*Article*

# Heuristic, parsimony and molecular analysis of variations between selected orb-weaver spiders from Mindanao, Philippines

**Ma. Theresa Pareno**, **Cesar G. Demayo**
Department of Biological Sciences, College of Science and Mathematics, MSU-Iligan Institute of Technology, Iligan City 9200, Philippines
E-mail: cgdemayo@gmail.com

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**Abstract**
Morphological structures and phenotypic characteristics define the status of organisms. It serves as their landmark for easy identification. Samples of orb-weaving spiders were surveyed in selected areas in Mindanao, Philippines, to describe the extent of morphological variations in body structures. Aside from their colorful and spiny abdomen, characters other than generally used to describe species were explored to be able to find specific landmarks to determine these spiders' identity. These characters were also used in comparing the described variationsbetween species using a heuristic method and parsimony analysis. DNA barcoding analysis using mitochondrial DNACOI sequences was also used to strengthen species delineation. Qualitative and quantitative morphological descriptions of body parts of the collected orb-weaver spiders showed a total of 7 species: 2 species under the genus *Thelacantha*, one species is under the genus *Cyrtarachne*, and four species were under the *Gasteracantha* genus. *Thelacantha brevispina* originally described as *G. mammosa* has shown features that are remarkably different from two other species *G. janopol*, and *G. diadesmia* based on the number and color of sigilla, including the sternum and color of the abdomen. Parsimony analysis of the morphological characters shows two separate groups of spiders showing homologous and analogous characters that are present on different species from their nodes. Results of the DNA barcoding revealed that *Gasteracantha* and *Thelacantha* orb-weaving spiders are not taxonomically close. The results of this study imply that the classification of organisms must include not only using morphological features but should also use other tools such as DNA barcoding to be able to have a clearer understanding of both phenotypic and molecular differentiation between species.

**Keywords** COI variances relationship; evolution; molecular analysis; parsimony; heuristic; NONA; WINCLADA; phonetics.

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## 1 Introduction

The basis for all phylogenetic relationship hypotheses of every organism is always based on morphological taxonomy. In determining morphological structures, the phenotypic characteristics of individual organisms are easily done based on the determination of the differences amongst species (Friedheim, 2016), including spiders.However, identification and classification of spiders are taxonomically challenging because first, morphological diagnosis is misleading due to the phenotypic and genetic variability and morphologically cryptic taxa; second, taxonomic expertise is collapsing (Hebert et al., 2003), and finally, there are still some debates about the agreement of the standard description of the morphological features that define that species because it is most of the time very subjective and changing. Likewise, striking sexual dimorphism and lack of information on sex identification lead to some of the impediments of identifying these organisms (Robinson et al., 2009). One of the most interesting groups of spiders to be studied is the orb-weaving spiny-back spiders. This group generally identified based on their characteristic hard, flat, spiny-backwith prominent spines on their abdomen, spinnerets,which are elevated, protruding, and enclosed by a thick rim (Yong and Ono, 2009). Records of orb-weavingspiny-back spiders in the Philippines are poorly known despite their ecological status and importance as potential biological control agents (Levi, 1978; Muma and Stone, 1971; Sharma, 2014; Reichert and Lockley, 1984; Nyffeler and Benz, 1987; Coddington and Levi, 1991). Some of these species have tremendous color polymorphism and body shape patterns. These may indicate genetic variation (White and Kemp, 2016) that may have played an important role in selection studies and the potential part for sympatric speciation and ecological function (McKinnon and Pierotti, 2010). Due to the existence of diversity in this group of spiders, we investigated the nature of variations in this group of species using a heuristic, parsimony, and DNA barcoding analysis. It is argued that describing morphological differences, including DNA barcoding (Hubert and Hanner, 2015; Ji et al., 2019), will help understand not only the nature of variability in this group of spiders but also their potential phylogenetic relationships.We limit our investigation on those species collected only in selected areas on the island of Mindanao, the Philippines.

## 2 Materials and Methods

Spiders were collected through opportunistic sampling in the area of Linamon, Iligan City, Mt. Agad-Agad, Iligan City, Mt. Timolan, Tigbao, Zamboanga del Sur, Ipil, Zamboanga Sibugay, Sungkilaw Falls, Dipolog City, Mt. Musuan, Kitanglad Range, and Impalutao, Bukidnon. The collection was done on in the area where there are a lot of shrubs and Mountains (Fig. 1), brought to the laboratory, described, andtook samples for DNA barcoding.

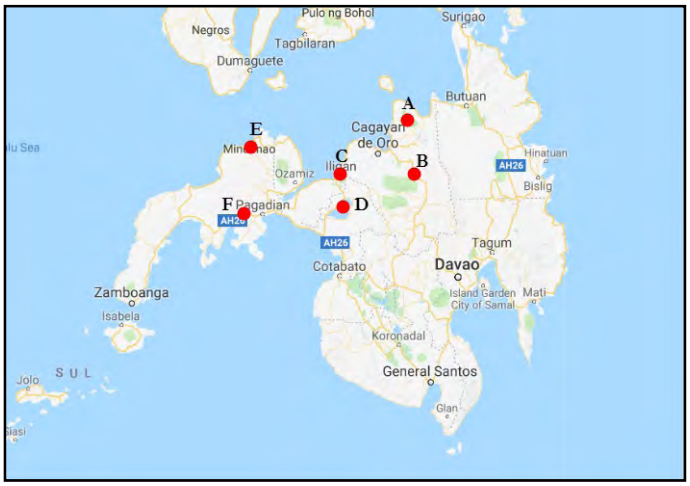


**Fig. 1** Study site (red square) in Northern Mindanao, the Philippines.

### 2.1 Morphological description and analysis

Specimens were recorded and photographed together with their webs in the field using Sony a6000, 16-50 mm kit lens. After recording them, one representative was dissected for morphological description and identification. The morphological characterization was conducted based on the works of Barrion and Litsinger (1995) with slight modifications (Fig. 2). All images were processed using ImageJ software.

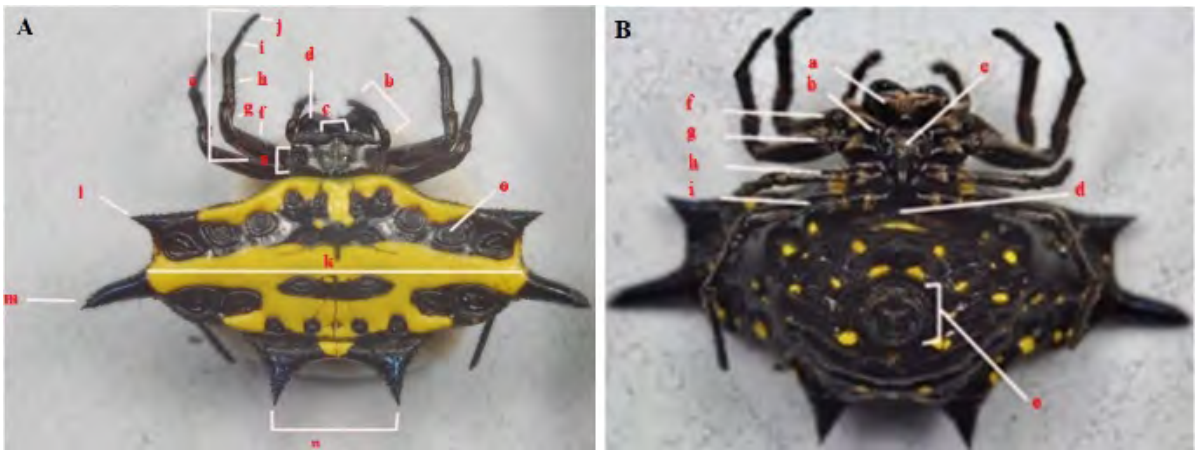


**Fig. 2** Morphological terms describing the dorsal view of *Gasteracantha diadesmia*. A: (a) cephalothorax/carapace; (b) pedipalp; (c) eyes; (d) chelicerae; (e) legs; (f) femur; (g) patella; (h) tibia; (i) metatarsus; (j) tarsus; (k) abdomen; (l) anterior; spine; (m) median spine; (n) posterior spine; (o) sigilla; B: ventral view; (a) maxilla; (b) coxa; (c) sternum; (d) epigynum; (e) spinneret; (f) 1st leg; (g) 2nd leg; (h) 3rd leg; (i) 4th leg.

A total of 52 characters were identified and use for the assessment of the spiders' morphology with additional characteristics based on the author's observations of all the collected species. The character states are based on the 'presence' or 'absence' represented by 0 or 1 or a pure description of their morphology, which is represented by numbers starting from 0 (Table 1). All of the codes from the dataset are transferred in WINCLADA (Nixon, 2002) software and analyzed by spawning to NONA (Goloboff, 1994). This program does the actual searching for the tree using a ratcheting technique (Giribet, 2007).

**Table 1** Morphological character and character states.

| ID Character | Character states | ID Character | Character states |
|---|---|---|---|
| **Cephalothorax** | | **Legs** | |
| 1. Cephalothorax | (0) Deep brown to reddish-brown (1) Yellow-brown & blackish brown in the thoracic region (2) Brown to dark brown, except a pair of black longitudinal parallel stripes in midcephalic area (3) Blackish brown clothed with white hairs (4) Sub squarish, dark reddish-brown, except pale yellow posterior margins of thorax (5) Yellow-brown at the edge and clothed with white hairs (6) Yellow brown and concave dorsally (7) yellowish-brown except black to from the MOQ, and from the lateral side starting from the lateral eyes going back. (8) | 24. Leg formula | (0) 1423 (1) 4123 |

| | | | |
|---|---|---|---|
| | yellow-brown to greenish | | |
| 2. Cephalic region | (0) Not elevated (1) elevated | 25. Leg color combination | (0) black and light brown (1) deep brown (2) All black (3) sebaceous deep brown (4) mostly yellow with small black spots (5) dark yellow-green to golden brown (6) mostly all black ¼ yellow |
| 3. Thorax position | (0) Not concealed under abdomen (1) Concealed under the abdomen | 26. Claw/s on Tarsal venter | (0) 1 claw (1) 2 claws (2) 3 claws |
| **Carapace** | | 27. Spines on the legs | (0) Absent (1) Present |
| 4. Hairs on the carapace | (0) Absent (1) present | 28. Pedipalp color combination | (0) black and light brown (1) deep brown (2) All black (3) sebaceous deep brown (4) mostly yellow with small black spots (5) dark yellow-green to golden brown (6) mostly all black ¼ yellow |
| 5. Carapace description | (0) Brown stripes in the lateral (1) black line in lateral and middle (2) gold (3) pure black/dark brown (4) rectangular but getting wider than the thorax | 29. Shape of abdomen | (0) Wider than long (1) octagon-like shape |
| 6. Clypeus height | (0) Same height as AME (1) same height as ALE (2) less than AME | 30. Spines | (0) Absent (1) present |
| **Sternum** | | 31. Color of the spine | (0) Black (1) yellow-brown (2) pink in the middle spine, the rest is black (3) combination of black and yellow (4) no spine |
| 7. Spots in sternum | (0) Absent (1) present | 32. No. of spine | (0) 2 (1) 6 (2) 4 (3) no spine |
| 8. Spots location | (0) Not center (1) center (2) scattered | 33. Hair on spines | (0) Absent (1) present (2) no spine |
| 9. Kind of spots | (0) Not connecting between coxae 1 (1) connecting between coxae 1 (2) no spots | 34. Anterior spine | (0) Absent (1) present (2) undeveloped (3) no spine |
| 10. Scope of spot | (0) Not covering 1,2 (1) covering 1,2 (2) on each side of coxae and tip (3) covering 1,2,3 (4)no spots | 35. Median spine | (0) Absent (1) present (2) undeveloped (3) no spine |
| 11. Spot location by coxae | (0) Both coxae 1,2 (1) both coxae 2,3 (2) coxae 1,2,3, 4 (3) covering 1,2,3 (4) at the center bet 1,2 (5) no spots | 36. Posterior spine | (0) Absent (1) present (2) undeveloped (3) no spine |
| 12. Spot on the tip | (0) Absent (1) present | 37. No. of color combination in the abdomen | (0) 2 (1) 3 |
| 13. Apex of sternum | (0) Concave (1) convex (2) straight | 38. Color Combination | (0) White and black (1) yellow and black (2) black gold and white (3) brown, black, chalk white (4) gold, brown, reddish brown |
| 14. Shape of sternum | (0) Triangularly pointed basally (v-shaped) (1) obovate (2) obovate with a more pointed tip (3) wider obovate with more pointed tip (4) heart shape | 39. Color pattern | (0) Horizontal (1) vertical (2) random |
| **Labium** | | 40. No. of sigilla | (0) 24 sigilla (1) 19 sigilla (2) 30 sigilla (3) 22 sigilla (4) 23 sigilla (5) 35 sigilla (6) 18 |

| | | | |
|---|---|---|---|
| | | | sigilla |
| 15.Shape of the labium | (0) Not wider than long (1) wider than long | 41. Color of sigilla | (0) all black (1) brown and black (2) gold and black (3) gold |
| 16. Coloration of the labium | (0) ¾ outer border (1) ½ outer border (2) ¼ outer border (3) plain | 42. Shape of sigilla | (0) Oval/obovate (1) not oval |
| **Maxillae** | | 43. Presence of spots in venter | (0) Absent (1) present |
| 17. Shape of maxillae | (0) As long as wide (1) wider than long (2) longer than wide (3) as long as broad | 44. Color of spots | (0) Yellow (1) white (2) no spots |
| 18. Color of maxillae | (0) ¾ outer border (1) ½ outer border (2) ¼ outer border (3) plain | 45. Spinnerets | (0) 4 equal sizes (1) Anterior spinneret larger (2) posterior spinneret larger |
| 19. Inner scapulae | (0) Black (1) yellow-brown (2) gold | 46. Extension like tip of spinneret | (0) absent (1) present |
| **Chelicerae** | | **Sex organs** | |
| 20. Color of chelicerae | (0) Black (1) reddish-brown (2) yellow-brown (3) golden brown (4) yellow-brown and black (5) dark brown | 47. The orientation of epigynum dorsal view | (0) bulging,convex (1) flat,concave |
| 21. Structure of chelicerae | (0) Short, small, robust (1) moderately small (2) stout and strong (3) long, robust (4) short and stout | 48. No. of epigynum (sperm entry) | (0) 2 (1) 1 (2) near almost 1 entry,concave |
| **Teeth** | | 49. Size of spermathecae | (0) 2 large equal (1) 2 small equal |
| 22. Retromarginal teeth | (0) 2 teeth (1) 4 teeth (2) 7 teeth (3) 6 teeth (4) 5 teeth | 50. Distance of spermathecae | (0) without space (1) with space |
| 23. Pro-marginal teeth | (0) Three teeth (1) 6 teeth (2) 7 teeth of equal sizes (3) 5 teeth (4) 4 teeth | 51. Shape of spermathecae | (0) g-shaped (1) spherical (2) globular with y shaped (3) spherical with straight duct |
| | | 52. Orifice | (0) absent (1) present |

## 2.2 DNA barcoding

The protocol for the DNA samples from each of the spiders includes DNA extraction, polymerase chain reaction, agarose gel electrophoresis, DNA clean up, qubit, and capillary sequencing (bidirectional). For the primer selection, we used LCO1490 5' - GGTCAACAAATCATAAAGATATTGG - 3' for the forward and HCO2198 5′ - TAAACTTCAGGGTGACCAAAAAATCA - 3′ for the reverse primers. These were done at the Philippine Genome Center (PGC), University of the Philippines in Diliman, Quezon City, the Philippines. The sequences were examined in BioEdit and FinchTV 1.4.0 software to view the quality of the chromatogram. This is necessary to check miscalls and cut the sequences when errors become too frequent. After editing, the sequences were converted into a fasta format and pasted in the National center for biotechnology information basic local alignment search tool (NCBI BLAST) to find the regions of sequences that have their similarity. All the sequences that have the nearest identity were also retrieved from BLAST for comparison. The Molecular Evolutionary Genetics Analysis Version 7.0 (Mega) software was used in the process. MUSCLE (Edgar, 2004a, b) was used specifically for the alignment. Analysis, namely, Neighbour-joining, was done for distance analysis.

## 3 Results

### 3.1 *Gasteracantha diadesmia* (Fig. 3)

Initial identification of the species was based on the descriptions of (Barrion and Listinger, 1995). Only

females were found and described. The samples were found inhabiting shrubs, most commonly seen in *Gliricidiasepium* plants or widely known as Madre de cacao and *Lantana camara* plants as well.

The total length of the spider is 8.125. The cephalothorax is 1.359 mm long, 3.304 mm wide, 1.05 mm high. Abdomen 6.667 mm long, 14.131 mm wide, 3.45 mm high. The cephalothorax is black. The cephalic region is rectangular, and the middle of the carapace is about 1 mm depressed, which is also called the cervical groove. The carapace is clothed with white hair. The thorax is located concealed underneath the anterior portion of the abdomen. This spider has eight eyes in two recurved rows; eye length (mm): AE = 4.232 and PE = 4.571. Eye diameter (mm): AME = PME = 0.180 and ALE = PLE = 0.14. Eye separation (mm): AME 0.271 eye diameter, AME – ALE 9.95 times AME diameter, LE one half AME diameter. PME 0.417 eye diameter, PME-ALE 8.95 times PME diameter. Median ocular quadrangle is an inverted trapezoid, narrower in the front than behind (0.579, 0.843 mm). The clypeus height is 1.05 mm AME diameter. The sternum is shaped like a heart shape, but more pointed basally and procurved apically with a yellow spot in the middle in line with leg 1.

The labium is pyramid-shaped with a rounded apex, which is reddish-brown from the bottom and transitioning to yellow, going to the apex. Maxillae also have the same color with labium but three times bigger than labium. The inner border of the maxillae is yellow with a thickness 0.367 mm, and the rest is reddish-brown. Chelicerae are robust and black. It also has 4 of the same size retromarginal and sixpromarginal teeth. The retromarginal teeth are more visible and more prominent compared to the promarginal teeth. Legs are brown to black and covered with white hairs, and all are sebaceous, with spines in the tarsal venter and three-clawed. The leg formula is 4123. The palp is black to brown, surrounded with hairs, yellow-brown basally, and ventrally with one claw (Table 1).


**Fig. 3** *Gasteracantha diadesmia*. a. dorsal view; b. ventral view; c. frontal view; of head; d. abdominal venter and spinneret; e. maxillae, labium, sternum; f. epigynum; g. spermathecae.

**Table 1** Length of leg segments (mm) of *G. diadesmia*.

| | Femur | Patella | Tibia | Metatarsus | Tarsus | Total |
|---|---|---|---|---|---|---|
| Leg 1 | 2.658 | 0.957 | 1.812 | 1.295 | 5:34 | 7.954 |
| Leg 2 | 2.716 | 1.02 | 1.667 | 1.384 | 1.075 | 7.862 |
| Leg 3 | 2.097 | 0.628 | 1.158 | 0.991 | 0.799 | 5.673 |
| Leg 4 | 3.191 | 0.843 | 1.997 | 1.897 | 1.175 | 9.103 |
| Pedipalp | 0.463 | 0.407 | 0.397 | 0.414 | 0.429 | 2.11 |

The abdomen is very attractive with black and yellow, which has a pair of the anterior spine, median spine, and posterior spine, which are located in the lateral side of the abdomen in the opposite direction pointing outwards. The abdomen is wider than long. It has a wide horizontal black band connecting the posterior spines. The abdomen contains 22 sigilla (10 big and 12 small), which are leaf-like in shape and black in color. Along with the horizontal back, the band includes six bigger sigilla, which is arranged three by three horizontally on both sides. In the middle of these bigger sigilla are small 6, which are also arranged per 3. Below the horizontal black bands are separate three small black bands that contain twosigilla in each band and four small sigilla connected to the posterior spines via the black bands. The shape and structure of the spines are robust and dark in color and serrated. The ventral side of the abdomen is black marked with scattered yellow spots and covered with small hairs. The epigynum is located below the bicleft and looks hollow on the top view but and concave when viewed laterally. The spinnerets are black with color and surrounded by a thick-bordered orifice. In the location where these spiders are found, it is observed that yellow individuals are more abundant than the white ones in the field. The spermathecae are twosignificant globular structures that are close to each other with the same size.

Variations: Mature females also have the same form and size with white coloration in the dorsal abdomen (Fig. 5). However, on the ventral side of the abdomen shows no white spots but yellow spots, which is the same as the other yellow-spotted individuals (Fig. 4). The collected samples range from the length of 6.96 - 9.38 mm, carapace length 1.077 – 2 mm, width 2.63 - 3.7 mm. The abdomen length 4.698 - 7.325, width 9.75 - 14.86 mm. The distance between spines taken tip to tip: anterior 10.96 - 15.88 mm, median 13.653 - 20.073 mm.

**Fig. 4** Variations of the abdomen of *G. diadesmia*. The distance between spines taken tip to tip: anterior 10.96 - 15.88 mm, median 13.653 - 20.073 mm.

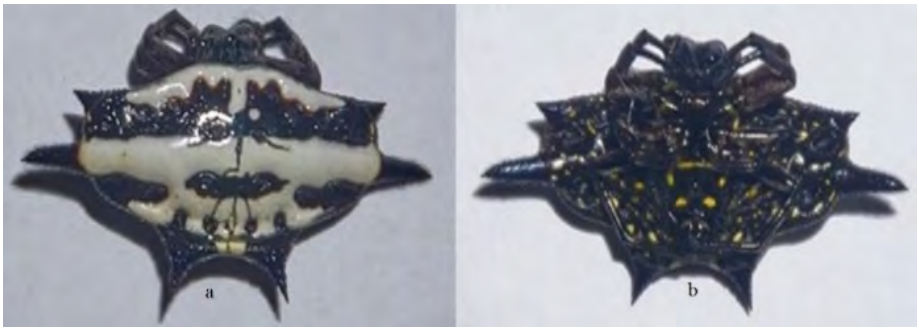


**Fig. 5** *G. diadesmia* (white). a. dorsal side b. ventral side with yellow spots.

### 3.2 *Gasteracantha kuhli* (Fig. 6)

Barrion and Listinger (1995) have described this species, and the current identification was based on their descriptions with some variations. These spiders were found in the shrubs and small trees. They set up their webs in the not so high elevation compared to other spiders and are most abundant in the dawn and evening.

The total length of the spider is 7.48 mm. The cephalothorax is 1.250 mm long, 2.5 mm wide, 2.3 mm high. The Abdomen was 6.23 mm long, 7.603 mm wide, 4.4 mm high. The cephalothorax is subsquares, dark reddish-brown, except pale yellow posterior margins of the thorax. The cephalic area is very high and subrectangular, with a pair of minute tubercles dorsally and posterior end abruptly sloped to a 45° angle. Thorax is low, concealed by the anterior part of the abdomen, with a straight depressed line in the center of the thorax. The fovea is distinctly Y-shaped. It has eight eyes in two recurved rows; eye length is AE nearly as long as PE (2.039 mm). Eye diameter is AME=0.140 mm, PME = 0.109 mm. Eye separation is AME-AME = 0.1.6 mm, AME-ALE= 0.873, PME-PME = 0.187, PME-PLE = 0.746. Median ocular quadrangle is wider than long, and wider behind than in front like an inverted trapezoidal shape. Clypeus height is small (0.66 mm). The sternum is obovate.

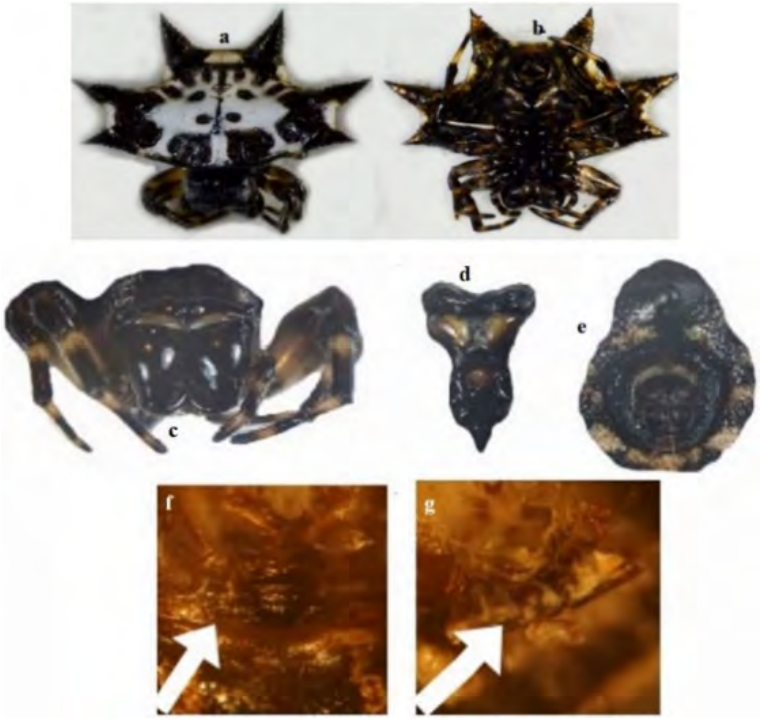


**Fig. 6** *Gasteracantha kuhli* (a. dorsal view; b. ventral view; c. frontal view of head featuring the eyes, clypeus, pedipalp and legs; d. epigynum and spinneret; e. spinneret; f. epigynum; g. spermathecae).

The labium is dark brown with a pale yellow distal border, slightly wider than long, arc-shaped, and pointed somewhat on the apex. Maxillae have a lighter color than the labium, yellow on the inner border, ½ yellow-brown color going to the inner part with small hairs at the border. Scopulae is fine black hair. Chelicerae are hard, strong, and black. Retromarginal teeth are 6, and promarginal teeth are 3. The leg for the formula is 4123. Legs are black except yellow brow in 1/2 posterior in tarsus, metatarsus, tibia, and femur in all legs. One claw in the tarsus. Pedipalp is all black except ½ of femur, tibia, and metatarsus (Table 2).

**Table 2** Length of leg segments (mm) of *G. kuhli.*

| | Femur | Patella | Tibia | Metatarsus | Tarsus | Total |
|---|---|---|---|---|---|---|
| Leg 1 | 2.105 | 0.83 | 1.345 | 1.25 | 0.776 | 6.306 |
| Leg 2 | 1.844 | 0.798 | 1.134 | 1.101 | 0.792 | 5.669 |
| Leg 3 | 1.407 | 0.51 | 0.757 | 0.862 | 0.605 | 4.141 |
| Leg 4 | 2.392 | 0.864 | 1.423 | 1.54 | 0.802 | 7.021 |
| Pedipalp | 0.84 | 0.298 | 0.545 | 0.457 | 0.425 | 2.565 |

The abdomen margin is serrated; black and white, and blackish sigilla. It contains six spines; a groove is present containing the two posterior spines projected outside. It has 23 sigilla. The spines are black with black hair. The shape and structure of the spine areserrated, short, but robust. Posterior spines are separated from the anterior and median spines by a groove. Ventral side black with scattered white spots. There's a prominent black tubercle anterior to the spinnerets and posterior to the epigastric furrow. An orifice surrounds spinneret with border.

**3.3 *Gasteracantha sp.*** (Fig. 7)

This species is found in the shrubs in Linamon, Iligan City, and Impasugong, Bukidnon. The total length of the spider is 6.55 mm. The cephalothorax is 1.640 mm long, 2.380 mm wide, 2.2 mm high. Abdomen 4.55 mm long, 7.967 mm wide, 3.4 mm high.

The cephalothorax is rectangular in size, clothed with white hairs, yellow-brown at the edge in the left and right side, and it has a distinctive protrusion at the top of the thorax. The cephalic region is on the same height level as the abdomen. Thorax is rectangular frontally and dorsally, with a distinct tubercle at the back of the thorax. The carapace is clothed with white hair. There are eight eyes in two very slightly recurved rows. The eye length is 2.506. Eye diameter is AME=0.113 mm, PME = 0.140 mm. Eye separation is AME-AME =0.154 mm, AME-ALE= 0.863, PME-PME = 0.225, PME-PLE = 0.901. 22. Median ocular quadrangle is wider than long and wider behind than in front. Clypeus' height is small as long as one AME diameter. The sternum is oval and pointed at the end with yellow spots at the upper center, yellow-brown spots in coxae II & III.

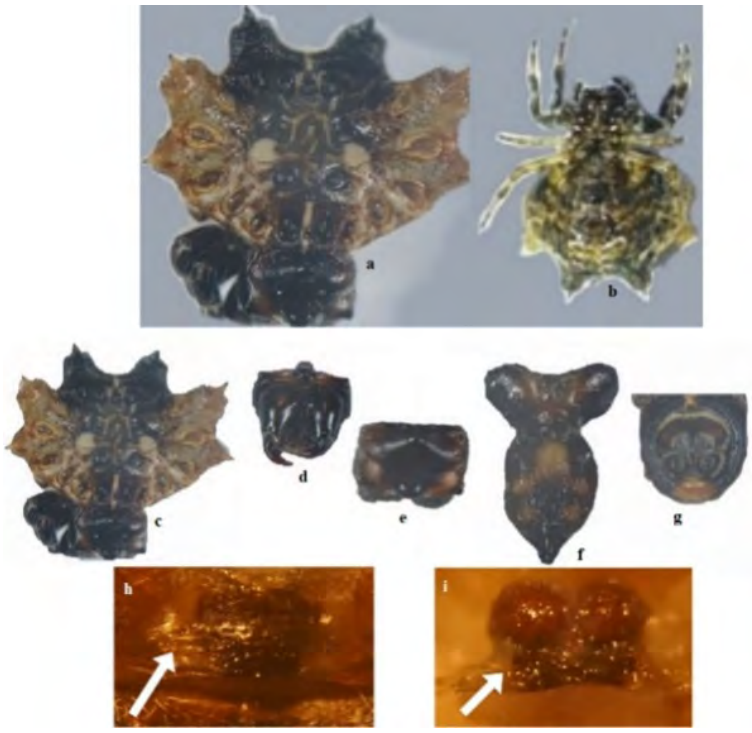

**Fig. 7** *Gasteracantha sp.* a. dorsal view; b. ventral view; c. dorsal view of the abdomen; d. frontal view of head featuring the eyes, clypeus, chelicerae, and fang; e. dorsal view of the carapace; f. maxilla, labium, sternum; g. spinneret; h. epigynum; i. spermathecae.

The labium is wider than long, halfway through the apex is yellow-brown. Maxillae are dark brown, except yellow in the later-inner margin, and as long as wide. Inner scapulae contain fine black hairs, with white hairs on the brown parts of the legs. The inner half of maxillae is 1/2 yellow-brown, going to the apex. Chelicerae are moderately small but robust. There are fourretromarginal teeth andsixpromarginal teeth. The Legs have the alternate color of black to yellow-brown; it's all black except the small portions of yellow-brown from the base of the coxae of femora I & II. Femora III & IV is mostly yellow-brown, occupying 1/2 from the coxae. Patella is all black in leg I & II. Tibia I & II is all black except a yellow-brown band, and 1/2 yellow-brown on patella III & IV. Metatarsus is all black except 1/4 on I & II, the metatarsus in III & IV is all black except 1/2 yellow-brown. Tarsus in all legs is black except 1/2 yellow-brown. Robust spines are present. The tarsal contains peg-like spines, with three claws. The leg formula is 4123. Pedipalp is all black except tibia and metatarsus, which includes a small band of yellow-brown coloration.

**Table 3** Length of leg segments (mm) of *G. sp*.

| | Femur | Patella | Tibia | Metatarsus | Tarsus | Total |
|---|---|---|---|---|---|---|
| Leg 1 | 1.822 | 0.826 | 1.366 | 1.388 | 0.894 | 6.296 |
| Leg 2 | 1.874 | 0.865 | 1.245 | 1.142 | 0.83 | 5.956 |
| Leg 3 | 1.385 | 0.645 | 0.85 | 0.875 | 0.754 | 4.509 |
| Leg 4 | 2.222 | 0.862 | 1.313 | 1.518 | 1.093 | 7.008 |
| Pedipalp | 0.565 | 0.477 | 0.697 | 0.727 | 0.397 | 2.863 |

The abdominal shape is concave in the anterior part, which is slightly higher than the posterior region, which is convex in shape. The abdomen is serrated. It contains twosignificant colors, golden yellow-brown, and black. There is a black coloration in the midsaggital section of the abdomen until the posterior spines. Lateral sides of the abdomen containing the anterior and median spines include the golden light-brown coloration visualizing like a wing. The abdomen consists of 35 sigilla with black and golden yellow-brown colorations depending on where they are situated. The dorsum contains 35 marginal ovoid sigillla with thicker borders at the edge. There are 25 black and ten golden sigilla. The orientation of the spines is slightly pointing 20° upward.

The shape and structure of the spines are small and pointy. There are located at the tip of the lobe-like structure in the abdomen. Colour of the spines in the anterior and median spines are golden yellow-brown with black at the tip of the spines. Posterior spines are entirely black. The description of the spines is robust, small, and pointy. The anterior spines are the smallest. It is pointing towards the anterior part and outside. Medial spines are the largest and are slightly pointing upward. The posterior spines are pointing upward. The venter is all black except some yellow-brown patches in random areas. The Spinneret is located at the center of the thick black orifice with an orange coloration at the bottom. The spinneret is protruding. The anterior spinnerets are noticeably larger than the posterior spinneret. Epyginum is transversely ovate with a small round looking structure at the tip. Spermathecae are small globular;also, they don't have space in between.

**3.4 *Thelacantha brevispina*** (Fig. 8)

This spider was initially identified as *G. mammosa* by Barrion and Litsinger (1995); however, according to the World Spider Catalog (2018, it is *Thelacantha brevispina* (World Spider Catalogue, 2018).

The total length of the spider is 7.76 mm. The cephalothorax is 1.81mm long, 2.74 mm wide, 3 mm high. Abdomen 6.2 mm long, 8.23 mm wide, 5 mm high. The cephalic region is yellow-brown except the black line from the MOQ running to the midsagittal plane dorsally as well as in the coronal plane running from the lateral eyes. The cephalic region is distinctly higher than thorax. Thorax is rectangular frontally and dorsally with a prominent tubercle in the center from the frontal view. The number of Eyes is 8 in two very slightly recurved rows. The eye length is 2.278. Eye diameter in mm is AME = ALE = 0.136, PME= 0.155. Eye separation in mm is AME-AME = 0.155, AME - ALE = 0.864, PME - PLE = 0.760. 22. Median ocular quadrangle is wider than long and wider behind than in front. Clypeus height is small as long as one AME diameter. The sternum is V-shaped and black except with a golden yellow-brown covering the sternum forming a continuous inverted U shaped from coxae I to III opposite to each other.

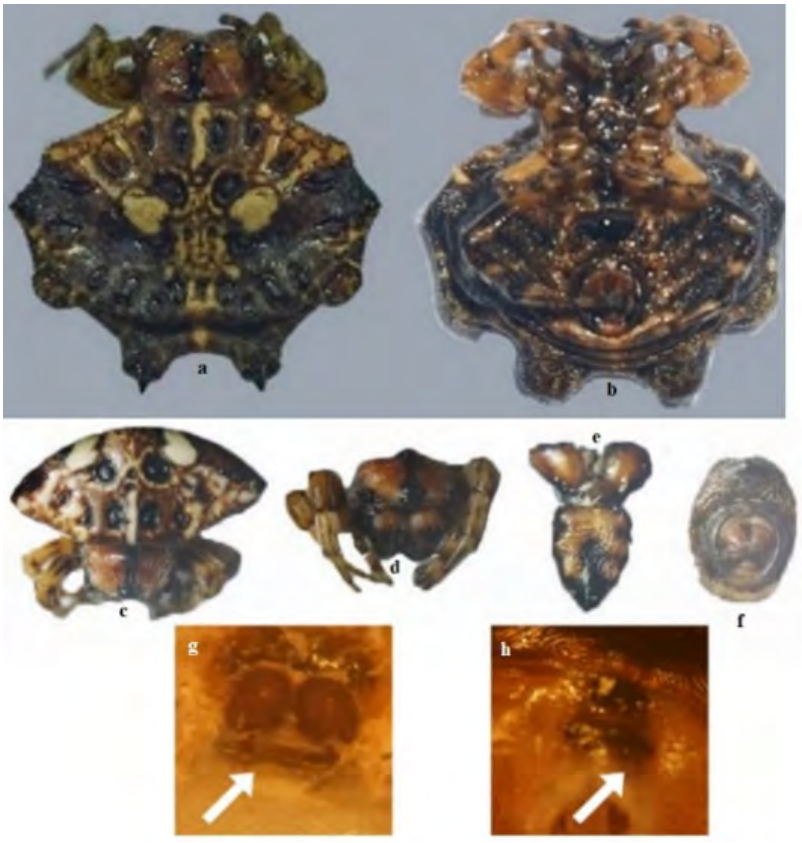


**Fig. 8** *Thelacantha brevispina*. a. dorsal view; b. ventral view; c. dorsal view of the cephalothorax and abdomen featuring black sigilla, midsagittal clack line from ocular quadrangle; d.frontal view featuring the chelicerae and eyes; e. sternum,l abium,maxillae; f. spinneret; g. spermathecae; h. epigynum.

The labium is wider than long, basal 1/4 dark brown and apical 3/4 yellow and narrow apically. Maxillae are dark brown, except yellow-brown latero-inner margin comprises 1/2 of maxillae, and as long as wide. Inner scapulae arefine black hairs. The inner half of maxillae is 1/2 yellow-brown. Chelicerae are robust but moderately small; there are fiveretromarginal and sixpromarginal teeth. Legs are yellow except, except lack base and apical 1/2 of femora III and IV, apical 4/5 of femora I and II, tibiae I-IV, and apices of all metatarsi and tarsi. Robust spines are absent; Tarsal contains fine hairs with one claw. Leg formula is 4123; Pedipalp is all black except yellow-brown on 1/2 of femur, tibia, patella, and metatarsus (Table 4).

**Table 4** Length of leg segments (mm) of *T. brevispina*.

| | Femur | Patella | Tibia | Metatarsus | Tarsus | Total |
|---|---|---|---|---|---|---|
| Leg 1 | 2.006 | 0.915 | 1.299 | 1.123 | 0.881 | 6.224 |
| Leg 2 | 2.016 | 1.015 | 1.176 | 1.176 | 0.878 | 6.261 |
| Leg 3 | 1.317 | 0.516 | 0.735 | 0.754 | 0.704 | 4.026 |
| Leg 4 | 2.361 | 0.69 | 1.285 | 1.677 | 0.98 | 6.993 |
| Pedipalp | 1.061 | 0.427 | 0.738 | 0.538 | 0.328 | 3.092 |

The abdomen is off-white, with scattered golden brown to dark brown patterns, it is an 11 sided polygon in form, wider than long, an anterior part strongly overlaps the thoracic area. There's a line in the midsaggital section dividing the dorsum with equal patterns; a groove is present containing the posterior spines. There are 19 marginal ovoid black sigilla. In the middle area includes three pairs (two large transverse and one small longitudinal) of sigilla in decreasing size towards the posterior and a couple of big white spots between the first pair of lateral tubercles. The posterior part of the abdomen is 1/2 of the abdomen, with two more pairs of tubercles projected vertically. The orientation of the spines is pointed 40 - 45° upward. The shape and structure of the spines are small and pointy, located at the tip of the tubercle in the marginal side of the abdomen. The color of the spines is black and black. The anterior spine is the smallest spine. Not prominent spine, median spine is second to the largest and is the most upright, posterior spine is the most extended spine. Venter is all black except some yellow-brown patches in random areas. Spinnerets are enclosed with a thick orifice, with anterior spinnerets larger than median and superior, Epyginum is transversely ovate with a small lip like structure. Spermathecae is spherical with a narrow external tube opening.

**3.5 *Gasteracatha parangdiadesmia* (**Fig. 9)

The total length of the spider is 8.636 mm. The cephalothorax is 1.53 mm long, 3.44 mm wide, 3.1 mm high. Abdomen 7.10 mm long, 15.27 wide, 5.1 mm high. The cephalothorax is rectangular with a black line in the mid cephalic area clothe with white hairs. The cephalic region is elevated and nearly 1/2 length of the carapace. Thorax is low concealed by the anterior part of the abdomen. The carapace is clothed with white hair. Several eyes are 8 in two recurved rows. The eye length is 3.301 mm. Eye diameter (mm) is AME = ALE = 0.11, PME= 0.134. Eye separation (mm) is AME-AME = 0.205, AME - ALE = 1.415, PME - PLE = 1.286. The median ocular quadrangle is narrower in front (0.20 mm) than behind (0.21 mm). Clypeus height is about 0.4 mm. The sternum is obovate and concave apically below labium, blackish brown with black hairs, except yellow dot on the center of the sternum. The labium is blackish brown, wider than long, and rounded at apex, with a yellow border at the tip. Maxillae are blackish-brown with yellow inner about 1/5 in the border. Barrion and Listinger (1997) originally described this spider. The current identification of this spider was based on their descriptions with some variations.

The copula is thick and black. The Inner maxillae are yellow located 1/5 away from the base. The chelicerae are strong, stout, and dark brown to black. There are sixretromarginal and promarginal teeth. Legs are all black covered with white hair. Robust spines are absent. Tarsal has three claws. The leg formula is 4123. Pedipalp is as long as femur I plus patella I (Table 5).

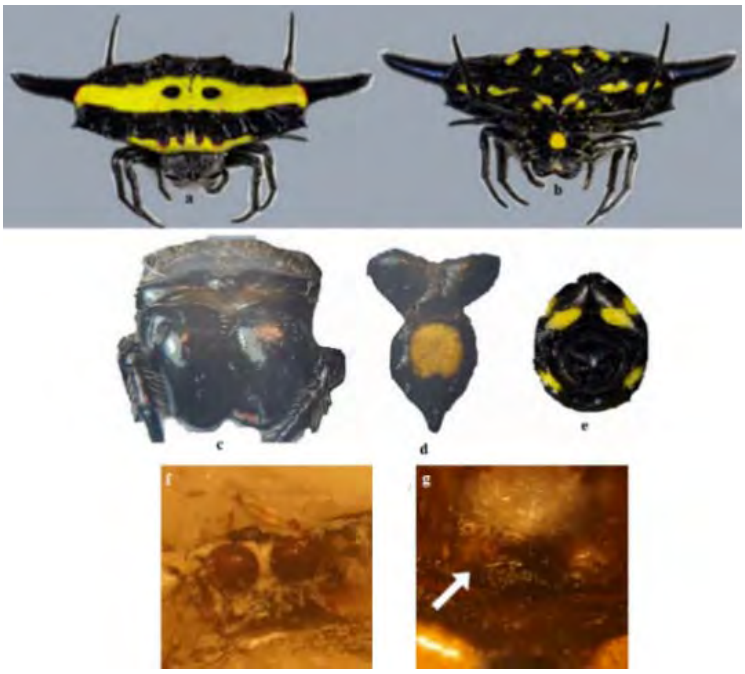

**Fig. 9** *Gasteracantha parangdiadesmia*. (a) dorsal; (b) ventral; (c) frontal view showing the eyes, clypeus, chelicerae, and pedipalp; (d) Sternum, labium, and maxillae; (e) spinneret; (f) spermathecae; (g) epigynum.

**Table 5** Length of leg segments (mm) of *G. parangdiadesmia*.

| | Femur | Patella | Tibia | Metatarsus | Tarsus | Total |
|---|---|---|---|---|---|---|
| Leg 1 | 2.472 | 0.466 | 1.0 | 1.0 | 0.877 | 5.815 |
| Leg 2 | 2.832 | 1.07 | 1.689 | 1.5 | 0.99 | 8.081 |
| Leg 3 | 2.086 | 0.835 | 1.877 | 1.971 | 1.048 | 7.817 |
| Leg 4 | 3.455 | 1.084 | 1.819 | 1.365 | 1 | 8.723 |
| Pedipalp | 0.771 | 0.397 | 0.78 | 0.575 | 0.423 | 2.946 |

The abdomen contains a pattern of transverse lines from the anterior (yellow, black, yellow, and black lines) the abdomen contains 23 sigilla. The 23 sigilla are in a pattern of 3 (big), 3(small), 3(small), 3 (big), all located in the black transverse line near to the anterior of the abdomen. 2 (big) in the yellow transverse line, and the last black transverse line near the posterior contains eight big sigilla and one small sigilla at the center. The orientation of the spines is projected outward. Spines are serrated, black covered with small black hairs and robust. The anterior spine is cleft at midlength. The laterals have a small apical spine and a large horn-like posterolaterally projected spine with two small tooth-like spines at the apex. The posterior spines are poorly developed, nipple-like. Venter contains yellow spots that are divided into two groups. In the 1/2 anterior part contains the ten spots like a transverse lane and, in the posterior region,includes seven yellow spots. The spinneret is all black and is surrounded by a thick orifice. Epigynum is a black nipple-like protuberance on a single space. The spermathecae are small globular with an inverted Y-shaped structure of ducts in between at the same time-space between is very noticeable.

**3.6 *Gasteracantha janopol*** (Fig. 10)

This species has been initially described by Barrion and Listinger (1995), and the current identification of this spider was also based on their descriptions with some variations. The total length of the spider is 8.917 mm.

The cephalothorax is 2.7 mm long, 4.0 mm wide, 2 mm high. Abdomen 7.317 mm long, 8.86 mm wide, 6.6 mm high.

The cephalothorax is a deep brown to reddish-brown. The cephalic region is rectangular and convex in the surface dorsally. The thorax is concealed underneath the anterior portion of the abdomen. It is wider with an M-shaped yellow marking below the fovea. The carapace is clothed with white hair. Several eyes are 8 in two recurved rows. Eye length (mm) is 2.973 mm, eye diameter (mm) is AME = PME= 0.172 and ALE = PLE = 0.155, eye separation (mm) is AME - AME = 0.47 and PME-PME is 0.189; AME-ALE=1.163 and PME-PLE is 1.131. Median ocular quadrangle is narrower in front than behind. Clypeus' height less than one AME diameter and clypeus is yellow-brown. The sternum is an inverted triangle in shape, procurved apically, and with the transverse yellow line between coxae I. There are yellow spots on coxae 1-4, covered with black hairs.

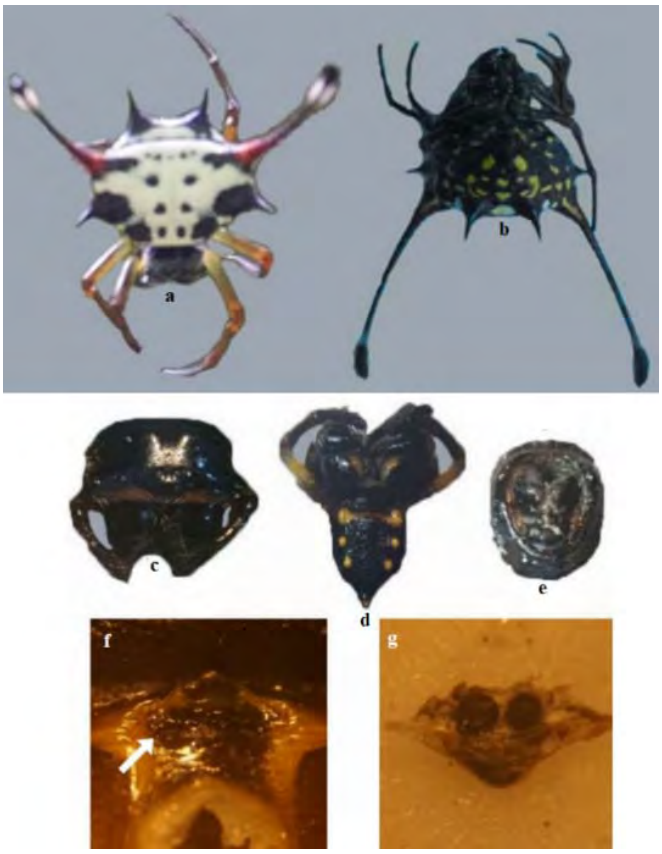


**Fig. 10** A. *Gasteracantha janopol*. (a) dorsal view; (b) ventral view; (c) frontal view of the head; (d) pedipalp, maxillae, fang, labium, and sternum; (e) spinneret; (f) epigynum; (g) spermathecae.

The labium is deep red-brown to black, more extensive than long, and slightly rounded at apex, with a yellow border. Maxillae have the same color with labium, as long as broad, with reducing base and yellow border on the inner part. Inner scapulae are yellow-brown, and the inner half of maxillae is yellow. The Chelicerae is short but robust and reddish-brown. There are fiveretromarginal and sevenpromarginal teeth of equal sizes. Legs are uniformly sebaceous, which ranges from light brown to reddish-brown. Robust spines are absent. Tarsal venter is a peg-like spine,three-clawed with eight teeth. The leg formula is 1423. Pedipalp is dark brown except yellow femur, single-clawed with nine teeth (Table 6).

**Table 6** Length of leg segments (mm) of *G. janopol.*

| | Femur | Patella | Tibia | Metatarsus | Tarsus | Total |
|---|---|---|---|---|---|---|
| Leg 1 | 3.034 | 0.906 | 2.504 | 1.811 | 1.54 | 9.795 |
| Leg 2 | 2.658 | 1.011 | 1.973 | 1.746 | 1.346 | 8.734 |
| Leg 3 | 2.138 | 0.658 | 1.055 | 1.044 | 0.85 | 5.745 |
| Leg 4 | 3.356 | 1.082 | 2.04 | 1.882 | 1.238 | 9.598 |
| Pedipalp | 0.375 | 0.393 | 0.634 | 0.489 | 0.575 | 2.466 |

The abdomen is concave, white with black marginal spines, black to reddish-brown. Sigilla is 22. Dorsum contains 22 ovoid sigilla arrange in patterns. The orientation of the spines in anterior and posterior are projected outward, and the median spine is projected upward. The shape and structure of the spines are thin and pointy. The color of the spines is black except the median spines, which have a red to violet color at the base and white color covered with white hairs at the distal tip. The median spine is very long and hairy, more than the height of the abdomen; it is clavate at the tip and arched inwards. The posterior spines are relatively longer than the anterior. Anterosubdorsalsigillaare nearly contiguous. Venter is black with highest along spinnerets and with scattered random yellow spots. Spinnerets are located inside an orifice with a thick border, and pointing inward Epigynum is not very noticeable. It is located almost below the sternum and is curved like two holes bordered with a black coloration. Spermathecae is small with little tail like, opposite each other.

**3.7 *Cyrtarachne sp*.** (Fig. 11)

This species was found in the shrubs in Impalutao, Impasug-long, Bukidnon at 9:00 pm. The total length of the spider is 9.786 mm. The cephalothorax is 3.495 mm long, 3.848 mm wide, 2.4 mm high. Abdomen 9.538 mm long, 14.817 mm wide, 5mm high. Cephalothorax yellow-brown in color and concave in shape dorsally. It is covered with small fine white hairs almost not visible. The cephalic region is located slightly under the abdomen. Transversely, the anterior part is subsquares and slightly rectangular; however, in the posterior part, the cephalothorax is becoming more rounded. The thorax is low concealed by the anterior part of the abdomen with an ovate structure at the center of the thorax right behind the posterior median eyes. The number of eyes is 8 in two very slightly recurved rows. The LE is AE = 2.15, PE = 2.286 eye diameter(mm):AME= 0.143, ALE= PLE = 0.127, PME=0.187. Eye separation (mm) is AME-AME = 0.241 mm, AME=ALE = 0.714 mm, PME-PME = 0.211 mm, PME-PLE = 0.795 mm. 22. Median ocular quadrangle is wider than long and wider behind than in front. The clypeus is short (133 mm). The sternum is heart-shaped.


**Fig. 11** *Cyrtarachne sp.* (a) dorsal; (b) ventral; (c) dorsal view of the cephalothorax; (d) frontal view featuring the chelicerae; (e) golden sigilla; (f) frontal view featuring the eight eyes and pedipalp; (g) sternum,labium,maxillae; (h) fangs of chelicerae.

The labium is very much wider than long; the border of the apex is lighter in color. The maxillae are concolorous with labium, very much wider than long, and have a yellow color at the tip. The inner scapulae have golden brown hairs. Chelicerae are long and robust. There are tworetromarginal teeth and threepromarginal teeth. Legs in the dorsal side are dark yellow-green and becoming lighter at going to the metatarsus and tarsus. The ventral side is dark green to black, and it doesn't have a robust spine. The tarsal contains three claws. The leg formula is 1423. Pedipalp has the same color as the legs with one claw (Table 7).

**Table 7** Length of leg segments (mm) of *Cyrtarachne sp*.

| | Femur | Patella | Tibia | Metatarsus | Tarsus | Total |
|---|---|---|---|---|---|---|
| Leg 1 | 3.734 | 1.502 | 2.55 | 1.789 | 0.782 | 10.357 |
| Leg 2 | 2.34 | n/a | 2.681 | 2.093 | 0.609 | 7.723 |
| Leg 3 | 2.363 | 0.757 | 1.407 | 1.241 | 0.547 | 6.315 |
| Leg 4 | 3.421 | 1.29 | 2.229 | 1.76 | 0.609 | 9.309 |
| Pedipalp | 0.983 | 0.539 | 0.733 | 0.684 | 0.326 | 3.265 |

The abdomen is triangular with a soft rounded edge in every angle; it doesn't have a spine. In the middle of the abdomen dorsally, there's a very noticeable two golden brown arc-shaped with a sigilla below, located in the middle of both arcs. The anterior portion of the abdomen, a golden brown band, is present just below the sigilla under the arcs. There are 18 sigilla. The dorsum contains 18 ovoid golden-colored sigilla. The spines are absent. The venter is concave and is covered by black color. It has a prominent wave-like vertical structure

near the margin of the abdomen. Spinnerets are slightly burrowed, and the anterior spinneret is larger than the posterior spinneret. Anterior spinneret has a darker color, whereas the posterior spinneret has a lighter yellow-brown coloration.

### 3.8 Parsimony analysis

Fig. 12 shows the consensus tree showing the characters of where the species have differed. The black circles represent the homologous characters, and the white circle represents the homoplasious characters. The characters and character-states that are unique in each species according to the parsimony analysis are shown in Table 8. *Thelacantha brevispina* has the following characters and states-black line in the lateral and middle carapace, clypeus height is the same height as AME, leg color combination is mostly yellow with small black spots as well as the pedipalp color combination, the color of the spine has the combination of black and yellow. They don't have the hairs in the spine. Gasteracantha has the following characters, and character states- the location of the spots are located at the center, there are also spots that are situated in on each side of coxae and tip, they just have a color combination of 2 in their abdomen, the color combinations are white and black as well as yellow and black, and the color of sigilla are all black. These are all homologous characters based on the parsimony analysis (Table 8).

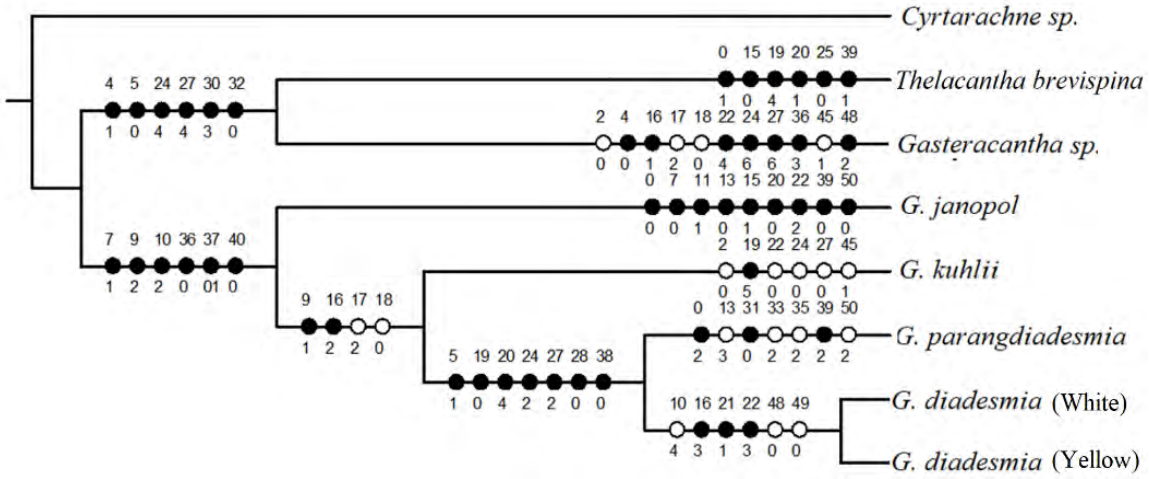


**Fig. 12** Parsimony analysis of the spiny orb spiders. (Consensus tree with length 198, consistency index with 67, retention index 48).

**Table 8** Characters and characters state that they are unique in each species according to the parsimony analysis.

| Group | Homologous characters | | Homoplasious characters | |
|---|---|---|---|---|
| | character | Character states | character | Character states |
| **Cluster 1** | 5. Carapace description<br>6. Clypeus height<br>25. Leg color combination<br>28. Pedipalp color combination<br>31. Color of the spine<br>33. Hairs on spines | 1. Brown stripes in the lateral<br>0. same height as AME<br>4. mostly yellow with small black spots<br>4. mostly yellow with small black spots<br>3. combination of black and yellow<br>0. Absent | | |
| *T. brevispina* | 1.Cephalothorax | 1. Yellow-brown & blackish | | |

| | | | | |
|---|---|---|---|---|
| | 16. Coloration of labium<br>20. Color of chelicerae<br>21. Structure of chelicerae<br>26. Claw/s on Tarsal venter<br>40. No. of sigilla | brown in the thoracic region<br>0. ¾ outer border<br>4. yellow-brown and black<br>1. short, small, robust<br>0. one claw<br>1. 19 sigilla | | |
| *Gasteracantha sp.*1 | 5. Carapace description<br>17. Shape of maxillae<br>23. Promarginal teeth<br>25. Leg color combination<br>28. Pedipalp color combination<br>37. No. of color combination in the abdomen<br>49. Size of spermathecae | 0. Brown stripes in the lateral<br>1. wider than long<br>4. 4 teeth<br>6. mostly all black ¼ yellow<br>6. mostly all black ¼ yellow<br>2. three colors<br>2. two small equal | 3. Thorax position<br>18. color of maxillae<br>19. Inner scapulae<br>46. An extension like the tip of spinneret | 0. Not concealed under the abdomen<br>The 2. ¼ outer border<br>0. Black<br>1. present |
| **Cluster 2** | 8. Spots location<br>10. Scope of spot<br>11. Spot location by coxae<br>37. No. of color combination in the abdomen<br>38. Color Combination<br>41. Color of sigilla | 1. center<br>2. on each side of coxae and tip<br>2. coxae 1,2,3, 4<br>0. two colors<br>0. White and black<br>1. yellow and black<br>0. all black | | |
| *G. janopol* | 1.Cephalothorax<br>8. Spots location<br>12. Spot on the tip<br>14. Shape of sternum<br>16. Coloration of the labium<br>21. Structure of chelicerae<br>23. Promarginal teeth<br>40. No. of sigilla<br>51. Shape of spermathecae | 0. Deep brown to reddish-brown<br>0. not center<br>1. present<br>0. triangularly pointed basally(v-shaped)<br>1. ½ outer border<br>0. short, small, robust<br>2. seven teeth of equal sizes<br>0. 24 sigilla<br>0. g-shaped | | |
| **Cluster 3** | 10. Scope of spot<br>17. Shape of maxillae | 1. not covering 1,2<br>2. longer than wide | 18. color of maxillae<br>19. Inner scapulae | The 2. ¼ outer border<br>0. Black |
| *G. kuhli* | 20. Color of chelicerae | 5. dark brown | 3. Thorax position<br>23.Promarginal teeth<br>25. Leg color combination<br>28. Pedipalp color combination<br>46. An extension like the tip of spinneret | 0. Not concealed under the abdomen<br>0. three teeth<br>0. black and light brown<br>0. black and light brown<br>1. present |
| **Cluster 4** | 6. Clypeus height | 1. same height as ALE | | |

| | | | | |
|---|---|---|---|---|
| | 20. Color of chelicerae<br>21. Structure of chelicerae<br>25. Leg color combination<br>28. Pedipalp color combination<br>29. Shape of abdomen<br>39. Color pattern | 0. black<br>4. short and stout<br>2. All black<br>2. All black<br>0. Wider than long<br>0. Horizontal | | |
| *G. Parangdiadesmia* | 1. Cephalothorax<br>32. No. of spine<br>40. No. of sigilla | 2. Brown to dark brown, except a pair of black longitudinal parallel stripes in midcephalic area<br>0. 2<br>2. 30 sigilla | 14. Shape of sternum<br>34. Anterior spine<br>36. Posterior spine<br>51. Shape of spermathecae | 3. wider obovate with a more pointed tip<br>2. undeveloped<br>2. undeveloped<br>2. globular with y shaped |
| **Cluster 5** | 17. Shape of maxillae<br>22. Retromarginal teeth<br>23. Promarginal teeth | 3. as long as broad<br>1. 4 teeth<br>3. five teeth | 11. spot location by coxae<br>49. Size of spermathecae<br>50. Distance of spermathecae | 4. at the center bet 1,2<br>0. two large equal<br>0. with space |
| *G. diadesmia* (yellow) | | | | |
| *G. diadesmia* (white) | | | | |

Based on neighbor-joining clustering analysis, two groups were formed that separated the genus *Thelacantha* [*T. brevispina*] from the genus *Gasteracantha* using *Cyrtarachne sp*. as the outgroup. Group 1 includes *T. brevispina* and *Gasteracantha sp*. Group 2 has *G. janopol*, *G. kuhli*, *G. parangdiadesmia*, *G. diadadesmia* (white), and *G. diadesmia* (yellow). In group 1, *T. brevispina* and *Gasteracantha sp*1 has 76% similarity. In group 2, *G. janopol* has only 33% of similarity with *G. kuhli. G. kuhli* has an 80% similarity with *G. diadesmia* (yellow and white). *G. diadesmia* (yellow) and *G. diadesmia* (white) has 92% similarity value (Fig. 13).

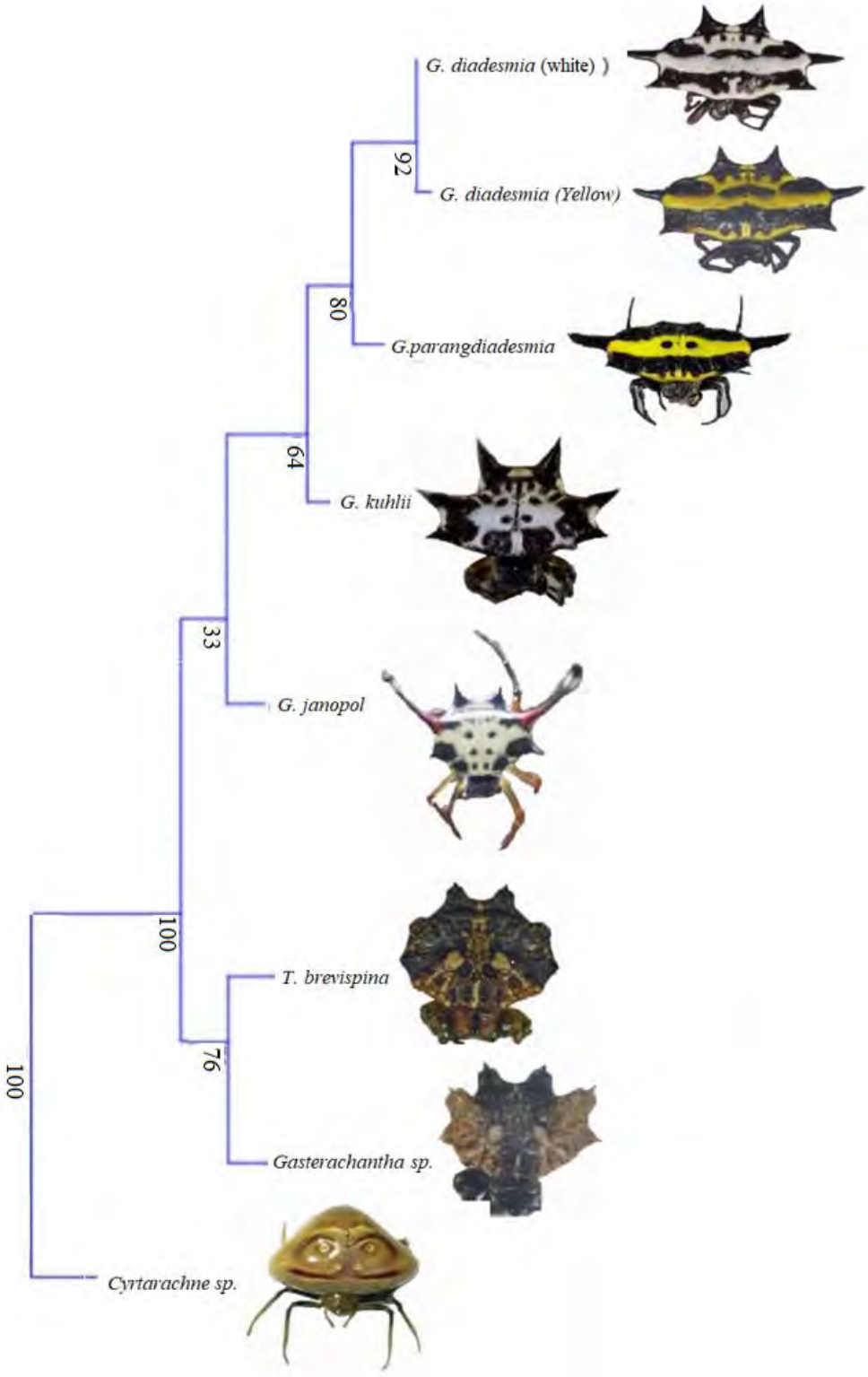


**Fig. 13** Neighbor-joining clustering of the spiny orb spiders.

It can be seen from the results of this study that variations between these orb-weaving spiders were mostly differences in colorations in the cephalothorax, legs, maxillae, and labium, especially the abdomen. It is argued that these variations in coloration are indicators of adaptation and speciation, leading to a clearer understanding

of evolutionary and ecological processes (Dyer et al., 2012). Several studies have shown that certain species of orb-web spiders can attract prey by their bright and conspicuous body-color (Hauber, 2002; Kemp et al., 2013, Tso et al., 2004). Many species of orb-web spiders demonstrate this sit and wait for strategy and body decorated with colors and patterns for prey attraction (White, 2015) but not really in conveying camouflage (Thery and Casas, 2008). There are arguments that these colorations suggest camouflage or show a form of aggressive mimicry (Oxford and Gillespie, 1998) to deceive the preys to approach them (O'Hanlon et al., 2014) although there are other species whose body color does not attract prey but only for thermoregulation, camouflage, aposematism, and intraspecific communication. (Nakata and Shigemiya, 2015).

**3.9 Molecular analysis**

There are a total of 6 species that were collected and identified and 14 COI sequences that have been aligned. Eight of these COI sequences were retrieved from NCBI BLAST. Phylogenetic analysis (Fig. 14) using MEGA7 (Kumar et al., 2015) shows the species *G. kuhli* has a 99% identity with *G. kuhli* from Taiwan (accession number of KJ957963 in NCBI). *G. parangdiadesmia* has only 97% identity with the morphologically identified *Gasteracantha sp.*1 (accession number of DQ127354) and potentially could be the same species just indicating phenotypic plasticity. *Gasteracantha sp.*1, on the other hand, has only 83% with *T. brevispina* despite their similarity in morphology and only 81% with *Gasteracantha kuhli*. *Cyrtarachne sp*. has 100% similarity with *Cyrtarachne yunoharuensis* (accession number of AB546975); thus, this species collected from Mindanao is *C. yunoharuensis* and the morphological variation observed is an indication of phenotypic plasticity.

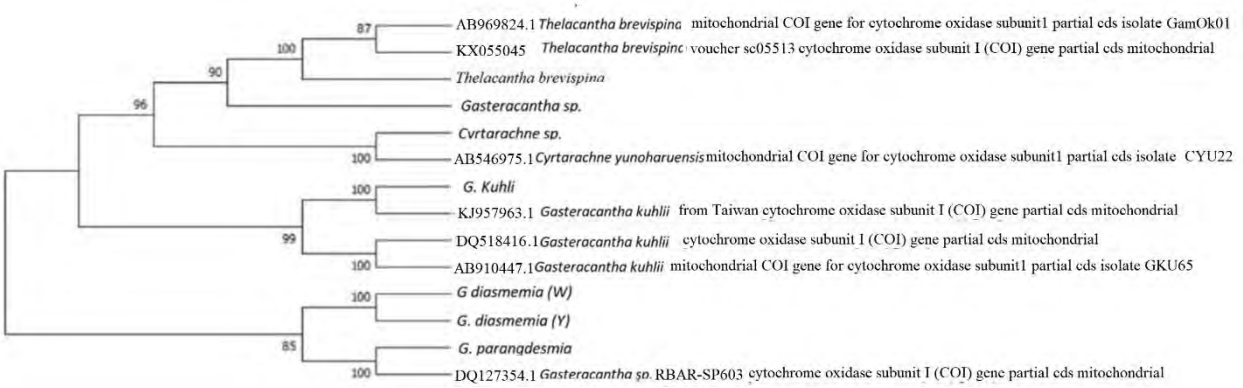


**Fig. 14** Phylogenetic tree showing the evolutionary relationships between species of the spiny orb spiders.

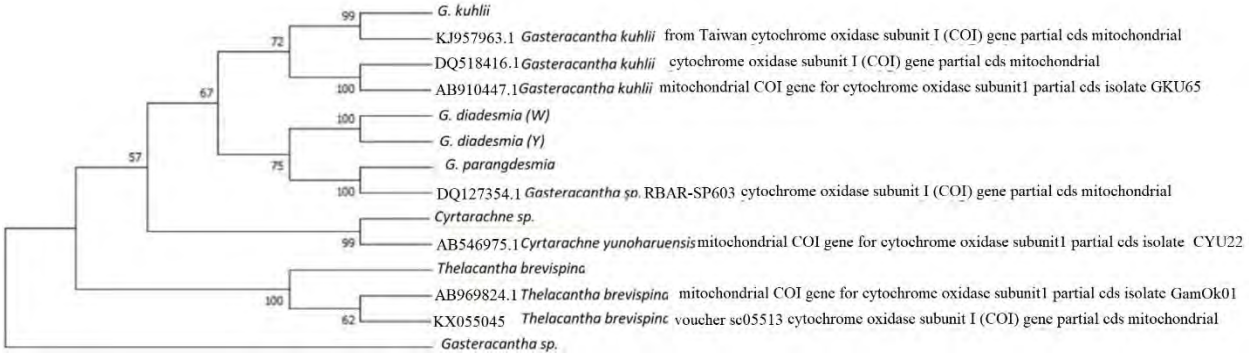


**Fig. 15** Maximum Parsimony analysis of the spiny orb spiders using the maximum parsimony method.

Fig. 15 shows the separations between the nodes of *Gasteracantha*, *Cyrtarachne*, and *Thelacantha* based on maximum parsimony analysis.

Maximum parsimony analysisshowsthe genera *Gasteracantha* and *Thelacantha*are closer morphologically compared to *Cyrtarachne.* Between these two genera, *Gasteracantha sp.,* which was observed to be morphologically similarly affiliated tothe previously identified *Gasteracantha mammosa* (later *T. brevispina*) was found to be more affiliated to *T. brevispina* based on mitochondrial DNA. *Gasteracantha sp.* might be a potentially new species of Thelacantha. It can, therefore, be argued that the DNA barcoding result was a good backup for the morphological analysis since it accelerates the process of identifying morphologically difficult taxa (Sun et al., 2012). In this study, parsimony analysis showed the clade of *G. kuhli* is monophyletic from *G. diadesmia, G. parangdiadesmia,* and *Gasteracantha sp*. (DQ1273541.1) (Fig. 15). While it was thought that most of the tribes of spiders consisting of spiny abdomens are close together (Coddington and Levi 1991), some studies proposed that *Gasteracantha* species are affiliated with other two different tribes of spiny orb-weavers, namely, *Xylethreae*, and *Micratheneae* (Micrathena). This idea, however, was shown to be unsupported since they were distantly related based on molecular evidence (Alvarez-Padilla, 2009; Dimitrov et al., 2012; this study). It is, therefore, safe to say that molecular methods of delineating the species arespeedy and helpful, especially for the puzzling morphology of these selected collected spidersserving as a good backbone for their taxonomic description (Jörger and Schrödl, 2013).

## 4 Conclusion

The results of this study have shown that the use of both phenotypic and molecular analyses are both criticalin providing information in delineating species of interest.*T.brevispina* initially identified as *G. mammosa* was confirmed by molecular data for its species status under Thelacantha. The white and yellow morphotypes of *G. diasdemia* were found to be the same species based on molecular data, thus indicating that it is phenotypically plastic. The morphologically different *Cyrtarachne* sp. was confirmed to be *C. yunoharuensis;*therefore, the observed morphological characteristics are but variant forms of the same species. *Gasteracantha sp*. was observed to be more affiliated to *Thelacantha* than *Gasteracantha* and was different from the rest of the species studied based on parsimony analysis; thus, this could be a new species of an orb-weaving spider under the genus *Thelacantha*.

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## References

Álvarez-Padilla F, Dimitrov D, Giribet G, Hormiga G. 2009. Phylogenetic relationships of the spider family Tetragnathidae (Araneae, Araneoidea) based on morphological and DNA sequence data. Cladistics, 25: 109-146

Barrion AT, Litsinger JA. 1995. Riceland Spiders of South and Southeast Asia. CAB International, Wallingford, UK

Coddington A, Levi HW. 1991. Systematics and evolution of spiders (Araneae). Annual Review of Ecof. Syst, 22: 565-592

Dimitrov D, Lopardo L, Giribet G, Arnedo MA, Álvarez- Padilla F, Hormiga G. 2012. Tangled in a sparse spider web: single origin of orb weavers and their spinning work unraveled by denser taxonomic sampling. Proceedings of the Royal Society of London B, 279: 1341-1350

Dyer AG, Boyd-Gerny S, McLoughlin S, Rosa MGP, Simonov V, Wong BBM. 2012. Parallel evolution of angiosperm color signals: common evolutionary pressures linked to hymenopteran vision. Proceedings of the Royal Society B: Biological Sciences, 279: 3606e3615

Edgar RC. 2004a. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics, 5: 113

Edgar RC. 2004b. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research, 32: 1792-1797

Friedheim S. 2016. Comparison of species identification methods: dna barcoding versus morphological taxonomy. Horizons, 1(1): 74-86

Giribet G. 2007. Efficient Tree Searches with Available Algorithms. Evolutionary Bioinformatics Online, 3: 341-356

Goloboff PA. 1994. NONA, version 1.5.1. American Museum of Natural History, USA

Hauber ME. 2002. Conspicuous coloration attracts prey to a stationary predator. Ecological Entomology, 27: 686-691

Hebert PDN, Cywinska A, Ball SL, de Waard JR. 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London B, 270: 313-321

Hubert N, Hanner R. 2015. DNA Barcoding, species delineation, and taxonomy: a historical perspective. DNA Barcodes, 3: 44-58

Ji T, Zi YC, Ching AO, Hoi SY. 2019. Phylogenetic relationships of *Actinacantha simon*, *Gasteracantha sundevall*, *Macracantha hasselt*, and *Thelacantha simon* spiny orbweavers (Araneae: Araneidae) in Peninsular Malaysia. Raffles Bulletin of Zoology, 67: 32-55

Jörger KM, Schrödl M. 2013. How to describe a cryptic species? Practical challenges of molecular. Frontiers in Zoology, 10(1): 59

Kemp DJ, Holmes C, Congdon BC, Edwards W. 2013. Color polymorphism in spiny spiders (*Gasteracantha fornicata*): Testing the adaptive significance of a geographically clinal lure. International Journal of Behavioral Biology, 119(12): 1126-1137

Kumar S, Stecher G, Tamura K. 2015. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution, 33: 1870-1874

Levi HW. 1978. The American orb-weaver genera Colphepeira, Micrathena, and Gasteracantha North of Mexico (Araneae, Araneidae). Bulletin of the Museum of Comparative Zoology at Harvard College, 148: 417-442

McKinnon JS, Pierotti ME. 2010. Colour polymorphism and correlated characters: genetic mechanisms and evolution. Molecular Ecology, 19(23): 5101-5125

Muma MH, Stone KJ. 1971. Predation of *Gasteracantha cancriformis* (Arachnida: Araneidae) eggs in Florida citrus groves by *Phalacrotophoraepeirae* (Insecta: Phoridae) and *Arachnophagaferruginea* (Insecta: Eupelmidae). Florida Entomologist, 54: 305-310

NakataK, Shigemiya Y. 2015. Body-color variation in an orb-web spider and its effect on predation success. Biological Journal of the Linnean Society, 116(4): 954-963

Nixon KC. 2002. Winclada, v. 1.00.08. Program and documentation available at www.cladistics.com

Nyffeler M, Benz G. 1987. Spiders in natural pest control: a review. Journal of Applied Entomology, 103: 321-339

O'Hanlon JC, Holwell GI, Herberstein ME. 2014. Pollinator deception in the orchid mantis. American Naturalist, 183: 126-132

Oxford GS, Gillespie RG. 1998. Evolution and ecology of spider coloration. Annual Review of Entomology, 43: 619-643

Reichert SE, Lockley T. 1984. Spiders as biological control agents. Annual Review of Entomology, 29: 299-320

Robinson EA, Blagoev GA, Hebert PDN, Adamowicz SJ. 2009. Prospects for using DNA barcoding to identify spiders in species-rich genera. ZooKeys, 16: 27-46

Sharma S. 2014. A Study on Spiders as Predators in the Agro Ecosystems. Munis Entomology and Zoology, 9(1): 80-83

Simon E. 1892-1903. Histoire naturelle des aragnées (2 edition). Paris, Encyclopedieroret, L. Mulo. T.I (1892-1895): 1-1084; T.II (1897-1903): 1-1080. http://www.insecta.bio.spbu.ru/z/nom/~Simon1892-1903.htm

Sun Y, Li Q, Kong L, Zheng X. 2012. DNA barcoding of Caenogastropoda along the coast of China based on the COI gene. Molecular Ecology Resources, 12(2): 209-218

Thery M, Je´roˆme C.2008. The multiple disguises of spiders: web color and decorations, body-color, and movement. Philosophical Transactions of the Royal Society of London B, 364: 471-480

Tso IM, Lin CW, Yang EC. 2002. Colorful orb-weaving spiders and web decorations through a bee's eyes. Journal of Experimental Biology, 207: 2631-2637

White TE, Kemp DJ. 2015 Technicolour deceit: a sensory basis for the study of color-based lures. Animal Behaviour, 105: 231-243

World Spider Catalog. 2018. World Spider Catalog. Version 19.0. Natural History Museum Bern. http://wsc.nmbe.ch. Accessed on May 15, 2018

Yong HS, Ono H. 2009. *Gasteracantha crucigera* (Araneae: Araneidae) from Maliau Basin, Sabah, Malaysia: an overlooked spiny-backed orb-weaver spider from Peninsular Malaysia. Journal of Science and Technology in the Tropics, 5: 101-103