.**9a). With one wide sheet shaped conductor that presents two projections, here called C1 and C2 (Fig. 5.9a; b). Embolus short and transparent located posteriorly to C; very difficult to see (Figs. 5.7c; 5.9a).

Figure 5.8.- ***Anapistula choojaiae*** sp. n. female: Habitus: **a** ventral view; **b** dorsal view. Epigynum: **c** ventral view; **d** dorsal view, cleared. Scale bars: 0.2 mm (**a, b**); 0.06 mm (**c**); 0.03 mm (**d**). A – Atrium; Cd – Copulatory duct; Co – Copulatory opening; MD – Epigynal median duct; Fd – Fertilization duct; Lb – lateral branch of the EMD; S – Spermatheca.

Vulva: Epigynal plate flat, without scape. Atrium semi-circular as wide as inner distance between S (Fig. 5.8c). Spermathecae spherical, heavily sclerotized in relation to the rest of the body (Fig. 5.8d). Cd easy to distinguish inside the EMD. LB diverging from the EMD forming a "Y" (Figs. 5.8d; 5.9c). Fertilization ducts very short and difficult to see, they appear as small bumps on the distal portion of Lb (Fig. 5.9c).

Male: Total length 0.4; carapace 0.2 long, 0.21 wide; clypeus 0.03; Chelicera 0.1 long, 0.06 wide; Leg I: femur 0.26, patella 0.1, tibia 0.17, metatarsus 0.09 tarsus 0.17; leg formula IV-I-II-III; abdomen 0.21 long, 0.21 wide.

Female: Total length 0.43, carapace 0.2 long, 0.21 wide; clypeus 0.3; Chelicera 0.1 long, 0.05 wide; Leg I: femur 0.20, patella 0.09, tibia 0.14, metatarsus 0.16, tarsus 0.1; leg formula IV-I-II-III; abdomen 0.24 long, 0.23 wide.

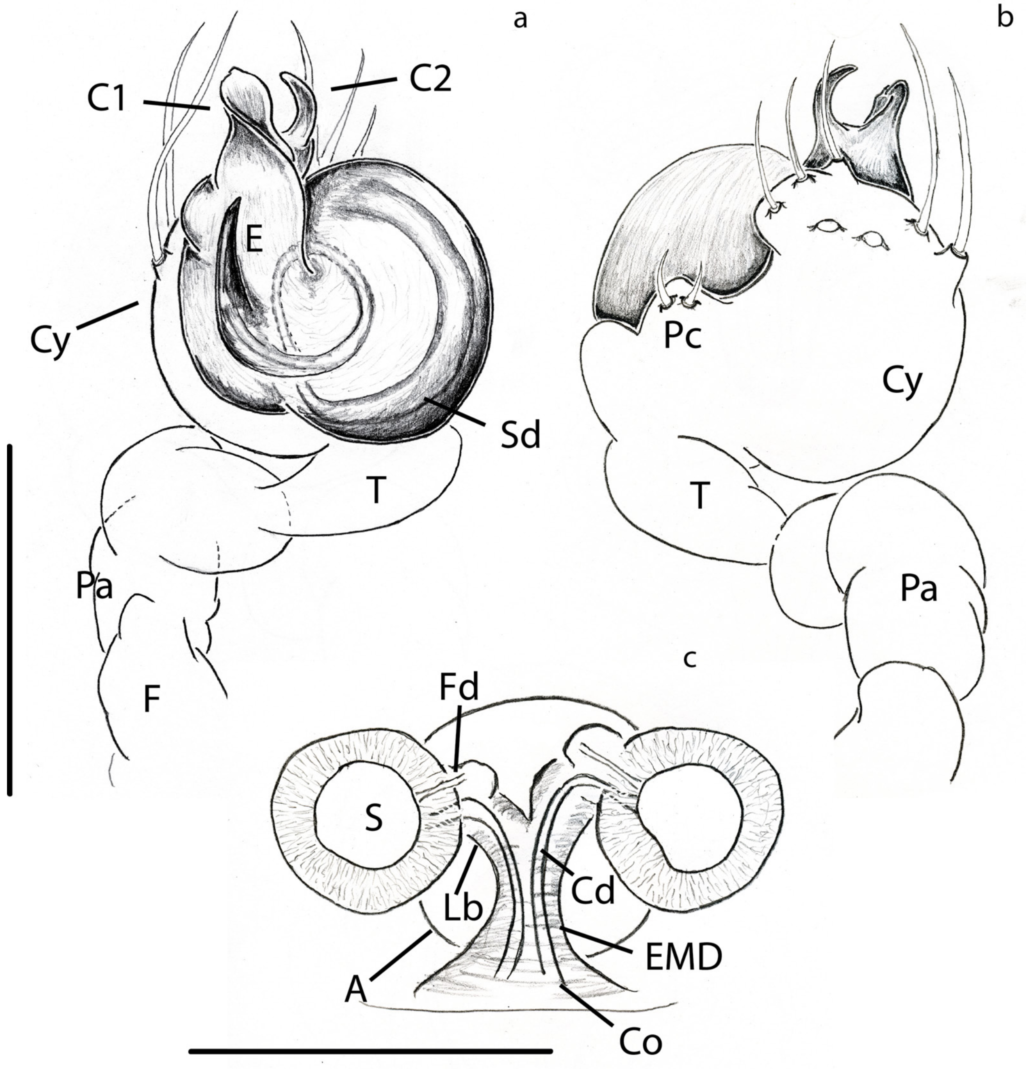

Figure 5.9.- ***Anapistula choojaiae* sp. n., genitalia**. Palp: a ventral view; b dorsal view. Epigynum, cleared: c dorsal view. Scale bars: 0.07 mm (a, b); 0.06 mm (c). A – Atrium; C1 – Conductor, anterior projection; C2 – Conductor, posterior projection; Cd – Copulatory duct; Co – Copulatory opening; Cy – Cymbium; E – Embolus; F – Femur; Fd – Fertilization duct; Lb – lateral branch of the EMD; MA – Median apophysis; MD – Epigynal median duct; Pa – Patella; Pc – Paracymbium; S – Spermatheca; Sa – Sececretory ampullae; Sc – Scape; Sd – Spermatic duct; T – Tibia.

Genus *Crassignatha* Wunderlich, 1995

Crassignatha Wunderlich, 1995: 547. Type species *Crassignatha haeneli* Wunderlich, 1995.

Crassignatha seeliam sp. nov.

Figs. 5.4a-c; 5.5b, d; 5.6a, b; 5.10–5.12.

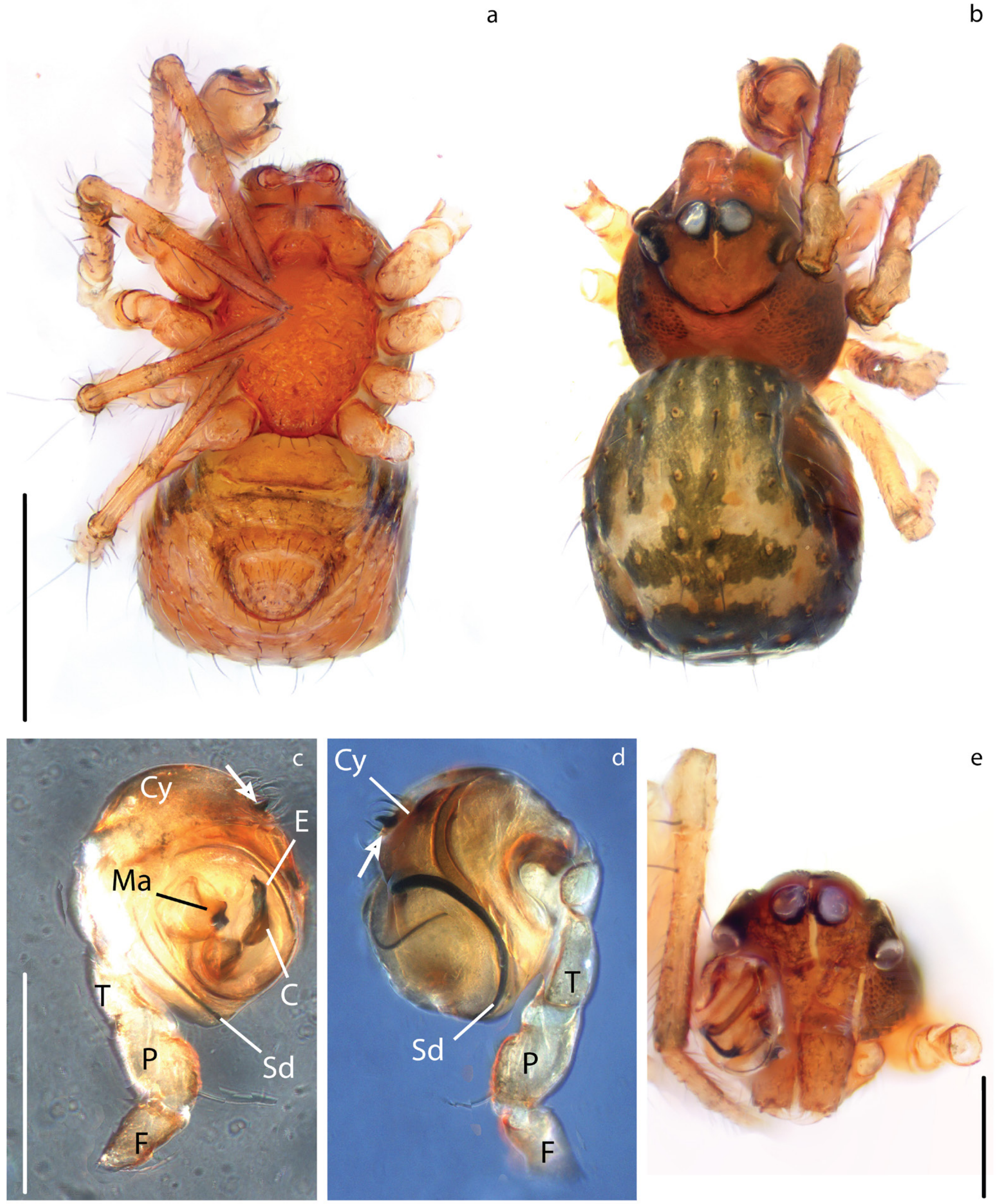

Figure 5.**10.**- ***Crassignatha seeliam*** sp. n., male: Habitus: **a** ventral view; **b** dorsal view. Palp: **c** ventral view; **d** retrolateral view. Prosoma: **e** anterior view. Scale bars: 0.3 mm (**a, b**); 0.15 mm (**c–e**). Arrow pointing at the Cymbial tooth. Ct – Cymbial tooth; Cy – Cymbium; C – Conductor; E – Embolus; Em– Embolic membrane; F – Femur; MA – Median apophysis; Pa – Patella; Sd – Spermatic duct; T – Tibia.

Holotype: THAILAND • ♂: Chiang Mai, Doi Inthanon National Park. Montane evergreen forest; 18°30.454’N, 98°30.584’E. 1605m; July 21-24, 2018;

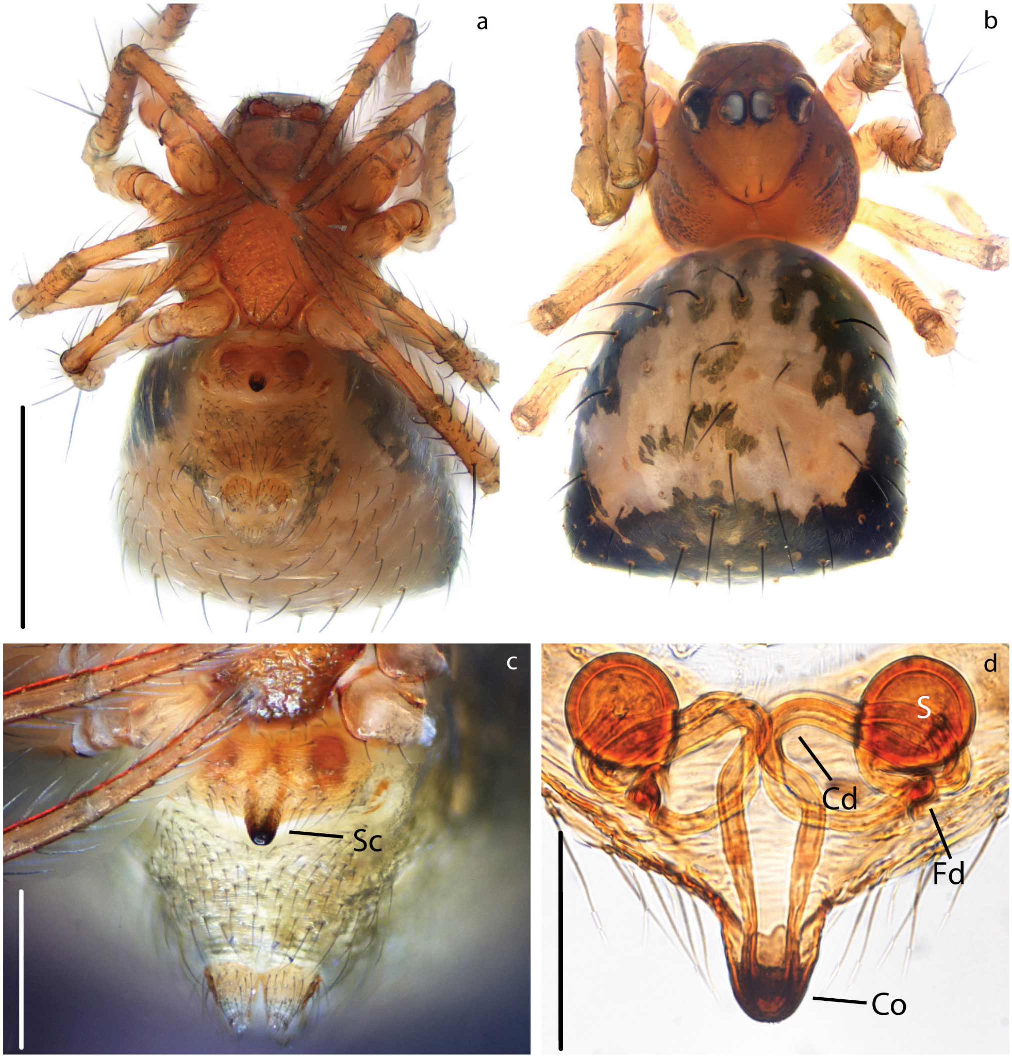

Figure 5.**11**.- ***Crassignatha seeliam*** sp. n. female: Habitus: **a** ventral view; **b** dorsal view. Epigynum: **c** ventral view; **d** dorsal view, cleared. Scale bars: 0.4 mm (**a, b**); 0.15 mm (**c**); 0.07 mm (**d**). Cd – Copulatory duct; Co – Copulatory opening; Fd – Fertilization duct; S – Spermatheca; Sa – Sececretory ampullae; Sc – Scape.

Booppa Petcharad, Jeremy Miller, F. Andres Rivera-Quiroz leg.; direct hand coll.; RMNH.ARA.18444. **Paratypes**: THAILAND • ♀ allotype; same data as holotype • 8 ♀; same data as holotype; RMNH.5106641• ♂ and ♀ Chiang Mai, Doi Suthep National Park. Montane evergreen forest with pine; 18º48.502'N, 98º53.528'E. 1409m; July 24-28, 2018; same collectors as holotype; pitfall traps. RMNH.ARA.18445.

Etymology: The species epithet is a derivation of the Thai *seeliam* (square), in reference to the shape of the abdomen in dorsal view.

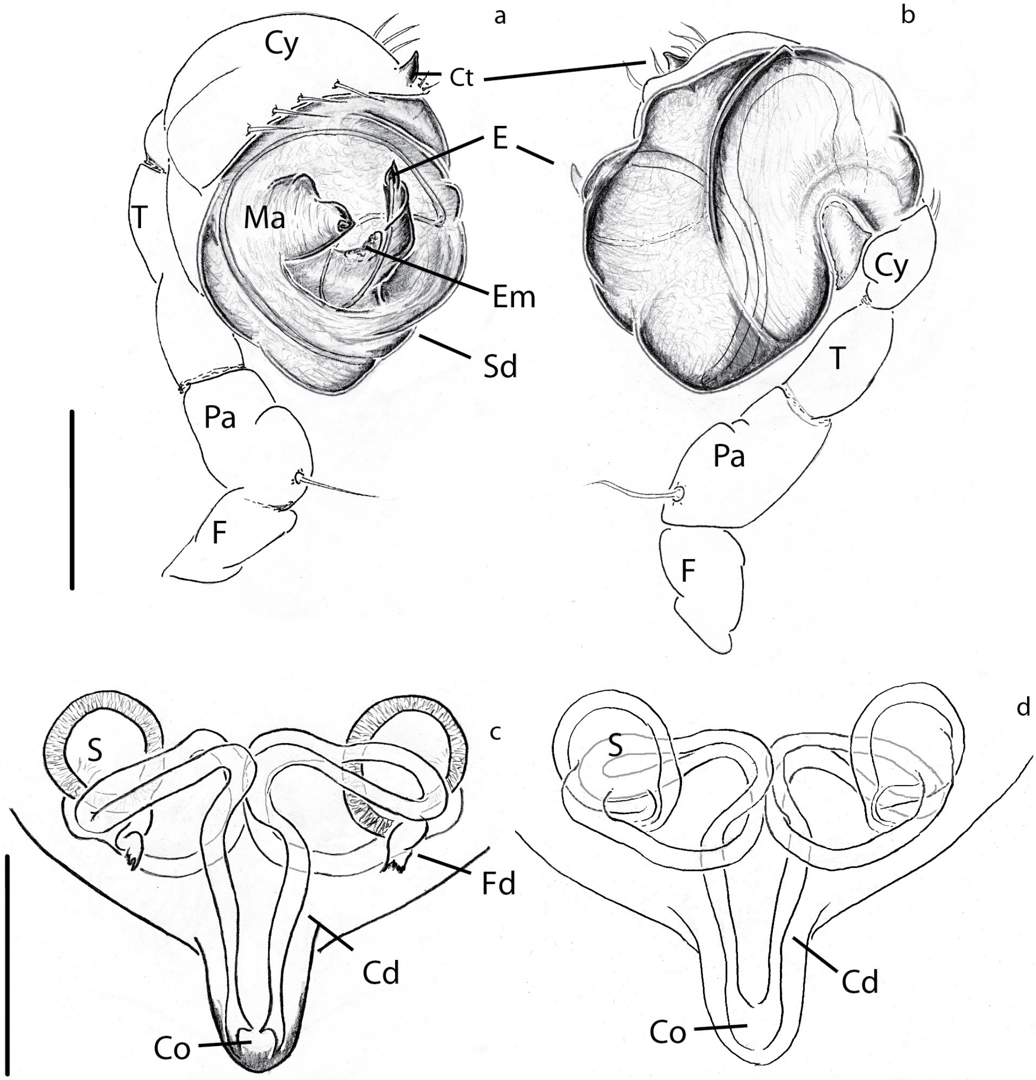

Figure 5.12.- *Crassignatha seeliam* sp. n., genitalia. Palp: a ventral view; b dorsal view. Epigynum, cleared: c dorsal view, d ventral view. Scale bars: 0.1 mm (a, b); 0.07 mm (c, d). Cd – Copulatory duct; Co – Copulatory opening; Ct – Cymbial tooth; Cy – Cymbium; E – Embolus; Em– Embolic membrane; F – Femur; Fd – Fertilization duct; MA – Median apophysis; Pa – Patella; S – Spermatheca; Sa – Sececretory ampullae; Sc – Scape; Sd – Spermatic duct; T – Tibia.

Diagnosis: Distinguished from other *Crassignatha* species except *Crassignatha quadriventris* [13] by the semi-squared posterior of the abdomen in dorsal view (Figs. 5.10b; 5.11b). Female can be separated from *C. quadriventris* by the coiling of the copulatory ducts in the epigynum (compare Figs. 5.11d and 5.12c, d to fig. 10: [13]). Male differs on the size of tegular sclerites and the cymbial tooth being short and stout instead of hook- shaped (compare Figs. 5.10c,d and 5.12a, b to fig. 8: [13]).

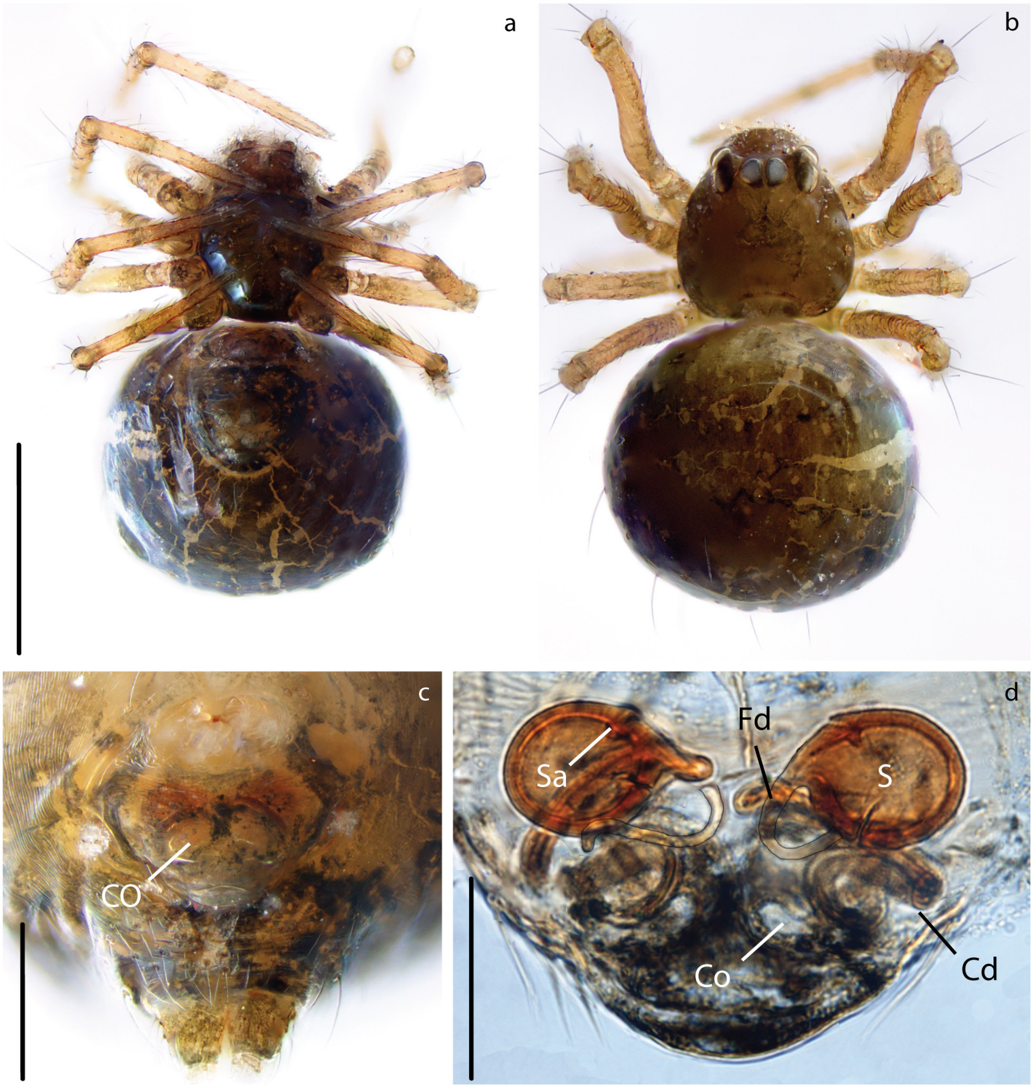

Figure 5.13.- ***Crassignatha seedam*** sp. n. female: Habitus: **a** ventral view; **b** dorsal view. Epigynum: **c** ventral view; **d** dorsal view, cleared. Scale bars: 0.3 mm (**a, b**); 0.1 mm (**c, d**); 0.05 mm (**d**). Cd – Copulatory duct; Co – Copulatory opening; Fd – Fertilization duct; S – Spermatheca; Sa – Sececretory ampullae.

Description: Carapace coloration orange-brown covered by small tubercles (Figs. 5.6a,b; 5.10a, b; 5.11a, b). Legs same color, slightly darker on distal portion its segments. Male Tibia II with two spines (mating claspers) (Fig. 5.5d). Abdomen black with light red patches; squared posteriorly, with sparse sclerotized patches, some bearing long setae (Figs. 5.10b; 5.11b). Male with posterior scutum wrapping the abdomen. Male palp: slightly less sclerotized than carapace. Semicircular from ventral view (Figs. 5.10c; 5.12a). Cymbium with distal tooth. Median apophysis as big as Ct (Fig. 5.12a). Embolus filiform, exposed when palp is expanded (Fig. 5.12c). Spermatic duct very long and coiling two times inside the bulb (Fig. 5.4b, c).

Vulva: Epigynum with wide scape directed ventrally, heavily sclerotized at the tip (Fig. 5.11c). Copulatory opening at the tip of scape (Figs. 5.11d; 5.12c, d). Spermathecae spherical, slightly more sclerotized than epigynum, separated by approximately two times their diameter (Fig. 5.11d). Copulatory ducts very long, coiling over themselves before connecting toS. Fertilization ducts as long as S width, projecting dorsally (Figs. 5.11d, 5.12c).

Male: Total length 0.68; carapace 0.36 long, 0.30 wide; clypeus 0.13; Chelicera 0.1 long, 0.07 wide; Leg I: femur 0.28, patella 0.12, tibia 0.37, metatarsus 0.17, tarsus 0.22; leg formula I-II-IV-III; abdomen 0.42 long, 0.38 wide.

Female: Total length 0.69, carapace 0.44 long, 0.39 wide; clypeus 0.12; Chelicera 0.15 long, 0.1wide; Leg I: femur 0.42, patella 0.15, tibia 0.53, metatarsus 0.22, tarsus 0.27; leg formula I-II-IV-III abdomen 0.44 long, 0.43 wide.

***Crassignatha seedam* sp. nov.**

Figs. 5.13; 5.15b, d

Holotype: THAILAND • ♀ Chiang Mai, Doi Suthep National Park. Montane evergreen forest with pine; 18º48.502'N, 98º53.528'E. 1409m; July 24-28, 2018. Booppa Petcharad, Jeremy Miller, F. Andres Rivera-Quiroz leg.; direct hand coll.; RMNH.5106640. **Male Unknown.**

Etymology: The species epithet is a derivation of the Thai *seedam* (black), in reference to the dark coloration of this species.

Diagnosis: *Crassignatha seedam sp. nov*. differs from other *Crassignatha* species by having a nearly round abdomen instead of triangular or squared, and having the epigynum bulging ventro-posteriorly but not forming an scape (compare Figs. 5.13d and 15b, d to Fig. 5.12c, and fig. 10: [13] and fig. 76d, h: [10]).

Description: Carapace brown with smooth texture (Fig. 5.13b). Legs light brown, slightly darker on the distal portion its segments. Abdomen sub-spherical, darker than carapace with sparse light patches (Figs. 5.13a, b).

Vulva: Epigynum weakly sclerotized but covered by small dark patches (Fig. 5.13d), bulging ventrally. Copulatory openings broad but not forming an atrium (Fig. 5.15b). Spermathecae spherical, much more sclerotized than epigynum, separated by 0.5 times their diameter (Fig. 5.13d). Copulatory ducts long, coiling over themselves before connecting to S. Fertilization ducts as long as S width, connecting very close to Cd and projecting dorsally (Figs. 5.15b, d).

Female: Total length 0.56, carapace 0.28 long, 0.26 wide; clypeus 0.06; Chelicera 0.1 long, 0.07 wide; Leg I: femur 0.3, patella 0.1, tibia 0.22, metatarsus 0.13, tarsus 0.19; leg formula I-II-IV-III; abdomen 0.47 long, 0.41 wide.

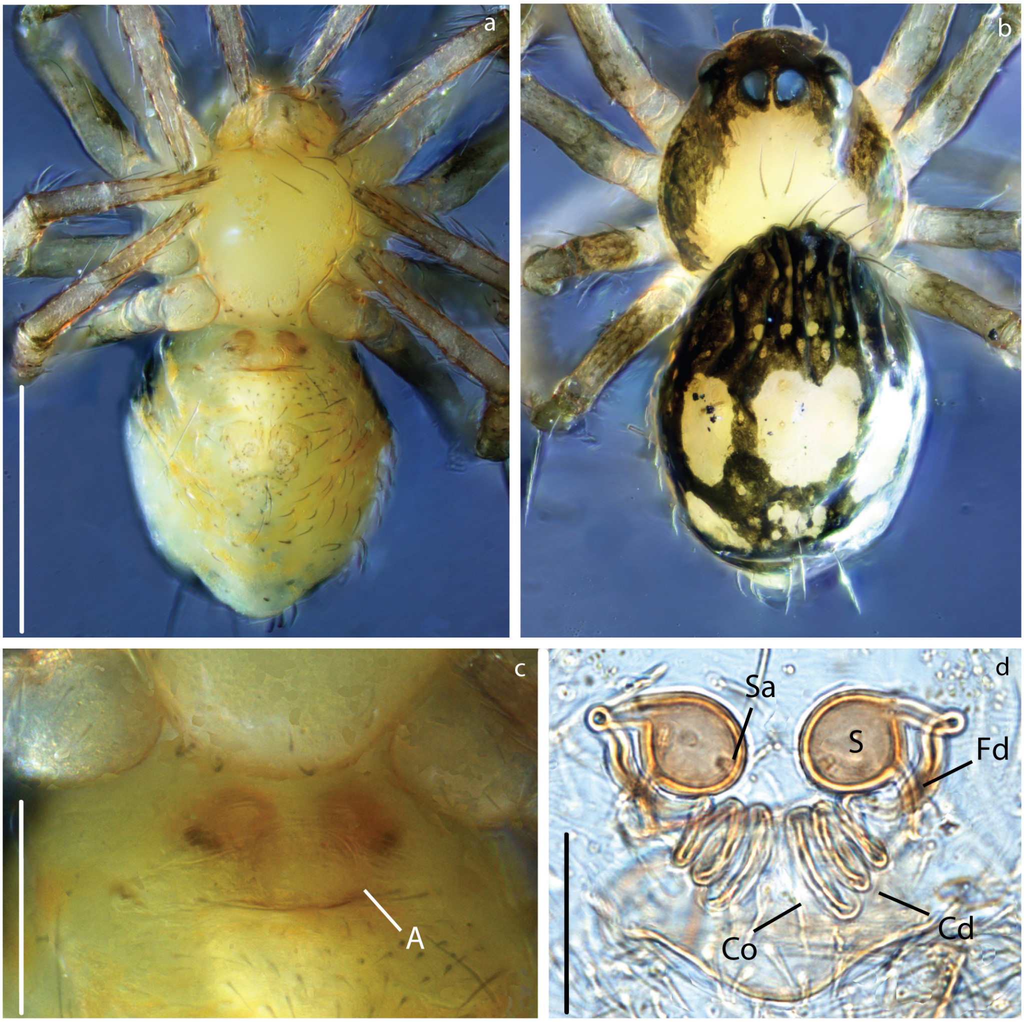

Figure 5.14.- *Patu shiluensis* **Lin & Li, 2009 female**. Habitus: **a** ventral view; **b** dorsal view. Epigynum: **c** ventral view; d dorsal view, cleared. Scale bars: 0.2 mm (**a, b**); 0.06 mm (**c**); 0.03 mm (**d**). A – Atrium; Cd – Copulatory duct; Co – Copulatory opening; MD – Epigynal median duct; Fd – Fertilization duct; Lb – lateral branch of the EMD; S – Spermatheca Sa – Secretory ampullae.

Crassignatha danaugirangensis **Miller et al., 2014**

Crassignatha danaugirangensis Miller et al., 2014: 4. f. 1a–f, 3, 4.Figs. 5.4d–f; 5.5a, c, e; 5.6c, d.

New records. BRUNEI • 2♂; Temburong, Huala Belalong Field Studies Centre; 4.545ºN 115.157ºE, 150m; September 26 – October 6, 2018; *Taxon Expeditions 2018* leg.; Winkler extractor; RMNH.5106643.

Genus *Patu* Marples, 1951

Patu Marples, 1951: 47. Type species *Patu vitiensis* Marples, 1951.

***Patu shiluensis* Lin & Li, 2009.**

Patu shiluensis Lin & Li, 2009: 59, f. 11A-B, 12A-B, 13A-D.

Figs. 5.14, 5.15a, c.

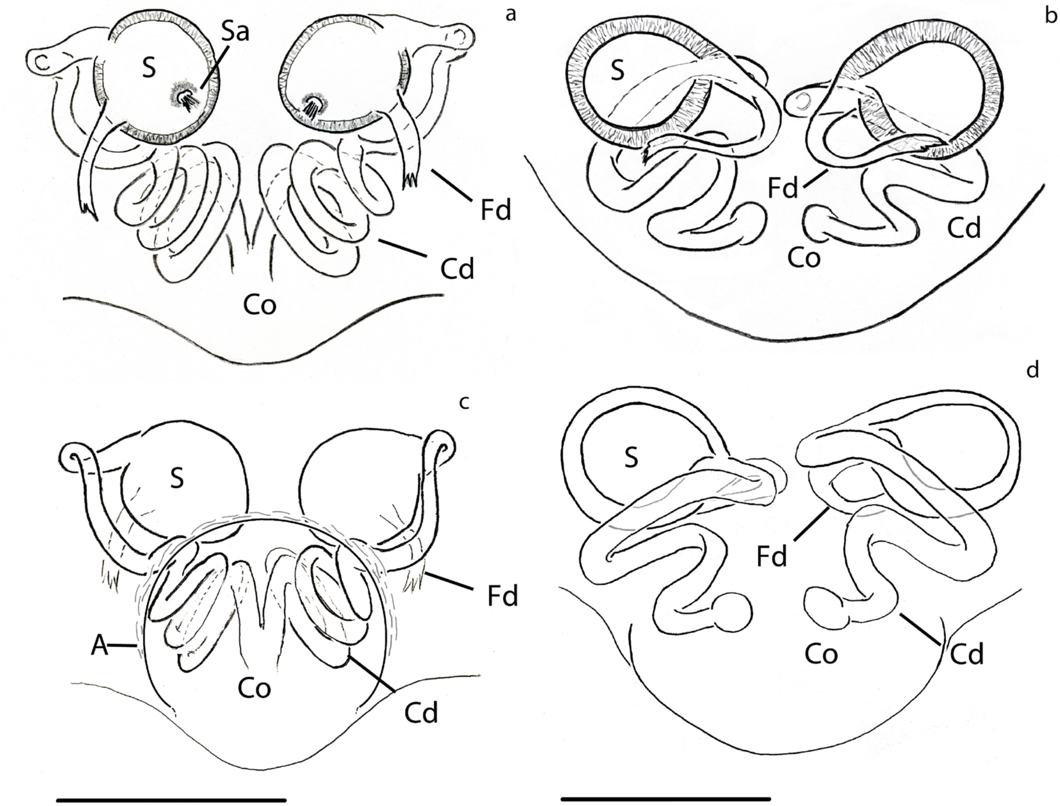

Figure 5.15.- Epigynum, cleared. a, c *Patu shiluensis* Lin & Li, 2009: **b, d** *Crassignatha seedam* sp. n. : **a, b** dorsal view; **c, d** ventral view. Scale bars: 0.03 mm (**a, c**); 0.05 mm (**b, d**). A – Atrium; Cd – Copulatory duct; Co – Copulatory opening; Fd – Fertilization duct; S – Spermatheca; Sa – Sececretory ampullae.

Collected material: THAILAND • 4♀; Phuket Province, Siray Island. Mixed tropical forest; 7º53.355'N, 98º26.083'E. 132m; August 02-06, 2018; Booppa Petcharad, Jeremy Miller, F. Andres Rivera-Quiroz leg.; Winkler extractor; RMNH.5106642.

Distribution: Known only from its type locality, Shilu Town, Hainan Province, China and the specimens collected for the present work.

Morphological remarks: Carapace pale yellow with black margin, smooth texture (Fig. 5.14b). Legs black and semi-transparent. Abdomen oval, longer than wide (Figs. 5.14a, b). Ventrally same color as carapace, dorsally, darker with pale yellow patches.

Vulva: Epigynum weakly sclerotized, semi-transparent (Fig. 5.14c). Atrium semi-circular slightly wider than inner distance between S (Figs. 5.14c; 5.15c). Spermathecae spherical slightly more sclerotized than epigynum, separated by 0.5 times their diameter (Fig. 5.14d). Copulatory ducts spring-like, spiraling three times over themselves.

Fertilization ducts as long as S width, projecting posteriorly (Figs. 5.14d; **5.**15a, c).

Female: Total length 0.52, carapace 0.21 long, 0.2 wide; clypeus 0.04; Chelicera 0.07 long, 0.05 wide; Leg I: femur 0.15, patella 0.07, tibia 0.1, metatarsus 0.07, tarsus 0.1; leg formula I-II-IV-III; abdomen 0.34 long, 0.28 wide.

Notes: Small somatic variations can be seen between the specimen we collected in Thailand and the ones previously described from China (compare Fig. 5.14b to fig. 11: [13]). However, we did not find any objective differences in the female genitalia.

Secretory ampullae (Figs. 5.14d, 5.15a) were very evident in our specimens; these glandular structures might be homologous to the accessory glands in Lopardo and Hormiga [31]. These structures were found in one anapid (*Tasmanaspis*) and several mysmenids, but scored as absent or unknown for all the symphytognathids.

The authors of this species mentioned it to be close to *Patu silho* Saaristo, 1996 from Seychelles. The possibility of *P. silho* not being a true *Patu* was discussed by its author [30,32] mentioning evident differences on somatic and sexual characters between *P. silho* and other *Patu* species. Nevertheless, the author deemed appropriate to place it in this genus. We also consider this species might be misplaced in *Patu* but would need further and more detailed analysis out of the scope of this work to clarify it (see discussion on *Patu* relationships below).

Discussion

The monophyly of the Symphytognathidae and its relations to other symphytognathoid spiders have resulted in complications and inconsistencies across different studies. The symphytognathoids were first recognized in a morphological study being formed by four putatively monophyletic families Anapidae, Symphytognathidae, Mysmenidae and Theridiosomatidae [2]. The monophyly of this clade has been tested several times using different molecular approaches targeting specific families [4,5,33], the Orbiculariae [34], and the whole order Araneae [6,7]. However, only a few representatives of the family Symphytognathidae have been used rendering their position and relations largely unexplored. Here, we built on two previous studies that used 9 species of Symphytognathidae to test the relations of the Mysmenidae [4,5]. Similarly to Feng et al. [5] low node supports were common in our trees, especially for MP and ML; still, the topologies we observed when including our 4 species are consistent with the results from these studies. All of our analyses showed a close relationship between the Symphytognathidae and the Anapidae (Figs. 5.1–**5.**3). This relationship has also been recovered in previous works [2,4–6]. Although tenuous due to the few terminals included, our study fails to recover the monophyly of the Anapidae and the position of micropholcommatids within this family. Our BI tree could not fully resolve the relations between the Anapidae and Symphytognathidae; similar issues have been observed before for the symphytognathoids [4,5,33–35]. This has been explained by either the limited set of loci and the relatively low taxon sampling [5] or an indication of the polyphyly of the "symphytognathoids" as suggested by three broad scoped phylogenies [6,34,35]. Nevertheless, Symphytognathoids were found to be a highly supported monophyletic group in a recent study that used ultraconserved elements (UCE) from 16 species across the four principal symphytognathoid families [7]

The internal relations of the Symphytognathidae in our analyses are still unresolved. Most of Lopardo's identifications (*pers. comm.*) are found in the *Crassignatha* + *Patu* clade. From these, SYMP_004_THAI (identified to *Crassignatha;* presumably conspecific to *C. seeliam*), and SYMP_002_MAD and SYMP_003_MAD (*Patu*) group together with the other representatives of the genera they were identified to. But the placing of two more, SYMP_006_AUS and SYMP_007_AUS (*Patu*), is more ambiguous being found outside of the *Crassignatha* + *Patu* clade rendering *Patu* paraphyletic. Thhis cladeand its internal relations are highly supported in all our trees (Figs. 5.1–5.3). Other two sequences, SYMP_008_DR (*Symphytognatha*) and Patu_SYMP_001_DR, are consistently grouped in another branch of the Symphytognathidae together with *Symphytognatha picta* and other unidentified symphytognathid (Figs. 5.1–5.3) suggesting that Patu_SYMP_001_DR might be misidentified. The position of *Anapistula* within the Symphytognathidae is also problematic. *Anapistula choojaiae* has a very long branch that is recovered as a sister to *Tasmanapis strahan* Platnick & Forster, 1989 with moderate to high support in the ML and BI (Figs. 5.2, 5.3). In these two analyses, this branch is related to other Anapidae having much higher support values in the BI than the ML (Figs. 5.2, 5.3). Nevertheless, the recent UCE study by Kulkarni, et al. [7] places this genus next to *Patu* in a highly supported but taxonomically limited Symphytognathidae. Solving the internal relations of the families Anapidae and Symphytognathidae, and clarifying their delimitations would need a much more detailed examination with a broader taxonomic sample.

The minute size of the symphytognathid spiders complicates the observation of diagnostic traits. Examination and interpretation of many characters require higher magnifications than those a dissection microscope can give. Therefore, SEM images have been previously used in the taxonomy of this family [8,10,29]. Unfortunately, the process for getting SEM images is destructive; therefore, rare specimens or short series are not usually prepared in this way and some characters cannot be properly observed. Here we used micro-CT scanning to overcome this issue and get clear views of important characters without damaging the specimens. 3D reconstruction has been used before to elucidate surfaces and internal structures of spider genitalia [36–38]. Nevertheless, ours are, to the best of our knowledge, the smallest palps that have been processed using this method. This was challenging in itself since we wanted to preserve the samples without critical point drying, a method commonly used in micro-CT scanning [37,39–41]. The tiny size of the palps, less than 0.2mm wide, did not allow to properly fix the dissected organ and keep it from moving during the scanning process. We attempted to fix the palp in agarose but the contrast of the resulting scans was too low to allow any observations. This problem was solved by scanning the entire spider (without dissecting the palp) in Et-OH 70% inside a modified 10ul pipette tip that was in turn inside a 0.5ml Eppendorf tube (Fig. 5.5f) in a similar fashion to Lipke, Hammel, and Michalik (2015), and Sombke et al. (2015). With this approach we were able to reconstruct the long and complicated internal ducts of the male genitalia (Figs. 5. 4b, c, e, f), as well as the surface of the external somatic and genital morphology (Figs. 5.4a, b; 5.5a-e; 5.6a-d; SM2, 3). Other internal structures of the male palp —probably glands— could be observed but would require more detailed examination out of the scope of the present work to accurately determine their nature; therefore, they are not shown in our 3D models. Images obtained through 3D reconstruction were used to interpret and discuss the diagnostic characters of the genus *Crassignatha* and compare them to other Symphytognathid genera in Table2.

Forster and Platnick [8] reviewed the Symphytognathidae and its component genera. Five of the eight currently recognized symphytognathid genera were included: *Anapistula* Gertsch, 1941, *Curimagua* Forster & Platnick, 1977, *Globignatha* Balogh & Loksa, 1968, *Patu* Marples, 1951, and *Symphytognatha* Hickman, 1931. *Crassignatha* Wunderlich, 1995 was described based on a single male specimen from peninsular Malaysia. This genus has been associated with several families (Synaphridae, Anapidae, Mysmenidae, Symphytognathidae; [9, 10, 31, 55]) and is currently considered a symphytognathid. Two other genera currently cataloged as Symphytognathidae, *Iardinis* Simon, 1899 *Anapogonia* Simon, 1905, are unrecognizable [8, 31, 46, 52]. Although spider taxonomy generally relies heavily on genitalia, little in the way of descriptive text or helpful depictions of genitalic characters was offered in Forster & Platnick's [8] revision. Table 2 summarizes some important diagnostic characters of the currently accepted symphytognathid genera in an attempt to clarify the taxonomic inconsistencies in this family.

Other than their small size, the characteristic that is perhaps most strongly associated with the Symphytognathidae was the fusion of the chelicerae [8]. But the degree of fusion is variable across the family and is particularly problematic in the genus *Patu*. The two species originally placed in *Patu* were reported as having the chelicerae fused for about half their length, but the degree of fusion was apparently less extensive in the genotype *Patu vitiensis* than in *Patu samoensis*, the other species described [48]. Subsequent authors have generally characterized *Patu* as having the chelicerae fused only at the base (Forster & Platnick, 1977). Curiously, Forster [54] made no mention of cheliceral fusion in *Patu*, but he did report basal fusion of the chelicerae in two genera (*Pseudanapis* and *Textricella*) that were subsequently transferred to Anapidae. So, assessing the presence or absence of basal cheliceral fusion is not always straight forward in practice. Some (but not all) *Patu* species known from males have a number of ventral distal macrosetae on tibia II, a characteristic scored as present in Lopardo's *Patu* specimens SYMP_002_MAD and SYMP_006_AUS and absent in Patu_SYMP_001_DR and *Symphytognatha picta* [31]. The this leg II clasper is otherwise found only in *Crassignatha.*

Genotype Crassignatha haeneli Wunderlich, 1995 features a textured carapace and a distinctive ventral spur on tibial II (Figs. 5.5d, e; figs. 14, 15, 17:[16]). The chelicerae are not conspicuously fused and are armed with a single bifid tooth (Fig. 5.5a); a character also scored for three species (SYMP_002_MAD, SYMP_006_AUS and SYMP_007_AUS, later on identified as Patu) used in Lopardo and Hormiga [31]. Miller et al. [10, 19] placed several additional species in Crassignatha, including the first

Table 2- Overview of diagnostic characters of the currently accepted genera of the Symphytognathidae.

	Anapistula Gertsch, 1941	*Anapogonia* Simon, 1905	*Crassignatha* Wunderlich, 1995	*Curimagua* Forster & Platnick, 1977
Sexes known	♀♂	♀	♀♂	♀♂
Species	25	1	9	2
Nomenclatural status	Valid	Valid	Valid	Valid
Female genitalia, internal	Pair of round spermathecae connected by t-shaped duct	--	Large spermathecae, convoluted duct path (Figs. 5.12c-d)	Ducts follow nearly straight path posteriorly from round spermathecae
Female genitalia, external	Transverse rounded lip overhanging furrow	--	Short robust scape (Fig. 5.11c-d)	Transverse rounded lip overhanging furrow
Tarsal claws	Homogeneous	--	Homogeneous	--
Cheliceral fusion	Near the base	Absent	Near the base	Near the base
Cheliceral teeth	Two (Fig. 5.7d)	--	Single asymmetrically bifid tooth, or two teeth (Fig. 5.5a)	Absent
Male tibia II clasper	Absent	N.A.	1-4 (Fig. 5.5d, e)	Absent
Male abdominal scutum	Absent except in *A. boneti*	N.A.	Surrounding the posterior part of the abdomen. Usually present, except in *C. haeneli*	Absent
Pars cephalica	Usually only slightly raised, strongly raised in *A. Boneti*	--	Strongly raised	Strongly raised
Eye arrangement	Usually four eyes (Fig. 5.8b), median eyes present in *A. boneti*	Six eyes in triads	Six eyes in diads (Figs. 5.10b, e; 5.11b)	Six eyes in triads
Female palp	Absent	--	Absent	Vestigial
Carapace texture	Mostly smooth	--	Generally covered with tubercles (Fig. 5.5b, c)	Mostly smooth
Abdomen shape	Subspherical	--	Subspherical, sometimes with postero-lateral lobes (Fig. 5.6)	Subspherical
Cymbium	With stong setae but without teeth or denticles	N.A.	With cymbial tooth (Fig. 5.4b, d)	With small bumps or denticles (figs. 66: [8])
Spermatic duct	Coiling 1.5 times over itself (Fig. 5.9a)	N.A.	Long, coiling several times around itself (Fig. 5.4b, e)	--
Embolus	Short less than 0.5 times the diameter of the bulb (Figs. 5.7c, 5.9a)	N.A.	Variable, short (Fig. 5.4 c) or long, about the diameter of the palp (Fig. 5.4 f)	Short about 0.5 times the diameter of the bulb (figs. 67, 68: [8])
Relevant literature	[8,17,29,43,44]	[45,46]	[9, 10, 31]	[8]

Number of species is based on the WSC [11].

Table 2- Overview of diagnostic characters of the currently accepted genera of the Symphytognathidae (Continuation).

	Globignatha Balogh & Loksa, 1968	*Iardinis* Simon, 1899	*Patu* Marples, 1951	*Symphytognatha* Hickman, 1931
Sexes known	♀	♂	♀♂	♀♂
Species	2	(2)	18	15
Nomenclatural status	Valid	Nomen dubium*	Valid	Valid
Female genitalia, internal	Spermathecae twisted anteriorly	N.A.	Spermathecae variable, sometimes elongate or reniform	Copulatory ducts loop around elongate spermathecae (figs. 1-6, plate 1, fig. 2: [47])
Female genitalia, external	Transverse rounded lip overhanging furrow	N.A.	Transverse rounded lip overhanging furrow, or a flexible scape (figs. 1d, 2e: [48])	Transverse rounded lip overhanging furrow
Tarsal claws	Homogeneous	--	Homogeneous	Multidentate only in anterior legs (figs. 6, 7: [8]; fig. 2: [47]; fig. 3: [15])
Cheliceral fusion	Almost entirely fused with no visible suture line (figs. 41, 42: [8])	--	Fused basally to about half their length	Fused for most of their length, with visible suture line
Cheliceral teeth	One large, two short (fig. 43: [8])	One (fig. 6: [49])	Usually a single large tooth with 1-3 peaks	Two sinuous teeth (figs. 3, 32, 36: [8]; Figs. 5.2B, 5.2C : [15]; fig. 122A: [31])
Male tibia II, clasper	N.A.	--	Sometimes 1-2	Absent
Male abdominal scutum	N.A.	--	Absent	Absent
Pars cephalica	Strongly raised	Strongly raised	Strongly raised	Strongly raised
Eye arrangement	Six eyes in diads	Six eyes in triads	Six eyes in diads (Fig. 5.14b)	Six eyes in diads
Female palp	Absent	N.A.	Absent	Absent
Carapace texture	Mostly smooth	--	Mostly smooth	Mostly smooth
Abdomen shape	Subspherical	--	Subspherical, sometimes with lobes	Subspherical
Cymbium	N.A.	--	--	--
Spermatic duct	N.A.	Coiling 1.5 times over itself (fig. 7: [49]; fig 135a: [31])	--	--
Embolus	N.A.	long, 0,5–1,5 the diameter of the bulb (fig. 7: [49]; figs. 1,2: [50])	long about 1 time the diameter of the bulb(figs. 1e, 1f: [48]; fig. 19: [51])	Short about 0.5 times the diameter of the bulb (figs. 8, 9: [8])
Relevant literature	[8]	[8, 31, 50–52]	[8, 30, 48, 53, 54]	[8,15,31,47]

◀Number of species is based on the WSC [11]. *Type species *Iardinis weyersi* Simon, 1899 considered *nomen dubium*; two species placed in this genus by Brignoli [48,51] remain cataloged here [11].

descriptions of females. In all of Miller's species where males are known, they possess a unique abdominal scutum surrounding the abdomen laterally and posteriorly. In most *Crassignatha* species, the female genitalia consists of a pair of robust round spermathecae separated by about their diameter, copulatory ducts that loop and switchback along their path, and a short, robust scape (figs. 76, 79, 89A-89D:[10]); only *C. longtou* and *C. seedam* sp. nov have a transverse bulge and not a scape (figs. 89E, 89F, 91F:[10]).

Wunderlich [16] stated that *Crassignatha haeneli* lacked an abdominal scutum, and among the Symphytognathidae, only *Anapistula boneti* and Miller's *Crassignatha* species have a scutum (but see *Patu spinathoraxi*, below). A dissection of *Crassignatha* chelicerae indicated that they were indeed fused at the base (fig. 78A:[10]). It is however worth noting that the 3D scan of *Crassignatha* presented here do not appear to indicate cheliceral fusion (Fig. 5.5a). It was also determined that most of these *Crassignatha* species have an asymmetrical split in the cheliceral tooth with a small peak on the mesal side of the tooth; only *C. longtou* has two subequal teeth. *Crassignatha* species known from the male all have a group of 1-3 strong ventral setae on male tibia II (figs. 74E, 77D, 80E, 83E:[10]). One species had the abdomen modified with a pair of posteriolateral lobes (figs. 86D-F:[10]), not as conspicuous in other species (Figs. 5.6b, d), or generally round or oblong.

Modern symphytognathid taxonomy in Asia – 2009 was a big year for little spiders in Asia. Four papers described a total of 18 symphytognathid species from China, Japan, and Vietnam [10, 13, 18, 56]. These were distributed across the genera *Anapistula*, *Crassignatha*, and *Patu*. Lin and Li [13] described five new *Patu* species from China. Again, fusion of the chelicerae only near the base was declared as a characteristic of *Patu*. Chelicerae of all species were illustrated as fused, but no details were provided in the text. Of these five species, three show characters that match the diagnostic characters of *Crassignatha* instead of *Patu*:

Patu bicorniventris Lin & Li, 2009, known from the female only, has an asymmetrically bifid cheliceral tooth (figs. 2C, 2D: [13]) resembling those typical of *Crassignatha* (fig. 78A: [10]). It also has modifications to the abdomen consisting of two posteriolateral lobes and a straight posterior margin, resembling *Crassignatha ertou* (figs. 86D-86F: [10]). The female genitalia of *Patu bicorniventris* resembles most *Crassignatha* females described in Miller et al. [10], featuring conspicuous spermathecae with convoluted copulatory ducts leading to a knob-like median scape.

Patu quadriventris Lin & Li, 2009 shares with *P. bicorniventris* an abdomen that is truncated posteriorly, but lacks the posteriolateral lobes. The female genitalia is consistent with *Crassignatha*. The cymbium of the male pedipalp has a distal apophysis (CS in fig. 9C: [13]) that strongly resembles the Ct in *Crassignatha* (figs. 9a; 13a, d; figs. 75, 77B, 81, 82B, 84, 87, 88: [10]).

Patu spinathoraxi Lin & Li, 2009 has distinctive spikey tubercles covering the carapace. It closely resembles (but is not conspecific with) *Crassignatha longtou* Miller, Griswold & Yin, 2009, which was described from the female only. The female genitalia of both species are similar, featuring round spermathecae with ducts that run ectally before turning back toward the middle and terminate in a pair of conspicuous posterior openings; they contrast with *Crassignatha* in that they lack a robust scape. The male has a medially split abdominal scutum, a single ventral macroseta on tibia II, and a distal apophysis of the cymbium similar to those found in *Crassignatha* (CS in fig. 16C: [13]). These two species are clearly congeneric; whether they are best placed together in *Crassignatha*, or in their own new genus, is debatable.

Current status and proposed changes – Of the eight valid symphytognathid genera, *Anapistula*, *Curimagua*, *Globignatha*, *Symphytognatha*, and *Crassignatha* seem morphologically coherent and recognizable; *Anapogonia* and *Iardinis* are currently unrecognizable; *Patu* remains problematic. However, some species currently placed in *Patu* show clear affinities with *Crassignatha*. We propose the following taxonomic changes: *Crassignatha bicorniventris* (Lin & Li, 2009) **comb. nov.**, *Crassignatha quadriventris* (Lin & Li, 2009) **comb. nov.**, and *Crassignatha spinathoraxi* (Lin & Li, 2009) **comb. nov.**

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Supplementary Material

Supplementary Material 1. List of primers used in our study, alignment of DNA sequence data used in phylogenetic analyses in nexus format, and Trace plot and histograms for both runs of the BI analysis observed in Tracer 1.7.1.

Supplementary Material 2. 3D reconstructions *Crassignatha seeliam* male pedipalp and habitus.

Supplementary Material 3. 3D reconstructions *Crassignatha danaugirangensis* male pedipalp and habitus.

(Included in the original publication in ZooKeys)

Chapter 6

Epilogue

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General discussion

The present thesis highlights the relevance and usefulness of taxonomic literature (old and new) and exemplifies the benefits of adopting an integral approach to the description of new taxa. As mentioned in **Chapter 1**, taxonomy as a science has accumulated data and knowledge for more than 250 years. The quality and usefulness of the facts recorded in taxonomic literature have greatly improved from the early (purely) descriptive texts to the modern works that are rich in detailed and integrated data. These developments have improved the rigor of phylogenetic inference, documented distribution patterns of taxa through time and space, and revealed broad evolutionary patterns and other interesting phenomena. My work illustrates some applications of legacy data contained in literature (**Chapters 2** and **3**), and also explores an integrative perspective that involves new taxonomic descriptions and generation of phylogenetic hypotheses integrating molecular and morphological data (**Chapters 4** and **5**).

Taxonomy has provided a system under which groups of natural entities can be catalogued and biological data can be aggregated by the use of a taxonomic name (e.g. genus or species). Nevertheless, slow pace of identification, description, and categorization, plus the huge number of unknown species and human impact on natural habitats and biodiversity have made traditional taxonomy obsolete and partially unreliable. As a response to this, several authors have made patent the urgent need for taxonomy to accelerate its description and knowledge accumulation process by the change of some practices and use of new technologies [1–9]. One of the main drivers of this change involves the access to primary taxonomic literature and specimen information [1, 6]. My thesis heavily relies on the utilization of some of these “e-taxonomic” products, like the BHL [10], WSC [11], Plazi [12], and GBIF [13]; and also helps to test and collaborates on the improvement of the program Golden Gate Imagine, the software used for data mining in **Chapter 2**. Electronic access to literature is one of the primary tools in my dissertation, being fundamental for acquiring primary taxonomic data used in **Chapter 2** to analyze the distribution of the *Teutamus* group; observing the patterns and formulating an evolutionary hypotheses for the origin of genital asymmetry in spiders (**Chapter 3**); and gathering taxonomic information that helped us identify and eventually describe new species and test the phylogenies of the the spider families Hahniidae (**Chapter 4**) and the Symphytognathidae **(Chapter 5**).

The approach used in my thesis for mining and gathering specimen data from taxonomic literature —used in **Chapter 2**— proved to be a powerful tool for analyzing species distributions. Here, I applied this knowledge to plan our fieldwork targeting adult specimens of one particular group of ground dwelling spiders; however, there are many other possible uses that remain to be explored in the future. Some examples of these applications include species estimations, catalog building and taxonomic inconsistencies identification, inclusion of underrepresented taxa in global datasets, species distribution patterns detection, among other applications. This potent tool has been gaining traction among taxonomic journals and publishers (like Pensoft, Zootaxa, Zookeys, among others) in its prospective approach [14, 15]; and is also being used in projects similar to the one presented here that have extracted data and analyzed the legacy taxonomic literature of groups as diverse as on damselflies [16] and *Tyrannosaurus rex* [17, 18].

Besides the aforementioned specimen data, taxonomic literature has also linked other types of information like illustrations, photographs, and molecular data to taxonomic names. In this way, I think of taxonomic literature as a massive (yet largely unstructured) data repository. This repository has accumulated biological data in the form of text called treatments [19–21]. The quality and usefulness of some of these data greatly varies depending on the time, author and even taxonomic group, and has —without any doubt — improved with utilization of modern techniques and technologies [22]. Spider taxonomy — probably one of the best curated bodies of taxonomic literature (see Chapter 1) — has accumulated and made available thousands of taxonomic documents that represent a huge collection of species, facts, and images. Similar to other arthropod groups, spider species delimitation greatly relies on the comparative morphology of the genital characters; therefore, it does not come as a surprise that sexual characters are some of the best studied and understood, having detailed images and descriptions that might allow for the detection of interesting phenomena and broad evolutionary patterns. One example of this is the evolution of asymmetry in spider genitalia [23, 24]. For **Chapter 3**, I conducted a casual but taxonomically broad study of the evolution of this character in spiders, assigning the identified cases to traditional categories of asymmetry [23–26] and suggested the evolutionary patterns and causes behind the development of this morphological character. Moreover, I attempted to test some of these hypotheses by examining the behavioral implications of the evolution of genital asymmetry in the species *Teutamus politus*; although I collected a fair number of live specimens during our fieldwork in an attempt to observe courtship and mating, the behavioral experiments were unsuccessful. Nevertheless, ours was the most comprehensive study of asymmetry in spiders encountering dozens of species in several families that show some kind of asymmetry and had been overlooked in the scarce previous reviews on the topic.

The sampling methods I used during our fieldwork — although intended for the collection of *Teutamus* group spiders — also captured much more material. Some of these specimens were used in this thesis for **Chapters 3**, **4** and **5**; but considerably more material remains to be studied. Our sampling collected thousands of specimens from several arthropod groups including insects, millipedes, mites, Opiliones, among many others. Together with my supervisor Jeremy Miller and our collaborator Booppa Petcharad, I collected more than 4,600 spider specimens of which 1,454 were adults representing 35 spider families. Only the families Liocranidae, Hahniidae and Symphytognathidae (the latter two both new records for Thailand) were identified to species level. Still, from this relatively small selection of taxa, I described a total of five new species following the integrative approach I mentioned in the introduction and including morphological and molecular data. All of the specimens collected for the present project are now deposited in the collection of Naturalis Biodiversity Center, where they can be archived and eventually contribute to other taxonomic, systematic, ecological and evolutionary studies.

In this context, **Chapters 4** and **5** focused on the generation of new taxonomic content using the integrative approach [27–33]. In these two chapters I described five new species and made the first reports of two families for Thailand. Both families — as in the aforementioned *Teutamus* group — are ground dwelling, mostly inhabiting leaf litter. They are relatively small to tiny, ranging from less than 1 cm down to less than 0.5 mm! In fact, one of the newly described species, *Anapistula choojaiae* n. sp., could be considered among the smallest spiders ever discovered together with a couple other species in the genus *Patu* [34, 35]. Both chapters are examples of the integration of new taxonomic descriptions with high resolution photographs, molecular data and character evolution, similar to what has been done previously in other spider studies [27, 29, 31, 33]. In the case of the Hahniidae (**Chapter 4**) by reviewing what is known about the eye size reduction and eventual eye loss within this family; and for the Symphytognathidae (**Chapter 5**) by using 3D modeling to accurately document and compare the genital morphology allowing us to better circumscribe the genus *Crassignatha*, and transferring there some species previously misplaced in the genus *Patu.*

I consider that this thesis demonstrates the use of many of the new taxonomic *e-tools* like specimen information databases (e.g. GBIF) and literature repositories, and data mining and management resources (e.g. BHL, WSC, PLAZI). In this way, the present work illustrates the re-use and re-analysis of specimen data and morphological images contained in literature, and also features the use of an integrative taxonomic approach for new descriptions that allow for species documentation, as well as the inference of evolutionary hypotheses. I feel that the common use of these tools will, without any doubt, help overcome the taxonomic impediment while collaborating on the effort of describing and understanding our (greatly endangered) biodiversity.

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Summary

Taxonomy as a science has accumulated data and knowledge for more than 250 years. The quality and usefulness of the facts recorded in taxonomic literature has greatly improved from the early descriptive texts to the modern data-rich, hypothesis-driven works. Our work illustrates the application of some of the "e-taxonomic" tools and the "New Taxonomy" thinking explored in the introduction. Here, we analyzed specimen data contained in legacy taxonomic literature in **Chapters 2** and **3** — to observe species distribution of one spider group and genital evolution, respectively — and also explored an integrative perspective that involves describing new taxa and testing phylogenetic hypotheses using molecular and morphological data, as done in **Chapter 4** and **5**.

In **Chapter 2** we extracted data from taxonomic legacy literature and analyzed the temporal and spatial distribution of the so-called *Teutamus* group — a group of spiders mostly distributed in Southeast Asia — based on the abundances of specimens reported in literature. These results were used to plan fieldwork that optimized the collection of this spider group. This sampling was carried out in six National Parks in Thailand during the summer of 2018. Some of the specimens collected during this expedition were used in the other three chapters that make up this dissertation. **Chapter 3** can be divided in two parts: a literature based survey of the rare cases of genital asymmetry in spiders; and a behavioral study that used the specimens caught in the field. The review of taxonomic literature allowed us to observe and formulate evolutionary hypotheses for the origin and evolution of spider genital asymmetry. Live specimens of the species *Teutamus politus* were observed to investigate male-female interactions in a species with Directional Asymmetry in both male and female genital morphology. Although we were not able to observe courtship or other mating behaviors, we were able to thoroughly document the genital morphology of this species and its intra-specific variation.

As a result of our fieldwork in Thailand, we collected specimens from 35 spider families; from these, two — Hahniidae and Symphytognathidae — had never been reported for the country. We described a total of five new species and one new genus from these families based on an integrative approach. **Chapter 4** documents and describes the genus *Hexamatia* gen. nov. and two new species *Hexamatia seekhaow* sp. nov. and *Hahnia ngai* sp. nov (Hahniidae) giving morphological and molecular information of these new and one previously described taxa. The multi-loci molecular data obtained from our samples was used together with available sequences from Genbank to test the phylogenetic relations of this family and the position of the newly described species. Also, this work briefly reviewed the taxonomy of the (apparently multiple) origins of eye loss and eye reduction in this family.

Finally, **Chapter 5** used a similar approach to describe and test the phylogenetic relations of three new species of the family Symphytognathidae: *Anapistula choojaiae* sp. nov., *Crassignatha seeliam* sp. nov., and *Crassignatha seedam* sp. nov. The male genital morphology of *C. seeliam* and *C. danaugirangensis* Miller et al. 2014 was documented using X-ray micro-CT scans allowing the observation of external and internal features and a better circumscription of the genus *Crassignatha.*

The integrative perspective used in this thesis gives evidence of the great deal of information that has been accumulated by traditional taxonomic work. When unlocked and analyzed properly, this data can allow the discovery and observation of biological patterns that range from taxa geographical distribution to interesting evolutionary phenomena. Furthermore, the application and analysis of multiple sources of information (e. g. molecules and morphology) favor the production of more robust phylogenetic hypotheses that can be easily tested and built on.

Samenvatting

Taxonomie als wetenschap verzamelt al meer dan 250 jaar gegevens en kennis. De kwaliteit en bruikbaarheid van de feiten die in taxonomische literatuur zijn opgetekend, zijn sterk verbeterd van de vroege beschrijvende teksten tot de hypothese-gedreven moderne werken die rijk zijn aan gegevens. Mijn proefschrift illustreert de toepassing van enkele van de “e-taxonomische” gereedschappen en het “nieuwe taxonomie”-denken die ik in de inleiding introduceer. In **hoofdstukken 2** en **3** analyseer ik gegevens van exemplaren die zijn opgenomen in oudere taxonomische literatuur — om respectievelijk de soortverdeling van één spinnengroep en genitale evolutie te bestuderen— en ik onderzoek die gegevens ook in integratief perspectief, wat inhoudt dat nieuwe taxa worden beschreven en fylogenetische hypothesen worden getest met behulp van moleculaire en morfologische gegevens, zoals in **hoofdstuken 4** en **5**.

In **Hoofdstuk 2** extraheer ik gegevens uit de taxonomische *legacy*-literatuur en analyseer ik de temporele en ruimtelijke verspreiding van de zogenaamde *Teutamus* groep —een groep spinnen die voornamelijk in Zuidoost-Azië voorkomt— op basis van de overvloed aan exemplaren die in de literatuur zijn gerapporteerd. Deze resultaten worden gebruikt om veldwerk te plannen waarmee ik het verzamelen van de spinnengroep kon optimaliseren. Deze bemonstering werd uitgevoerd in zes nationale parken in Thailand in de zomer van 2018. Een deel van de exemplaren die tijdens deze expeditie werden verzameld, werd gebruikt in de andere drie hoofdstukken waaruit dit proefschrift bestaat. **Hoofdstuk 3** bestaat uit twee delen: een literatuuronderzoek naar de zeldzame gevallen van genitale asymmetrie bij spinnen en een gedragsstudie waarbij gebruik werd gemaakt van de in het veld gevangen exemplaren. Het overzicht van taxonomische literatuur stelde mij in staat om evolutionaire hypothesen over de oorsprong en evolutie van genitale asymmetrie van spinnen te formuleren. Ik onderzocht seksuele interacties aan levende exemplaren van de soort *Teutamus politus* (een soort waarbij gemitale asymmetrie bestaat bij zowel mannetjes als vrouwtjes). Hoewel ik geen balts of andere paargedrag kon waarnemen, was het wel mogelijk om de genitale morfologie van deze soort en zijn intra-specifieke variatie grondig te documenteren.

Als resultaat van mijn veldwerk in Thailand heb ik exemplaren verzameld van 35 spinnenfamilies, waarvan twee —de Hahniidae en Symphytognathidae—nooit eerder waren gerapporteerd voor het land. In totaal beschrijf ik vijf nieuwe soorten en één nieuw geslacht uit deze families op basis van een integratieve benadering. In **Hoofdstuk 4** beschrijf ik het genus *Hexamatia* gen. nov. en de twee nieuwe soorten *Hexamatia seekhaow* sp. nov. en *Hahnia ngai* sp. nov (Hahniidae) en geef ik ook morfologische en moleculaire informatie over deze nieuwe en de ook eerder beschreven taxa. De multi-locus moleculaire gegevens verkregen uit onze monsters werden samen met beschikbare sequenties van Genbank gebruikt om de fylogenetische relaties van deze familie en de positie van de nieuw beschreven soorten te toetsen. Ook wordt in dit hoofdstuk kort de taxonomie besproken van de (schijnbaar meerdere) oorzaken van oogverlies en oogvermindering binnen deze familie.

Ten slotte gebruikte ik in **Hoofdstuk 5** een soortgelijke benadering om de fylogenetische relaties van drie nieuwe soorten van de familie Symphytognathidae te beschrijven en te toetsen: *Anapistula choojaiae* sp. nov., *Crassignatha seeliam* sp. nov., en *Crassignatha seedam* sp. nov. De mannelijke genitale morfologie van *C. seeliam* en *C. danaugirangensis* Miller et al, 2014 werd gedocumenteerd met behulp van micro-CT scans die de observatie van externe en interne kenmerken mogelijk maakten daardoor een betere omschrijving van het geslacht *Crassignatha*.

Het integratieve perspectief dat in dit proefschrift wordt gebruikt, laat zien hoe groot de hoeveelheid informatie is die is verzameld door traditioneel taxonomisch werk. Wanneer zulke gegevens op de juiste manier worden ontsloten en geanalyseerd, kunnen ze de ontdekking en observatie van biologische patronen mogelijk maken, variërend van geografische spreiding van taxa tot interessante evolutionaire verschijnselen. Bovendien bevordert de toepassing en analyse van meerdere informatiebronnen (bv. moleculair en morfologische databases) de productie van robuustere fylogenetische hypothesen die gemakkelijk kunnen worden getoetst en waarop kan worden voortgebouwd in vervolgstudies.

Curriculum Vitae

Andrés was born in Mexico City, Mexico on March 7th 1990. He started pursuing his career in Biology when he joined the National Autonomous University of Mexico (UNAM) in 2009. He began working on spiders for a short project in 2010, after which he joined Fernando Alvarez-Padilla's Lab in the Faculty of Sciences UNAM. Between 2012 and 2013 he worked on his Bachelor's thesis, earning his B.Sc. diploma in May 2013. During this project he described the richness of cob-web spiders (Theridiidae) in a remnant tropical forest in a mountain range on the Center-East state of San Luis Potosí, Mexico. Also, during this time, he did a three month' internship at the California Academy of Sciences, San Francisco, US. Andrés joined the graduate school of the Faculty of Sciences, UNAM in 2013 and got his M. Sc. by the end of 2015. This time, he worked on the diversity of a two families of wandering spiders (Anyphaenidae and Corinnidae) from three sub-tropical mountain forests in the eastern states of San Luis Potosí and Veracruz, Mexico. During this period he presented his partial results in one American Arachnological Society Meeting in Ohio, US and was awarded with the Ernst Mayr travel grant to revise collection material in the Museum of Comparative Zoology MCZ, Harvard, US.

Andrés moved to the Netherlands in October 2016 to start his Ph.D. at Naturalis Biodiversity Center, and enrolled at the University of Leiden. He continued to work on spiders, but this time, looking at their systematics and sexual character evolution, mainly focusing on the family Liocranidae, under the supervision of Jeremy Miller and Menno Schilthuizen. Jeremy and Andrés carried out a one month' fieldwork in Thailand, sampling in several National Parks and natural reserves and also conducting experiments on the sexual behavior of one group of liocranid spiders. The studies that make up this thesis are mainly the result of the analysis of data extracted from legacy taxonomic literature, as well as analysis, material and data gathered during fieldwork. Collection material deposited in Naturalis, along with material borrowed from other international collections (CAS, FMNH, SMF, MCZ, among others) was also analyzed. Different stages of the data presented here were also presented in international meetings and congresses in Mexico, New Zealand, the Netherlands, and the US. During his time at Naturalis, Andrés was involved in teaching activities being twice teaching assistant on the Integrative Taxonomy M.Sc. course at Leiden University, co-organizing the "Spider identification day" at Naturalis in Nov 2019 and being supervisor of one M.Sc. project. Andres was also an active member of the student community co-organizing sports activities, organizing for a short period the Endless Forms Journal club, and participating in the Ph.D. Council from 2018 to 2020.

List of publications

- **L. van der Jagt, F. A. Rivera-Quiroz, H. Ortega-Salas and Jeremy A. Miller.** "Magnitude, Richness and Uniqueness: an analysis of lestid damselfly (Insecta: Odonata) records in GBIF". (*In prep.*).
- **F. A. Rivera-Quiroz and Jeremy A. Miller.** "Systematics of the spider family Liocranidae (Araneae Araneomorphae)". (*In prep.*).
- **F. A. Rivera-Quiroz, Petcharad, B. and Jeremy A. Miller. 2020.** "First records and a new genus of Comb-tailed spiders (Araneae: Hahniidae) from Thailand with comments on the six-eyed species of this family". *European Journal of Taxonomy*. 724:51-69. doi:10.5852/ejt.2020.724.1157
- **F. A. Rivera-Quiroz, Petcharad, B. and Jeremy A. Miller. 2021.** "First records and three new species of the family Symphytognathidae (Arachnida: Araneae) from Thailand, and the circumscription of the genus *Crassignatha* Wunderlich, 1995". *ZooKeys* 1012: 21–53. doi.org/10.3897/zookeys.1012.57047
- **F. A. Rivera-Quiroz, Petcharad, B. and Jeremy A. Miller. 2020.** "Mining data from legacy taxonomic literature and application for sampling spiders of the *Teutamus* group (Araneae; Liocranidae) in Southeast Asia". *Scientific Reports* 10:15787. doi:10.1038/s41598-020-72549-8
- **F. A. Rivera-Quiroz, Schilthuizen, M., Petcharad, B. and Jeremy A. Miller. 2020.** "Imperfect and askew: A review of asymmetric genitalia in araneomorph spiders (Araneae: Araneomorphae)". *PLoS One*. 15(6, e0220354): 1-26. doi:10.1371/journal.pone.0220354
- **F. A. Rivera-Quiroz, and F. Alvarez-Padilla. 2019.** "Description of five new *Wulfila* (Araneae, Anyphaenidae) species from Mexico with comments on the taxonomy of the genus". *Zootaxa*. 4712(2): 269-289. doi:10.11646/zootaxa.4712.2.6
- **F. A. Rivera-Quiroz, and Jeremy A. Miller. 2019.** "Extracting Data from Legacy Taxonomic Literature: Applications for planning field work". *Biodiversity Information Science and Standards* 3: e37082. doi: 10.3897/biss.3.37082
- **F. A. Rivera-Quiroz, U. Garcilazo-Cruz and F. Alvarez-Padilla. 2016.** "Spider cyberdiversity (Araneae: Araneomorphae) in an ecotouristic tropical forest fragment in Xilitla, Mexico". *Revista Mexicana de Biodiversidad*. 87 (2016) 1023–1032. doi: 10.1016/j.rmb.2016.07.011
- **F. A. Rivera-Quiroz, and F. Alvarez-Padilla. 2015.** "New species of the genus *Trachelas* (Araneae: Trachelidae) from an oak forest inside a biodiversity hotspot in Mexico". *Zootaxa* 3999(1) 95-110. doi: 10.11646/zootaxa.3999.1.6

International meetings

- **November 2020.** Magnitude, Richness and Uniqueness: a global analysis of lestid damselfly (Insecta: Odonata) records in GBIF. (Oral presentation). Entomological Society of America. Virtual Meeting.

- **June 2020.** Mobilizing data from taxonomic literature: process, tools and applications. (Oral presentation). American Arachnological Society. Virtual Symposium.

- **October 2019.** Extracting Data from Legacy Taxonomic Literature: Applications for planning field work. (Oral presentation). Biodiversity Next International Conference. Leiden, The Netherlands.

- **February 2019.** Asymmetric genitalia in spiders, an overview of this rare phenomenon with emphasis on *Teutamus politus* Thorell 1890 (Araneae; Liocranidae). (Oral presentation). International Congress of Arachnology. Christchurch, New Zealand.

- **July 2017.** Old papers, new data: Application of document mark-up to liocranid spiders (Liocranidae Araneae). (Oral presentation). American Arachnological Society. Queretaro, Mexico.

- **June 2014.** Spider diversity of three families of the Dionycha clade (Araneae: Anyphaenidae, Corinnidae and Salticidae) in two mountain ecosystems of Mexico. (Poster presentation). American Arachnological Society. Ohio, USA.

- **June 2014.** Faunistic inventories of Araneomorphae (Arachnida: Araneae) in Mexico: A comparative approach. (Oral presentation). American Arachnological Society. Ohio, USA.

- **November 2013.** Cave Arachnids. (Oral presentation). XIII Semana de Cuevas, Facultad de Ciencias, UNAM. Queretaro, Mexico.

- **June 2013.** Spider (Araneae Araneomorphae) diversity in the Edward James Garden tropical forest, Xilitla, San Luis Potosí. (Oral presentation). Mexican Society of Entomology (Sociedad Mexicana de Entomología). Guerrero, Mexico.

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