*Zootaxa* 4722 (3): 241–269
https://www.mapress.com/j/zt/

**Article**

ISSN 1175-5326 (print edition)
**ZOOTAXA**
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.4722.3.2
http://zoobank.org/urn:lsid:zoobank.org:pub:6AC6E70B-6E6A-4D46-9C8A-2260B929E471

# A protocol for online documentation of spider biodiversity inventories applied to a Mexican tropical wet forest (Araneae, Araneomorphae)

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## Abstract

Spider community inventories have relatively well-established standardized collecting protocols. Such protocols set rules for the orderly acquisition of samples to estimate community parameters and to establish comparisons between areas. These methods have been tested worldwide, providing useful data for inventory planning and optimal sampling allocation efforts. The taxonomic counterpart of biodiversity inventories has received considerably less attention. Species lists and their relative abundances are the only link between the community parameters resulting from a biotic inventory and the biology of the species that live there. However, this connection is lost or speculative at best for species only partially identified (e. g., to genus but not to species). This link is particularly important for diverse tropical regions were many taxa are undescribed or little known such as spiders. One approach to this problem has been the development of biodiversity inventory websites that document the morphology of the species with digital images organized as standard views. Their main contributions are the dissemination of phenotypic data for species difficult to identify or new with the assignment of species codes, allowing species comparisons between areas regardless of their taxonomic status. The present paper describes a protocol to produce these websites almost automatically. This protocol was successfully applied to 237 species from a tropical primary forest in Mexico. The time and infrastructure required for the documentation of these species are discussed. Taxonomic information in terms of identification challenges, possible new species, and potential nomenclatural issues is described. In addition, the conventional community parameters (e. g., inventory completeness, species richness estimations, sampling intensity) are also calculated and compared through time and between methods. An optimized version for sampling allocation effort per season is presented and compared with protocols optimized for other tropical forests.

**Keywords:** Arachnida, COBRA-TF, Faunistics, Neotropics, Taxonomy

## Introduction

Biodiversity inventories with highly diverse taxa must use standardized or optimized sampling protocols; inventories that do not use these protocols provide only fragmented data and exclude themselves for meaningful comparisons. A protocol can be defined as a set of rules for collecting data with the objective of maximizing the number of species captured, the analytical tools applicable, and to allow for accurate estimation of community parameters such as: inventory completeness, species richness, collecting method efficiency, sampling effort allocation, beta diversity of communities, and more (Agosti *et al.* 2000; Brown *et al.* 2018; Coddington *et al.* 1991; Colwell & Coddington 1994; DeVries & Walla 2001; Erwin *et al*. 2005; Gotelli & Colwell 2001; Longino *et al.* 2002; Malumbres-Olarte *et al.* 2018; Scharff *et al.* 2003; Sorensen *et al.* 2002).

Sampling protocols can be characterized in four categories: standardized, optimized, quasi-optimized, and *ad hoc* collecting. Standardized protocols define explicit units of collecting effort and area delimitation and can be applied to any ecosystem. Optimized protocols distribute the collecting effort differentially, allocating more samples to those methods that yield more species and are either site or season specific. Quasi-optimized protocols also use

*Accepted by G. Hormiga: 16 Oct. 2019; published: 14 Jan. 2020*

an optimized distribution of samples per method but are extrapolated to more than one similar ecosystem or seasons. *Ad hoc* collecting can or cannot include any measurement of effort or area delimitation, and is planned for a specific place, taxon or season. If measurements of effort and area delimitation are not established, *ad hoc* collecting must be avoided for biodiversity inventories; however, it is useful when searching for a particular group in the context of taxonomic revisions or phylogenetic analyses (Cardoso *et al*. 2009b; Miller *et al*. 2009; Malumbres-Olarte *et al*. 2017).

For spiders (Araneae), —a highly speciose arthropod clade found in nearly all environments—, these protocols already exist, have been tested in many ecosystems and represent the best option to sample this species community. Most spider sampling protocols use as basic unit of sampling effort the "one hour" of collecting per person and for area delimitation the one-hectare plot. However, they are flexible enough to vary these two basic units as long as they are analytically comparable, and can select among the traditional collecting methods or incorporate new ones depending on the habitats intended to be sampled (Cardoso *et al*. 2008a, 2008b, 2009a; Carvalho *et al*. 2012; Castanheira *et al*. 2016; Coddington *et al*. 1991; Coddington *et al*. 1996; Coddington *et al*. 2009; Muelelwa *et al*. 2010; Scharff *et al*. 2003; Silva-Davila & Coddington 1996; Sørensen *et al*. 2002; Toti *et al*. 2000). Furthermore, optimized versions have been proposed for several ecosystems along temperate and tropical latitudes (Cardoso 2009; Cardoso *et al*. 2017a; Emerson *et al*. 2017; Malumbres-Olarte *et al*. 2017, 2018).

These protocols have provided useful data for inventory preparation and resource allocation. It can be expected for one hectare of tropical forest to yield 241 (standard deviation +/- 126.35) species on average with an estimated richness two to three times higher (Bonaldo & Dias 2010; Castanheira *et al*. 2016; Coddington *et al*. 1991, Coddington *et al*. 2009; Miller & Pham 2011; Ricetti & Bonaldo 2008; Rivera-Quiroz *et al*. 2016; Silva-Davila & Coddington 1996; Sørensen *et al*. 2002; data from Table 3). The samples will be dominated by rare species (singletons) and the proportion of adult specimens will oscillate around one third of the several thousands of spiders collected with an average completeness of 71.77 % (+/-8.4). Optimized protocols provide the minimum collecting effort required to estimate these parameters accurately. In addition, these optimizations allow to explore larger areas with the same resources, given that sampling per each plot does not need to be so intensive (Malumbres-Olarte *et al*. 2017). In terms of conservation, they contribute to the rapid acquisition of data regarding how species distributions change in space and time identifying taxa or areas for environmental protection or restoration (Cardoso *et al*. 2011, 2017a).

The taxonomic counterpart for spider biodiversity inventories has received considerably less attention. For community parameters calculation, the species list could be an array of random letters as long as they are constant among specimen phenotype or genotype data. The species list creates a connection between these parameters with the biological information of that community and the evolution of these species. Taxonomically, proper documentation of phenotypic information is usually presented within the context of taxonomic revisions, monographs or new species descriptions; however, for new species or partially identified taxa this connection is lost or speculative at best through comparisons among relatives. This is particularly important for tropical regions inhabited by many undescribed and described species (Miller *et al*. 2014).

The online resources provided by The World Spider Catalog have enormously contributed to the distribution of taxonomic information (World Spider Catalog 2019). It has made possible species identifications for taxa with good quality original or subsequent descriptions and it makes evident where taxonomic work is needed. In addition to the World Spider Catalog, there are several websites sharing thousands of digital images to deal with phenotypic data. Salticidae websites provides taxonomic resources and digital images for described and undescribed species (Jerzy Prószyński, https://www.peckhamia.com/salticidae/ salticidae.php?adres=permision.php; Wayne Maddison, http://salticidae.org/salticidImages/). Phenotypic data also have been distributed through websites to coordinate taxonomic revisions in Oonopidae resulting in an exponential increase in new species descriptions and revisionary work (The Goblin Spider PBI 2019), and several independent sites exist that provide local species list and digital images (LinEpig 2019; Jørgen Lissner, http: //www.jorgenlissner.dk/families.aspx; Pierre Oger, https://arachno.piwigo.com/).

Spider biodiversity inventories with species documented online are just beginning and only three websites exist. The first website was developed in Argentina to document the species found in an inventory in Thailand done by the Spider ATOL team during 2003 in the locality of Doi Inthanon (Martin Ramirez, http://aracnologia.macn.gov.ar/ThaiPlot/index.htm). The second website is also on South East Asian spiders and has documented several inventories; in addition, it incorporates links for taxonomic, genetic and geographic data (Jeremy Miller, http: //www.digitalspiders.org/). The third website documents Araneomorphae species for inventories in Mexico (Fernando Alvarez-Padilla, http: //www.unamfcaracnolab.com/). The main features that unite these websites are the use of standard views and the assignment of species codes. Their main contribution is allowing comparisons by sharing phenotypic data for species difficult to identify or new independently of Linnaean names (Miller *et al*. 2014). These resources have been proved useful for new species descriptions, species identification, collaborations in several taxonomic revisions (Campuzano & Ibarra-Núñez 2018; Chamé-Vazquez *et al*. 2018; Maya-Morales & Jiménez 2016) and could assist in conservation efforts regardless of the taxonomic status of the animals compared.

The goal of the present paper is to describe a protocol to make websites almost automatically for spider inventories immediately after the image acquisition process is completed. This set of rules was successfully applied at the Tropical Biology Station Los Tuxtlas IB-UNAM (Veracruz, Mexico). The time required, logistics and infrastructure for this inventory documentation are also discussed. Relevant information is also presented in terms of taxonomic identification challenges and possible nomenclatural issues. In addition, the species richness of one hectare of this primary tropical wet forest is estimated. The differences between collecting methods and seasonality are evaluated, and an optimized version for sampling this type of forest is presented.

## Methods

**Area of study.** The study area is located within the Reserva de la Biofera Los Tuxtlas with an extension of 155,122 hectares and is divided into three core zones corresponding to the volcanoes: San Martín Tuxtla, Santa Marta and San Martín Pajapan. These are located at the eastern edge of the Mexican Volcanic Belt in the State of Veracruz and correspond to the Veracruzan province according to the Biogeographic regionalization based on terrestrial taxa (Morrone 2014). These volcanoes range in altitude from 0 to 1,680 m and present six types of ecosystems: Tropical Cloud forest, Oak forest, Pine forest, Mangrove forest, Grasslands and Tropical wet forest, where the present inventory was conducted. This last type of ecosystem is the most common below 700 m (Guevara *et al*. 2004). Human occupancy in the area dates to 6,000 years ago with the establishment of the Olmec culture (Gonzalez-Soriano *et al*. 1997). More recent changes by humans in land use policy have reduced its surface to 5 % distributed in approximately 60 fragments (Guevara *et al*. 2004; Von Thaden *et al*. 2018).

The Los Tuxtlas Tropical Biology Field Station is part of the Instituto de Biología and it is operated by the Universidad Nacional Autónoma de México (IB-UNAM). It is located within the core zone which corresponds to the San Martín Volcano with an extension of 640 ha. Scientific research by UNAM has been carried out here since the 1967 recording over 2,697 plant and 2,096 animal species, in particular mammals, birds and several orders of insects. It has a maximum average temperature of 27.3 °C, a minimum of 21.5 °C with the coldest days around 10 °C. The total annual precipitation is 4,725.2 mm of rain. The dry season is from March to May. The rainy season starts in June concluding in February with most precipitation from September to November accounting for the 85% of the year's average. The other 15% of rainfall occurs from September to February and is characterized by violent storms with winds of 100 Km/hr. (Gonzalez-Soriano *et al*. 1997). The vegetation is composed by species form Lauraceae and Fabaceae, with trees that reach 30 m in height with trunks of up to 2 m in diameter. Lower strata are dominated by Euphorbiaceae with smaller legumes and laurel trees. The forest undergrowth is dominated by palms, particularly *Astrocaryum mexicanum* Liebm., in combination with vines, shrubs and weeds, among other herbaceous plants (Villaseñor *et al*. 2018).

**Sampling methods.** Four expeditions were conducted for two years. They were intended to cover the locality seasonality with one field trip during the dry season (16-22.V.2016), two field trips during the rainy seasons (16-22.VIII.2016 and 20-27.XI.2017) and the last one at the end (9-16.II.2018). Spiders were collected in a 1 ha square plot with central coordinates 18°34'56.1"N, 95°4'32.1"W and 205 m of average elevation (range from 172 to 217 m). The plot was traced with the following compass orientation, 300 to 120 and 210 to 30 degrees. The undergrowth vegetation was heavily dominated by *A. mexicanum*, as well as species of Araceae. The upper canopy vegetation included several tree species of over 30 m in height and with buttresses several meters wide, as well as other relatively large trees of over 15 – 20 m in height which formed a dense middle canopy (R. Coates, pers. com.). A creek that runs through the plot was included to represent this common habitat throughout the tropical forest. This plot was subdivided in sixteen 625 $m^2$ subplots to organize 48 pitfall traps as a grid, using a mixture of 10% glycerin and 90% alcohol to reduce evaporation. Originally, an alternating pattern between subplots was intended, but terrain features imposed some changes. The logic behind this grid was to distribute the pitfall traps as homogenously as possible throughout the plot (Fig. 1).

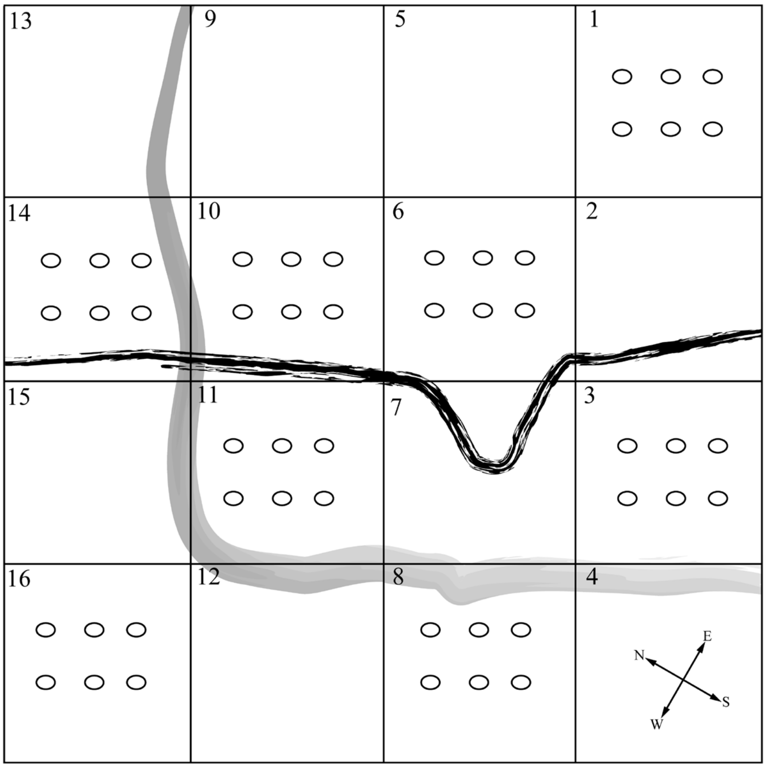


**FIGURE 1.** Diagram for the one-hectare plot sampled and its 16 subdivisions. Grey path indicates the creek that ran throught. Black path indicates the access trail. Ovals indicate the position of the pitfall traps.

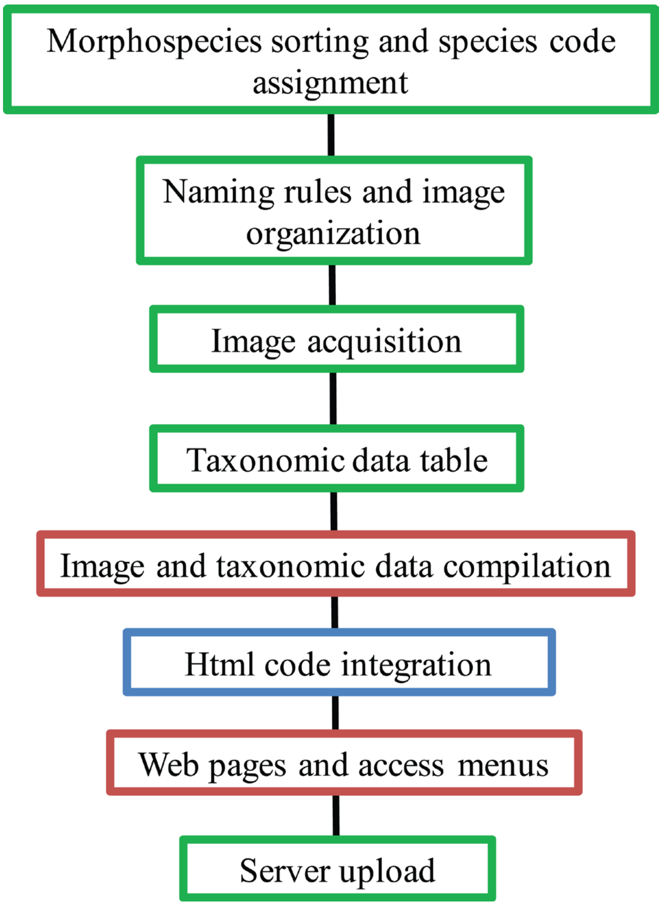


**FIGURE 2.** Schematic diagram for website protocol. Green squares indicate that the data are provided by the user. Red squares indicate data produced by Perl. Blue squares indicate data produced by Excel.

Six sampling methods were applied to cover most of the microhabitats: looking up (LUP), looking down (LUD), cryptic searching (CRP), beating (BEAT), Berlese funnels (BERL) and pitfall traps (PF) following Scharff *et al*. (2003) definitions. Berlese funnels extracted specimens from a volume of 1.5 L of sifted leaf litter, left to dry under incandescent light bulbs of 60 watts for four days. A total of 48 pitfall traps were installed in each fieldtrip. Five to six collectors participated on each expedition working six to seven hours per day inside the plot. The effort unit per sample was one hour / person for looking up, looking down, cryptic searching, and beating. The first two methods were carried out at night and the remaining four during the day. A total of 220 samples were planned for each season distributed in 40 replicates for the direct collecting methods (160 samples), 48 pitfall traps and 12 Berlese funnels. The obtained number of samples is presented in Table 2. Samples were labeled with expedition code, collector, method, replicate number and preserved in 96% ethanol. It is important to mention that 10 samples per season of the looking down method were done during the day to capture jumping spiders and other ground species not active at night, but not differentiated from the total. Beating at night, a recommended method for some tropical forests (Cardoso 2009; Malumbres-Olarte *et al*. 2017) was not applied.

All spider specimens were collected within the Los Tuxtlas Tropical Biology Field Station premises and will be deposited in the Colección Nacional de Arácnidos which is part of the Instituto de Biología at UNAM. All specimens of described species will be deposited upon publication; putative new taxa will also be donated to this collection after formal descriptions are published.

**Biodiversity website protocol.** The objective of this protocol was to make inventory websites almost automatically. It systematizes the acquisition of standard views to create web pages by combining data from three sources: the image file names, the species folder organization, and a table with taxonomic data for these species or morphospecies. It uses a MS-DOS batch file that coordinates two scripts written in Perl that transform these data into a format that Excel formulas read and incorporate the HTML code. The last and third script uses the result of the Excel formulas to make the species individual pages and their access menus. Pages not done automatically are the Welcome page for the inventory and two more html files for website control. All scripts, Excel templates, control pages and the user manual are available at http: //www.unamfcaracnolab.com/cyberINT.html. The user manual provides an extensive explanation of how to modify these scripts for different inventories. The user needs to understand MS-DOS, HTML and Excel at a basic level and only five functions in Perl to modify all code as desired. The workflow for website construction can be summarized in the following seven steps (Fig. 2).

*1) Morphospecies sorting*: A morphospecies is defined as a set of specimens deemed to belong to the same species based on similar genital features, therefore excluding immature specimens. Specimens of different sexes are matched by taking into account somatic features and/or phenologies. The following sequence of steps reduces considerably the time doing this task: the first sample is revised, and the immature specimens accounted for. The adult specimens are separated to a first morphospecies approximation with splitting as the desired rule for sorting given that it is easier to fuse vials with the same morphospecies in a jar, rather than revise a given vial with many lumped taxa. At this first morphospecies approximation all specimens are identified only to family. Each consolidated family is submitted to a second morphospecies approximation and the vouchers are assigned. At this point no images have been taken as the most important step here is to thoroughly compare each specimen side by side with the designated voucher morphotypes (or a sample of them). This second approximation result in the total number of morphospecies for each family. At the end of this part, the species codes are assigned, documented with images and identified to genus or species. For families rich in species and/or specimens, a series of rough sorting can be applied (e. g., by color) before the second morphospecies approximation.

*2) Naming rules and image organization*: These rules use the number and relative position of the characters in the image file name to code the standard views metadata. They were created by the Planetary Biodiversity Inventory (PBI) of the spider family Oonopidae (The Goblin Spider PBI 2019) that with only four letters (three coding the body part and the fourth giving the surface orientation) unambiguously named 810 "standard views" of all exhaustively surveyed anatomy. Fifteen of these standard views were selected to maximize the acquisition of morphologic data and at the same time reduce the number of images required. The specimen general morphology was covered with four standard views, one for the prosoma anterior surface and three for the habitus ventral, dorsal and lateral sides. Epigyna were documented with one to five images covering the ventral, lateral and posterior surfaces; with the last two images recording either the dorsal or apical views. The male pedipalp was documented with three to six standard views recording the ventral, prolateral and retrolateral surfaces; the last three images documented either the apical, dorsal or posterior views if required. These metadata were encoded in the file name as follows:

‘Mysmesp002mppalrTXV087_200X_E2B.jpg’ would be equal to *Mysmena* sp02, male specimen, digital picture of a palp in retrolateral view from the specimen voucher number TXV087 at 200X, taken with the workstation E2B and it is a jpg file. The image folder organization contains the names for families, genera and species presented in the web pages; for example: ./Mysmenidae/Mysmena/sp02 corresponding to this file name example. The user can modify these items with the required modifications in the Perl scripts as explained in the User Manual. Beyond the family folders structure there are two more directory layers: one that includes all the high-resolution images and the other is the main folder for the inventory website.

*3) Image acquisition*: All images were taken with the following Nikon equipment. The first workstation has two digital cameras a DS-Fi1 connected to the dissecting microscope SMZ1270 and a DS-Fi2 connected to the glass slides microscope E200, both cameras are controlled with a desktop Dell Inspiron 660s. The second workstation has a SMZ1000 and E200 microscopes, both sharing a DS-Fi3 connected to a HP Slimline 270-a0xx. Illumination was provided with custom made lamps made of two 1.5 watts LEDs of 2 cm in diameter. The LEDs are mounted at the end of flexible tubes on aluminum heads. For complex structures measuring less than 0.2 mm, the E200 microscopes were used with illumination coming from the top. For magnifications of 200X, tiny structures were fixed to an excavated glass slide, closed with a glass slit and placed over a white paper sheet. For 100X magnification, a deeper plate was used without cover slit. All individual pictures at different focal depths were acquired manually in TIFF format. The compound digital images were stacked with Helicon Focus 6.8.0 using the following parameters: radius15, smoothing 4 and rendering method B (depth map). Individual number of images varied from 12 to 60 (25 average) increasing on number with the depth, magnification and complexity of the structures.

*4) Taxonomic data table*: This information is provided by the user. The table dimensions are the number of observed species as rows and twelve columns with the following data: family name, species name, species code formed by the first six letters of the family name and a unique number for each taxa, World Spider Catalog unique reference number (LSID) of the taxonomic rank identified, if identified to species the author and year, specimen image voucher codes, locality and collecting data for these vouchers, taxonomic identification author, number of female specimens, number of male specimens, taxonomic notes and image acquisition author. The species names inside this file must match the family, genus and species names in the folders described above. The species codes are kept independent of these Linnaean ranks identifications to allow comparisons of new or taxonomically challenging morphospecies with other studies.

*5) Image and taxonomic data compilation*: The image metadata are extracted by the names and folder organization with a MS-DOS batch file. The first Perl script reads the file IMGN.txt produced and creates the first 24 columns that the Excel sheet will use to incorporate the html code. Compilation of the taxonomic data table is done by a second Perl script that produces the last 11 columns for the same Excel sheet.

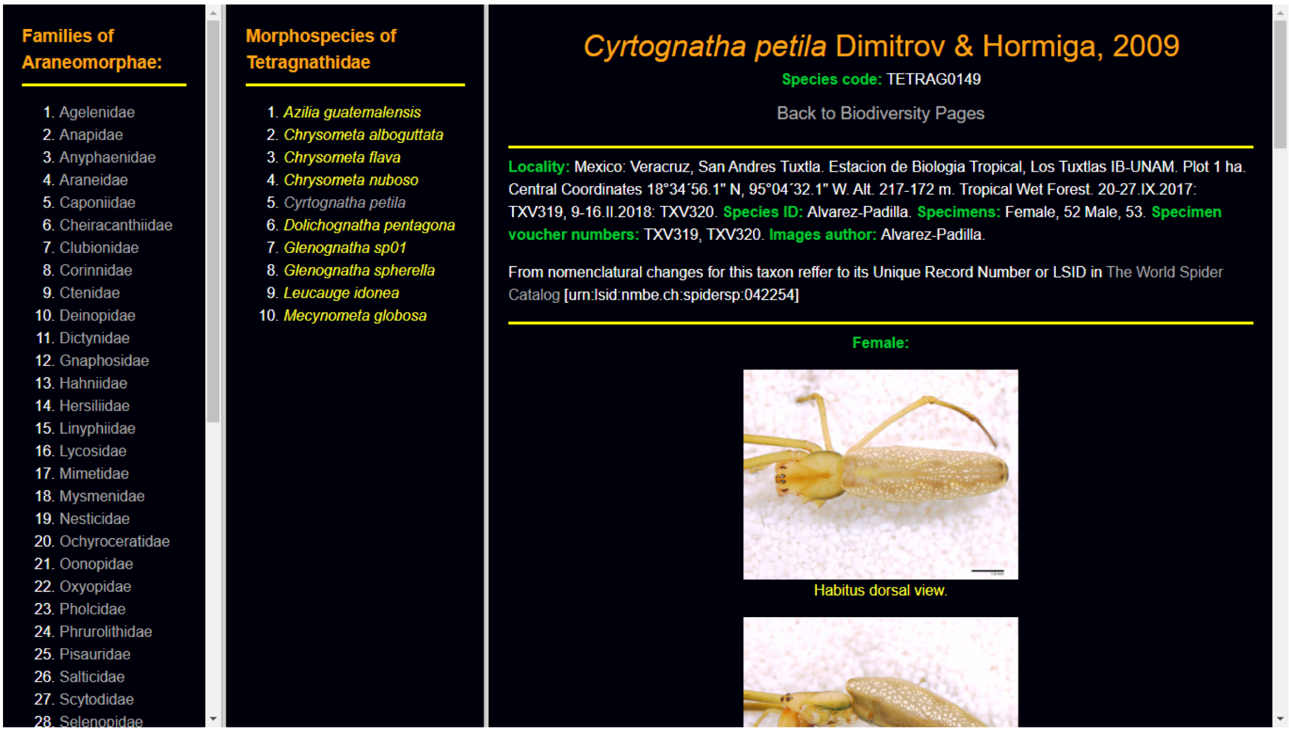
*6) HTML code integration*: The HTML code is written in an Excel workbook that contains four sheets connected with formulas. The data produced in the steps described above is pasted inside the “image data sheet”. The HTML code integration is done inside another sheet with 41 formulas most of them OR, AND or IF statements. The other two sheets make the species and family access menus with the same process.

*7) Species pages and server upload*: The results of the Excel formulas are read by the last Perl script to make the individual species pages and access menus. Thumbnail images are created in a copy of the high resolution (HR) folder differentiated only by the letters LR (or equivalent). All pages are static html files inside the folder structure described above and called by the index.html file in the server. The web site appearance is controlled by a single CSS file.

**Data analyses.** The species diversity of local community (alpha diversity) parameters were calculated and estimated with the following programs. Species diversity indices and non-parametric species richness estimations were calculated with 500 replicates in EstimateS 9.1.0 (Colwell 2019). The same non-parametric estimators; and fits for the Clench, Exponential, Rational and Weibull species extrapolation curves were calculated with R using the packages BAT (Cardoso *et al*. 2015), command *alpha.accum* and functions “nonparametric” and “curve” with 1000 replicates each, and the slopes for these curves were calculated with the command *slope*. The Preston’s lognormal model was used to calculate parametric species richness estimations (Longino *et al*. 2002; Scharff *et al*. 2003) with the R package vegan with two functions (Oksanen *et al*. 2017). The *prestonfit* function exploring the parameters tiesplit = TRUE that splits the taxa among adjacent octaves, and tiesplit = FALSE that take octaves frequencies without this split. The second function was *prestondistr* with the parameters truncate = -1 and truncate = 0 that move the modal octave (So) of the distribution form right or left from the center. This function can also accept values between and beyond this range; however, these variations make the estimated species vary from negative numbers to infinity. Completeness was calculated dividing the number of observed species over the average of all estimations. All these estimations were calculated for the total samples of the inventory, each of the four expeditions and the six collecting methods. Sampling intensity was calculated dividing the number of individuals over the number of species. Similarity was measured with the Chao abundance-based Jaccard index as implemented in SpadeR (Chao *et al*. 2005, 2015). The estimated proportion of shared species between all pairs of methods and expeditions was represented with two UPGMA trees. Shannon-Weaver diversity index and the Hutchison *t* test were obtained to assess if the pair wise differences in diversity between seasons and methods were statistically significant (Hutchison 1970). Optimal allocation of effort per methods was measured with BAT with 1000 replicates and the function *optim.alpha* (Cardoso *et al*. 2015). These last analyses were done for the total inventory and each of the expeditions. All analyzes were done with the datasets in Excel format available at www.unamfcaracnolab.com/WPGS_TUXV/tuxv.html.

## Results

**Los Tuxtlas Biology Station spider biodiversity website.** This website documented 237 species of Araneomorphae with 2,514 images organized by 281 web pages (Fig. 3). The number of pages corresponds to the sum of observed species, number of families and four pages for website control; therefore, the number of html files for any inventory can also be calculated. Each species had on average 10.5 standard views ranging from five, for species with only females, to 18 for species with both sexes present and complicated anatomy. The body regions more documented were the habitus (1,162), genitalia (953) and prosoma (384). The other 15 images correspond to nine species where the chelicerae (3), sternum (1), prosoma (1), and legs I, II and IV (10) presented diagnostic characters; these cases elevated the total number of standard views to 27.



**Families of Araneomorphae:**

1. Agelenidae
2. Anapidae
3. Anyphaenidae
4. Araneidae
5. Caponiidae
6. Cheiracanthiidae
7. Clubionidae
8. Corinnidae
9. Ctenidae
10. Deinopidae
11. Dictynidae
12. Gnaphosidae
13. Hahniidae
14. Hersiliidae
15. Linyphiidae
16. Lycosidae
17. Mimetidae
18. Mysmenidae
19. Nesticidae
20. Ochyroceratidae
21. Oonopidae
22. Oxyopidae
23. Pholcidae
24. Phrurolithidae
25. Pisauridae
26. Salticidae
27. Scytodidae
28. Selenopidae

**Morphospecies of Tetragnathidae**

1. *Azilia guatemalensis*
2. *Chrysometa alboguttata*
3. *Chrysometa flava*
4. *Chrysometa nuboso*
5. *Cyrtognatha petila*
6. *Dolichognatha pentagona*
7. *Glenognatha sp01*
8. *Glenognatha spherella*
9. *Leucauge idonea*
10. *Mecynometa globosa*

*Cyrtognatha petila* Dimitrov & Hormiga, 2009

**Species code:** TETRAG0149

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**Locality:** Mexico: Veracruz, San Andres Tuxtla. Estacion de Biologia Tropical, Los Tuxtlas IB-UNAM. Plot 1 ha. Central Coordinates 18°34´56.1" N, 95°04´32.1" W. Alt. 217-172 m. Tropical Wet Forest. 20-27.IX.2017: TXV319, 9-16.II.2018: TXV320. **Species ID:** Alvarez-Padilla. **Specimens:** Female, 52 Male, 53. **Specimen voucher numbers:** TXV319, TXV320. **Images author:** Alvarez-Padilla.

From nomenclatural changes for this taxon reffer to its Unique Record Number or LSID in The World Spider Catalog [urn:lsid:nmbe.ch:spidersp:042254]

**Female:**

Habitus dorsal view.

**FIGURE 3.** Example of a species page documented with standard views *Cyrtognatha petila* Dimitrov & Hormiga 2009. http://www.unamfcaracnolab.com/WPGS_TUXV/tuxv.html

Documentation of all species was completed in 141 days. Working time was divided in five days a week and six hours a day. The images were taken by three persons sharing two workstations. On average, one person (after two weeks of practice) documented 2.4 species a day represented by both sexes with 24 standard views. These 4.7 months were not continuous working time, continuous time was 87 days. Specimen sorting was on average 1.2 months per expedition (4 in total), adding to ca. ten months for sorting and species documentation. Regarding server space this website used 5.95 GB of memory. High resolution images used 95%, low resolution images or thumbnails 4.8% and html files 0.2%. The folder with the high-resolution images (equivalent to standard views) in addition includes most of the data and structure to make all these pages.

**Taxonomic section.** A total of 237 species in 40 families were collected and sorted. Of these, 146 taxa were identified to species (Appendix 1), collecting the complementary sexes for 17 of these taxa currently described only by one sex. Taxa only identified to morphospecies represent either new species or the taxonomy of that group is in disarray and reliable identifications were impossible. The species code is of particular importance for these taxa when comparisons with other inventories are intended. The World Spider Catalog online resources were fundamental to identify these species (World Spider Catalog 2019) with the additional help of five more arachnologists that used the standard views. The biodiversity website for this inventory was essential for Salticidae generic identifications, other families that also benefited from this website were Thomisidae and Trechaleidae. The time that took these identifications is included within the days spend in documenting these species with images.

**Spider community measurements.** These 237 species were identified from 8,655 adult specimens that represented 39% of all collected individuals. The total number of samples was 823 with an average of 205.75 samples per season (+/- 17.35) (represented by month names) and 137.17 samples per method (+/- 53.58). May was the most intensively sampled with 213 samples and February the least with 181; similarly, Berleses funnels and cryptic searching accounted for 34 and 164 samples, respectively. Abundances per month were on average of 2,164 specimens. May and February presented the lowest and highest abundances, respectively. Methods had 1,442.7 specimens on average with Berlese funnels and pitfall traps sharing the lowest values and looking up, beating and cryptic the highest with more than 2,000 specimens each. The species richness by method and season had averages of 163.75 (+/- 9.53) and 98.33 (+/- 54.48), respectively. The observed species versus seasonality were fairly homogeneous, contrary to the methods that can be divided in three categories: low richness methods with Berleses funnels (35) and pitfall traps (45), medium richness methods with cryptic searching and looking down with values around 95 species and looking up and beating as the high richness methods collecting 160 species on average (Table 1). Looking up and beating were the most efficient with 2.24 and 1.83 species per sample on average, followed by cryptic searching (1.38). The least efficient methods were looking down (0.72), pitfall traps (0.49) and Berlese funnels (0.22).

**TABLE 1.** Diversity Parameters related to Seasonality, Methods and Total

| | MAY | AUG | NOV | FEB | BEAT | BERL | CRP | LUD | LUP | PF | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Abundance | 1712 | 1965 | 2208 | 2770 | 2078 | 373 | 2004 | 1049 | 2650 | 501 | 8655 |
| Samples | 213 | 221 | 208 | 181 | 160 | 34 | 164 | 128 | 154 | 183 | 823 |
| Species observed | 151 | 164 | 166 | 174 | 147 | 35 | 98 | 92 | 173 | 45 | 237 |
| Singletons | 36 | 38 | 30 | 46 | 36 | 17 | 28 | 33 | 55 | 17 | 45 |
| Singletons overall | 6 | 13 | 9 | 17 | 11 | 1 | 1 | 2 | 26 | 4 | 45 |
| Doubletons | 23 | 20 | 28 | 17 | 16 | 2 | 16 | 19 | 24 | 1 | 17 |
| Completeness | 0.82 | 0.82 | 0.86 | 0.75 | 0.78 | 0.52 | 0.79 | 0.74 | 0.73 | 0.55 | 0.83 |
| Sampling intensity | 11.34 | 11.98 | 13.30 | 15.92 | 14.14 | 10.66 | 20.45 | 11.40 | 15.32 | 11.13 | 36.52 |
| Shannon | 4.12 | 4.22 | 4.31 | 4.22 | 4.08 | 2.4 | 3.48 | 2.95 | 4.11 | 2.82 | 4.44 |
| Alpha | 39.94 | 42.55 | 41.6 | 41.2 | 36.12 | 9.46 | 21.58 | 24.28 | 41.45 | 11.98 | 45.02 |

The percentage of singletons for the complete inventory was 19% and 7.17% of doubletons (Table 1). May, August and November singletons values oscillated around 34.67 (+/- 6.61) and February showed the highest value with 46. Doubletons values oscillated around 22 (+/- 4.69) for these three months with February presenting the lowest value with 17. The methods had on average 33.14 (+/- 13.88) singletons and 15.4 (+/- 8.17) doubletons. The methods targeting the ground resulted in more singletons in relation to the species that they collected. Berlese funnels had 48.57%, followed looking down with 35.87% and pitfall traps with 37.78%. The methods focused on vegetation had 24.5% for beating and 31.79% for looking up. Cryptic searching had the second lowest singleton proportion at 28.57%. The collecting methods that resulted in more doubletons were looking up (24), followed by looking down (19), and cryptic searching and beating with 16. Pitfall traps and Berlese funnels had one and two doubletons, respectively (Table 1). These results may imply that more effort is needed for three of the ground methods; however, their contribution to the overall singletons is considerably lower. These values clearly separate the methods in those directed to the vegetation with a contribution of 11 and 26 singleton species globally, contrary to the ground methods with a maximum of four. The overall singletons highest values were for August and February with 13 and 17, respectively. May and November presented the lowest values with 6 and 9 specimens of this kind (Table 1).

Inventory completeness evaluated with non-parametric estimations was at 83%, however if the lognormal estimations are considered this value dropped to 79%. Seasonality values varied from 86% in November to 82% for May and August and 75% for February. The results split in two groups the methods with values around 50% for Berlese funnels and pitfall traps, and 75% on average for the other methods. Sampling intensity for the total inventory was 36.52 for adult specimens. The methods and months have individually lower sampling intensity values ranging from 11.34 to 15.92, with the highest value for cryptic searching with 20.45. Shannon and Alpha diversity indices were homogeneous across months, while methods were separated in high diversity values above 4.08 for vegetation and 2.73 on average for ground methods except for cryptic searching with an intermediate value of 3.48 (Table 1). Nonparametric species estimations for the complete inventory varied from 311.89 species for Jack 2 based on incidence with 321.18 species as its 95% confidence interval, to 257.29 from Bootstrap. Parametric estimations based on the normal distribution oscillated from 261.5 to 423.1 predicted by the Preston lognormal fit with the parameter truncate -1 and 0, respectively. The average of all these estimations was 285.41 species. Non-parametric estimators and fits to distribution curves converged on similar values for all months except February and for most of the methods excluding looking up, Berlese funnels and pitfall traps. Jack 2 based on incidence presented the highest non-parametric estimations for all, except Chao 2 in February and Chao 1 for pitfall traps and Berlese funnels. Estimations based on the lognormal distribution were very sensitive to values entered in the variables "tiesplit" and "truncate". Depending on these values they estimated from few species above the observed values, to several hundred (Fig. 4, values with *) or even infinite taxa (Fig. 4, red triangles at the bottom line). For this reason, the Preston lognormal model was not averaged for the completeness measurements. Lognormal estimations in the months of May and August also over estimated the number of species, regardless that these seasons had the highest stability for the other estimations, following a similar pattern for cryptic and looking down methods (Fig. 4).

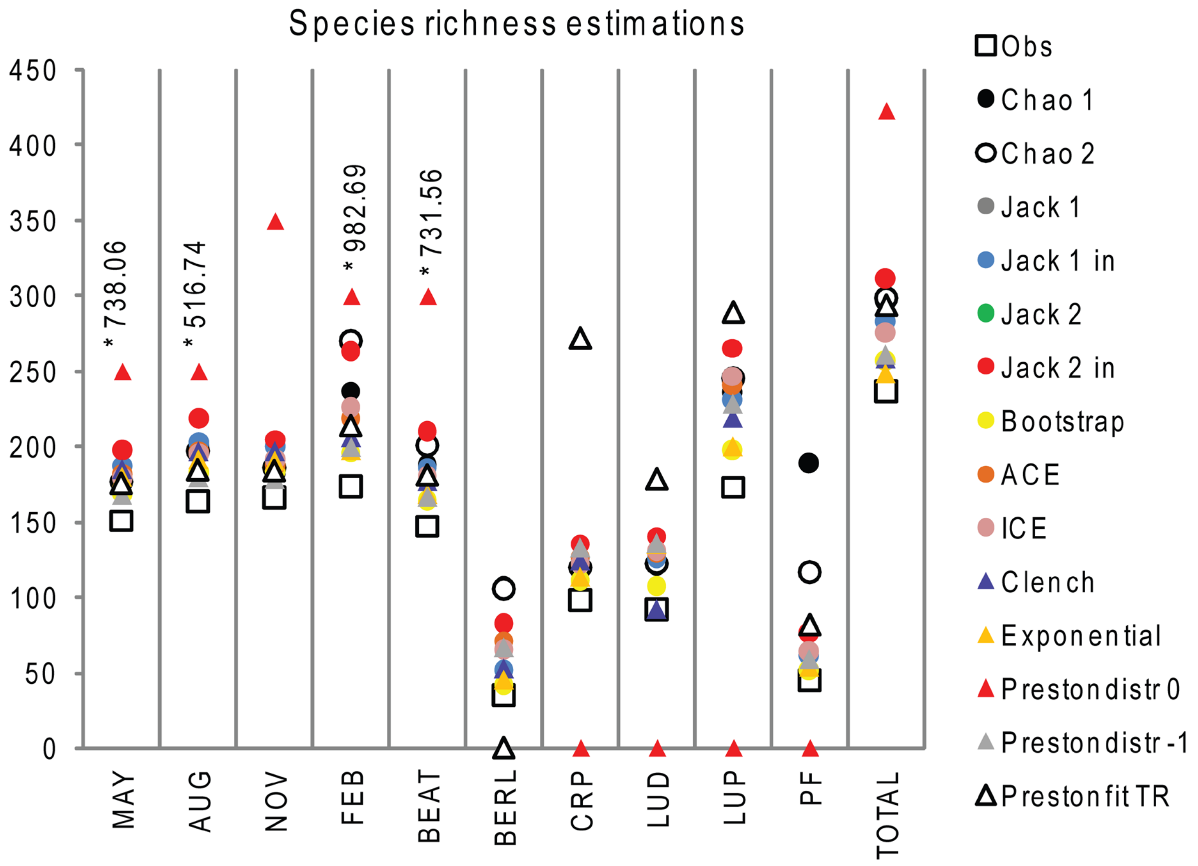


**FIGURE 4.** Species richness estimations. Red triangles with values indicated with (*) correspond to the species estimations for those treatments fitted to a lognormal distribution. White and red triangles over the x axis indicate higher overestimations.

All richness estimations slopes for the complete inventory were on average 0.005. Slopes below 0.01 were concentrated in the months of November for all estimations. August and May slopes also were on average below this threshold; but the Jack 1 estimations in May and the parametric estimators for both months presented grater values than 0.01. February presented considerably steeper slopes varying from 0.014 to 0.045 (Fig. 5, abundance and incidence slopes are combined and referred as Chaos). As for the methods cryptic searching was the only in which slope values oscillated closer to 0.01. The steepest slopes were for pitfall traps with its peak at 0.529 for the Chao estimators and an average of 0.167, followed by Berlese funnels with an average of 0.082, beating and looking up share a slope of 0.23. Looking down slopes oscillate from negative values in the Chaos estimations, to 0.25 as its maximum and 0.14 on average (Fig. 5).

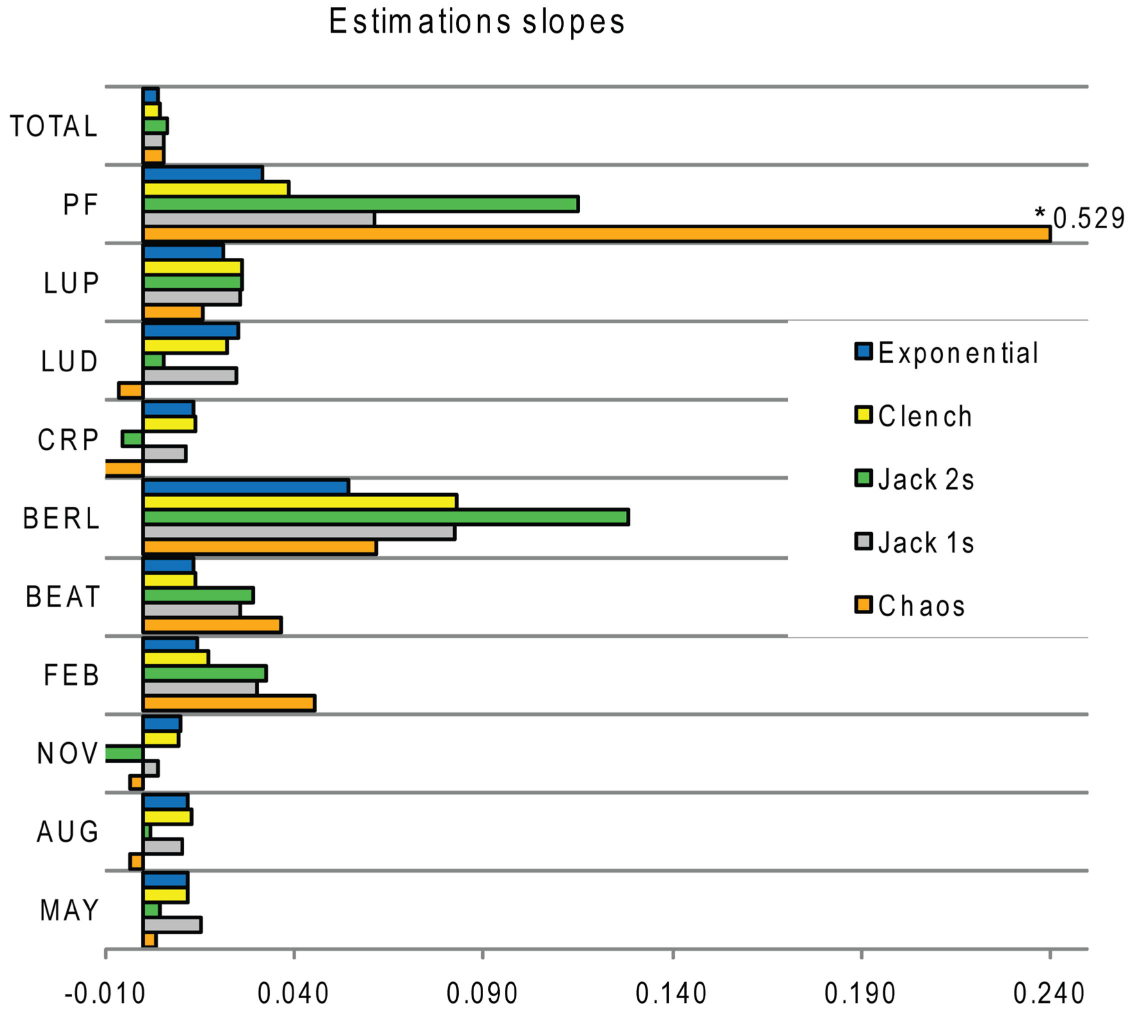


**FIGURE 5.** Species richness estimations slopes. Jack 2s indicates the slope average for these estimations based on incidence (BAT) and abundance (Bat and Estimates). Same averages were calculated for Jack 1s and Chao 1 and 2 estimation slopes. Column indicated with (*) correspond to the slope value for pitfalls under Chao's estimations.

Species similarity analyses with the Jaccard differences were depicted as UPGMA distance trees. For the seasonality the total length difference was 0.0825 with 106 species present through the year (* in Appendix 1). Two season clusters (May-Aug and Nov-Feb) were recovered with 0.027 differences of length (p value 0.002) and Shannon diversity values of 4.29 and 4.37, respectively. The monthly differences ranged from 0.02 to ca. 0.06 (Fig. 6B). The methods similarity analyses had a length of 0.7070 with only five species shared by all (+ in Appendix 1). Two clusters clearly separated vegetation from ground targeting methods with 0.52 of difference and Shannon diversity indices of 3.66 and 4.24 (p value 0.00). However, the differences dropped to 0.073 on average within the ground species and 0.018 between looking up and beating. The overall methods differences were from 0 to 0.883 (Fig. 6A). Shannon index for Xilitla and Los Tuxtlas was 4.04 and 4.44 (p value 0.00), respectively, with differences of

0.77 in length. Forty-seven species are shared between both localities, of which six morphospecies were possible to compare because both inventories have biodiversity websites.

**Number of samples and optimization of collecting effort:** The present inventory was defined as a standardized protocol since the sampling units of effort and area delimitation were applied to every season without any variation among them. The samples obtained per season differed from the planned number (220) whether adult specimens were collected (cases below 40) or changes in the planned logistics (cases above 40). February was the only month that differed considerably in its number of samples, in particular for looking down (15), cryptic searching (46) and Berlese funnels (NA) (Table 2). The low numbers of looking down samples were due to the saturated soil during the rainy season; consequently, more effort was allocated to cryptic searching. Despite that the number of obtained samples per method per season varied, no significant differences were observed among them (ANOVA, p value 0.509 between rows and 0.079 for columns excluding Berlese funnels). The sample average per method was 37.78 (+/- 11.63) and for season 205.75 (+/- 17.35, p value 0.142).

**TABLE 2.** Obtained samples and optimization of collecting effort.

| **Total samples** | **BEAT** | **BERL** | **CRP** | **LUD** | **LUP** | **PF** | **Samples** | **SPP** |
|---|---|---|---|---|---|---|---|---|
| MAY | 39 | 12 | 40 | 37 | 37 | 48 | 213 | 151 |
| AUG | 41 | 10 | 40 | 41 | 42 | 47 | 221 | 164 |
| NOV | 39 | 12 | 38 | 35 | 35 | 48 | 207 | 166 |
| FEB | 40 | NA | 46 | 15 | 41 | 40 | 182 | 174 |
|  | 159 | 34 | 164 | 128 | 155 | 183 | 823 | 237 |
| **36 samples** | **BEAT** | **BERL** | **CRP** | **LUD** | **LUP** | **PF** | **SPP** | **% of Obs.** |
| MAY | 5 | 2 | 9 | 2 | 18 | 0 | 93.17 | 61.7 |
| AUG | 8 | 0 | 9 | 1 | 18 | 0 | 105.52 | 64.33 |
| NOV | 13 | 0 | 7 | 2 | 14 | 0 | 111.09 | 66.92 |
| FEB | 6 | NA | 10 | 1 | 19 | 0 | 117.79 | 67.7 |
| **96 samples** | **BEAT** | **BERL** | **CRP** | **LUD** | **LUP** | **PF** | **SPP** | **% of Obs** |
| MAY | 28 | 12 | 14 | 5 | 37 | 0 | 133.61 | 88.5 |
| AUG | 20 | 0 | 27 | 7 | 42 | 0 | 148.91 | 90.11 |
| NOV | 34 | 2 | 16 | 7 | 35 | 2 | 151.59 | 91.32 |
| FEB | 30 | NA | 23 | 2 | 41 | 1 | 158.96 | 91.36 |

The distribution of optimized samples among methods (36 or 96 Table 2) was almost homogeneous for all months allocating most effort to looking up that the other, except November in which the beating samples were almost equal. Beating and cryptic searching were the other methods that concentrated more effort, however, the number of samples between them oscillated depending of the season or the optimizations; allocating more samples to cryptic searching than beating when 36 samples were optimized and vice versa when 96 samples were considered. Looking up could require even more effort, given that the optimization values at 96 samples were the same as the total number (35 to 42). The optimizations for looking down resulted from one to seven whether 36 or 96 samples were considered. Berlese funnel samples are low except during May and 96 samples optimized (12), all other optimizations were either zero or two. Pitfall traps were almost excluded. The number of species collected have on average 106.89 (+/- 10.43) with 36 samples and 148.27 (+/- 10.66) with 96 samples, similarly, the percentage of observed species varied from 64.92 (+/- 3.15) to 90.32 (+/- 1.35) (Table 2).

## Discussion

**Los Tuxtlas Biology Station spider biodiversity website.** One of the main problems for tropical biodiversity inventory comparisons are the partially identified and new species. All those "sp01, 02, 03" are impossible to compare without examination of the specimen reference or asking to the specimen holder for images. The main contributions of these websites to biodiversity, taxonomy, community ecology and conservation are allowing comparisons of undescribed species with high quality data, helping taxonomists doing revisionary work to identify new species and request the specimens, and providing evidence of the identification accuracy. Furthermore, these digital images organized as standard views and linked to species codes allow species comparisons between geographic areas regardless of their taxonomic status (Miller *et al*. 2014) assisting in conservation efforts and beta diversity comparisons.

There are only three websites that document spider biodiversity inventories with standard views, of which two remain live. The first for South East Asia with its base in the Netherlands (Miller http: //www.digitalspiders.org/) and the second for araneomorph spiders from Mexico (Alvarez-Padilla, http: //www.unamfcaracnolab.com/).

The objective for this protocol was to expedite the image acquisition process and make almost automatic the website creation for any spider inventory. This protocol uses data provided by the user while taking the images and organize it with scripts in standard views and html static pages. The taxonomic data table, naming rules and image organization are different ways for encoding the image metadata that these scripts will use. However, these types of metadata encoding and their transformation with Perl scripts can be compiled in a program with a user interface such as EstimateS (Colwell 2019). These will reduce the protocol steps to only four and would simplify greatly the species renaming process. Currently, name updates need to be changed in three places: the taxonomic data table, the folder names, and if a new name is added, the folder organization. Following these changes, the scripts have to be run again for all species, or selected taxa, to make the new pages.

The most labor and time intensive steps of this protocol are specimen sorting and image acquisition. These two steps accounted for almost ten months of work and they require most of the care and concentration. The image acquisition step is also intended to demonstrate that no expensive equipment is needed to achieve excellent image quality. Each workstation described in the Methods section is equivalent in price to a mid-size car in Mexico. The step of naming rules and folder organization takes only a few seconds per species and standard view but is of particular importance to avoid typos in the image names. Such mistakes can be discovered and fixed as explained in the user manual, the present inventory had 2.6% of names with errors and were easily fixed; however high percentages of errors may defeat the purpose of doing automatically the html code and image metadata integration.

These results, in addition to the four weeks for field collecting, represent an example that in less than two years and with economically accessible equipment it is possible to thoroughly document 237 species of spiders from a tropical wet forest hectare.

**Taxonomic section.** The discovery of new species is expedited for those taxa that have taxonomic revisions; on the contrary, species groups in disarray will introduce ambiguity between new and already described taxa slowing the discovery process. Since 2014, the online resources provided by The World Spider Catalog have enormously expedited species identification for taxa with good quality original or subsequent descriptions (World Spider Catalog 2019). Species identification in this context can be considered as a first approximation of the taxonomic organization in a particular taxon; however, all this information is not published as part of biodiversity inventories. We consider that the inclusion of these data increases considerably the utility of biodiversity inventories for the taxonomic community. For that reason, we discuss the issues found while identifying the genera and species within the 40 families collected (Appendix 2).

**Spider community measurements.** A comparison of 19 spider inventories worldwide (Table 3) indicate that the richest forests are in the Neotropics with an average of 270.43 observed species per hectare; however, they present a large standard deviation +/- 118.70 with Peru and Brazil as the richest places with 498 and 391 species, respectively. The completeness of these inventories has on average 68% with a standard deviation of +/- 12%; with the most complete inventories at 83% (Bonaldo & Dias 2010; Castanheira *et al*. 2016; Coddington *et al*. 1991, Coddington *et al*. 2009; Ricetti & Bonaldo 2008; Rivera-Quiroz *et al*. 2016; Silva-Davila & Coddington 1996). Paleotropical forests have on average 215.03 +/- 121.71 species, with a completeness of 75.29% +/- 5.9% (Malumbres-Olarte *et al*. 2017, 2018; Miller & Pham 2011; Sørensen 2004; Sørensen *et al*. 2002). African forests are richer (215.45 species) that the South East Asian counterparts (104) (Miller & Pham 2011). However, these numbers should be taken cautiously because the spider biodiversity of many tropical regions remains poorly explored and in urgent need to be systematically sampled, described and measured. Holarctic forests present the lowest richness with 115.24 +/- 51.39 species, but the highest completeness values of 81.856% +/- 8.72 (Cardoso *et al*. 2008a, 2008b, Cardoso *et al*. 2017b; Coddington *et al* 1996; Scharff *et al*. 2003). These values are also in agreement with recent analyses based on taxonomic data for spiders (Piel 2018).

**TABLE 3.** Comparison of 18 spider standardized inventories.

| STUDY | REGION | VEGETATION | AREA | PLOTS | SPP | SPP/ AREA | EST. MEAN | COMPL. |
|---|---|---|---|---|---|---|---|---|
| Bonaldo & Dias 2010 | Neotropical | Tropical forest | -- | -- | 393 | -- | 639 | 61.50% |
| Cardoso *et al*. 2008a | Holarctic | *Quercus* forest | 10,000 | 1 | 150 | 150 | 168 | 89.29% |
| Cardoso *et al*. 2008b | Holarctic | *Quercus* forest | 10,000 | 1 | 185 | 185 | 214 | 86.45% |
| Cardoso *et al*. 2017b | Holarctic | Coastal forest | 2,500 | 4 | 104 | 104 | -- | -- |
| Castanheira *et al*. 2016 | Neotropical | Tropical forest | 400** | 1 | 353 | -- | 425 | 83.06% |
| Coddington *et al*. 1991 Estación Beni | Neotropical | Tropical forest | 10,000 | 1 | 191 | 191 | 315 | 60.63% |
| “” Cerro Uchumachi | Neotropical | Tropical forest | 10,000 | 1 | 151 | 151 | 232 | 65.09% |
| “” Rio Tigre | Neotropical | Tropical forest | 10,000 | 1 | 259 | 259 | 440 | 58.86% |
| Coddington *et al*. 1996 | Nearctic | Hardwood forest |  | 1 | 89 | 89 | 128 | 69.53% |
| Coddington *et al*. 2009 | Neotropical | Tropical forest | 10,000 | 1 | 352 | 352 | 464 | 75.86% |
| Coddington *et al*. (unpub data) | Paleotropical | Tropical forest | 10,000 | 1 | 230 | 230 | 347 | 66.28% |
| “” Cameroon | Paleotropical | Tropical forest | 10,000 | 1 | 55 | 55 | 68 | 80.88% |
| Coddington *et al*. (unpub data) | Paleotropical | Tropical forest | 10,000 | 1 | 249 | 249 | 320 | 77.81% |
| “” Madagascar | Paleotropical | Tropical forest | 10,000 | 1 | 195 | 195 | 237 | 82.28% |
| Hormiga & Coddington 1994 | Neotropical | Tropical Forest | 10,000 | 1 | 98 | 98 | -- | -- |
| Malumbres-Olarte *et al*. 2017 Plot I | Paleotropical | Montane forest | 2,500 | 1 | 125 | 500 | 185 | 67.57% |
| “” Plot II | Paleotropical | Montane forest | 2,500 | 1 | 92 | 368 | 119 | 77.31% |
| Malumbres-Olarte *et al*. 2018 High | Paleotropical | Montane forest | 12,500 | 5 | 311 | 248.8 | 386 | 80.57% |
| “” Low | Paleotropical | Lowland forest | 12,500 | 5 | 305 | 244 | 398 | 76.63% |
| “” Medium | Paleotropical | Submontane forest | 12,500 | 5 | 307 | 245.6 | 418 | 73.44% |
| Miller *et al*. 2014 Cat Ba | Paleotropical | Tropical forest | 10,000 | 1 | 108 | 108 | 154 | 70.13% |
| “” Cuc Phuong | Paleotropical | Tropical forest | 10,000 | 1 | 76 | 76 | 98 | 77.55% |
| “” Vu Quang | Paleotropical | Tropical forest | 10,000 | 1 | 128 | 128 | 194 | 65.98% |
| Ricetti & Bonaldo 2008 | Neotropical | Tropical forest | -- | -- | 427 | -- | 614 | 69.54% |
| Rivera-Quiroz *et al*. 2016 | Neotropical | Tropical forest | 10,000 | 1 | 205 | 205 | 265 | 77.36% |
| Scharff *et al* 2003 | Palearctic | Beech Forest | 10,000 | 1 | 66 | 66 | 81 | 81.48% |
| Silva & Coddington 1996 | Neotropical | Tropical forest | 10,000 | 1 | 498 | 498 | 773 | 64.42% |
| Sørensen *et al*. 2002 | Paleotropical | Montane forest | 10,000 | 1 | 148 | 148 | 181 | 81.77% |
| Sørensen 2004 | Paleotropical | Montane forest | 906** | -- | 149 | -- | 183 | 81.42% |
| This study | Neotropical | Tropical forest | 10,000 | 1 | 237 | 237 | 286 | 82.87% |

Footnote: Inventories marked with double dashes (--) did not mention the area sampled, or the area was delimited only for some of the sampling methods. Inventories marked with * did not make clear the sampled area, but one hectare was established by comparison with other studies from the same authors. Inventories marked with ** make the sampled area explicit, but linear extrapolations to one hectare were impossible due to their small size. SPP = observed species, SPP/AREA number of observed species fitted to one hectare, EST. MEAN = Average for the species richness estimations for that study, COMPL = Inventory completeness calculated as SPP/EST. MEAN.

As expected for the Neotropical region, the present inventory collected 237 species with a completeness of 82.87%; however, this percentage must be considered only for a volume of 20,000 m$^3$ considering two meters as the maximum sampling height. The species richness estimations per sampling methods predict that the remaining 25 to 186 will be canopy dwellers. These results are also consistent with the high numbers of overall singletons found in the methods targeting the vegetation (Table 1). These singletons correspond to 12 families that usually are present on canopy fauna from different tropical forests: Araneidae, Salticidae and Theridiidae are the most diverse (Battirola *et al*. 2016; Hofer *et al*. 1994; Sørensen 2004; Zheng *et al*. 2015). Therefore, the implementation of additional sampling techniques such as canopy fogging (e. g., Allison *et al*. 1997; Erwin *et al*. 2005) would sample the missing vegetation strata. An example of canopy fogging is the spider inventory conducted in a Tanzanian montane forest, in which 149 species with 81.42% of completeness (Sørensen 2004). These species were captured using eleven samples placed around large trees selected within 25 hectares, and with a combined area of 906 m$^2$. A very crude and speculative guess to the present study will be around 380 species of spiders for one hectare of a low altitude tropical wet forest including the canopy.

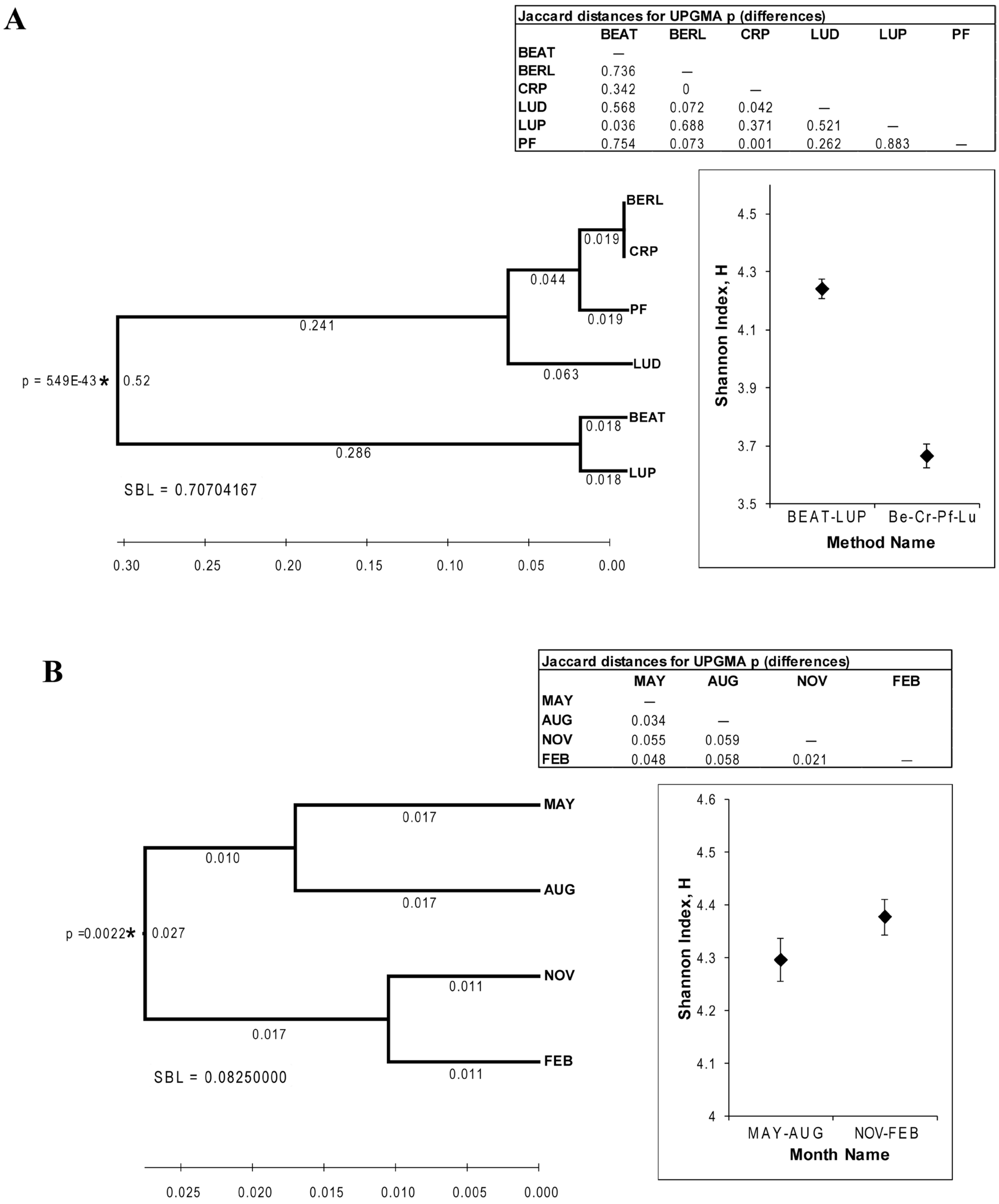
Similarity analysis for sampling methods revealed variations in taxonomic composition between methods applied to the ground and vegetation. The Hutchinson *t* test resulted in significant differences for most of the comparisons, except looking up-beating and looking down-pitfalls assessments. Nevertheless, the most evident dissimilarities are those between the ground and vegetation methods, in which the Shannon index values were also statistically distinct. The high differences between these two clusters (0.52) are because only five species were shared by all methods (Fig. 6A, Appendix 1). This is consistent with other inventories applied to temperate and tropical ecosystems where the samples targeting vegetation and the methods focused in the ground or cryptic habitats are generally separated in two groups (Coddington *et al*. 1996; Miller & Pham 2011; Toti *et al*. 2000).

The recommended time of the year for collecting spiders has been after the rainy season. The ecosystem productivity dynamics and evidence provided by these studies strongly support this argument (Malumbres-Olarte *et al*. 2017; Sørensen *et al*. 2002). In Los Tuxtlas, a similar result was found with February the more species rich month just at the end of the rainy season; however, we would recommend collecting at least another time through the year based on the seasonality of the area. The similarity results showed that the species composition through time is barely different between November and February. The species composition between the dry season and the beginning of the rainy season forms a second cluster (Fig.6B: 0.027). If only one month within these two-time clusters is sampled, then approximately 95% of the observed species would be present; however, if only February is sampled then this number drops to 73% (Table 1).

**Number of samples and optimization of collecting effort:** A quasi-optimized protocol for tropical forests named COBRA-TF has been recently proposed and successfully tested (Malumbres-Olarte *et al*. 2017, 2018). This protocol distributes the collecting effort by method in function of its efficiency to capture species and recommended 36 samples to achieve accurate community measurements in plots of 0.25 ha. The COBRA-TF protocol fixed the number of samples per method, allocating most of the sampling effort to vegetation methods, referred by these authors as "aerial night" and "beating during the day", but with a chance to incorporate additional samples to any other method. Beating during the night was also recommended with an effort similar to that of looking down or ground collecting at night. Cryptic searching during the day was the most efficient method to sample ground vegetation, leaf litter, under rocks and small crevices. The contribution of pitfall traps, looking down at day, cryptic searching at night and looking up at day was very small for the analyzed tropical ecosystems; however, these methods are necessary to compare with other studies, mainly because they collect species missed by other methods (Malumbres-Olarte *et al*. 2017, 2018).

The optimized samples per season were considering 36 or 96 samples. The results of the optimization with 36 samples was very similar to those obtained by COBRA-TF for tropical ecosystems (Malumbres-Olarte *et al.* 2017) in terms of proportion and distribution of the samples per method and provided 60.71% to 67.7% of the observed species per month. Three quarters of the sample distribution rows found in Los Tuxtlas seasonality (Table 2) were the same to those of the Guyana forest (Malumbres-Olarte *et al.* 2017: 500, table 2) with the exception of more samples for cryptic searching. Also, the samples distribution of November was more similar to the African forests suggesting a similar species turnover through time as in space. The 96 samples version resulted in similar collecting effort distributions except in the beating/cryptic searching and Berlese funnels proportions and collected 88.5% to 91.36% of the observed species (Table 2). In both cases, these samples would be enough to collect the 106 species present year-round if the similarity analyses are considered among seasons (Fig. 6B, Appendix 1).

The optimized samples for the methods targeting the vegetation concentrated most of the effort as in previous studies. Likewise, the optimization results for the ground methods indicated that cryptic searching during the day and looking down during the night were the most efficient methods. Pitfall traps were almost excluded in other tropical forests (Malumbres-Olarte *et al.* 2017: 500, table 2); but optimized as the most efficient method in oak forests (Cardoso 2009). Pitfall traps and Berlese funnels were considered as the lowest efficiency methods, but they collected five overall singletons in the equal effort sampling (Table 1). Berlese funnels were optimized as an efficient method with 12 samples only during the dry season (May), therefore it is important to consider this method or replace it with more samples of cryptic searching at this time of the year when most of the leaf litter accumulates (Table 2). This replacement is also supported by high similarity of both methods differing only in one species (Fig. 6A).



Jaccard distances for UPGMA p (differences)

| | BEAT | BERL | CRP | LUD | LUP | PF |
|---|---|---|---|---|---|---|
| BEAT | — | | | | | |
| BERL | 0.736 | — | | | | |
| CRP | 0.342 | 0 | — | | | |
| LUD | 0.568 | 0.072 | 0.042 | — | | |
| LUP | 0.036 | 0.688 | 0.371 | 0.521 | — | |
| PF | 0.754 | 0.073 | 0.001 | 0.262 | 0.883 | — |

Jaccard distances for UPGMA p (differences)

| | MAY | AUG | NOV | FEB |
|---|---|---|---|---|
| MAY | — | | | |
| AUG | 0.034 | — | | |
| NOV | 0.055 | 0.059 | — | |
| FEB | 0.048 | 0.058 | 0.021 | — |

**FIGURE 6.** Methods and seasonality diversity indices and Jaccard similarity analyses. A, similarity and Shannon diversity indices for methods. B, similarity and Shannon diversity indices for the collecting months representing seasonality.

## Conclusions

The goal of the described protocol is to facilitate the online documentation of tropical spider inventories. Currently only two active websites that document this biodiversity exist. This protocol aims to increase this number by systematizing the standard view acquisition and making almost automatic the website creation. This is particularly important to compare the biodiversity across tropical regions where most of the species are either new or difficult to identify. The contributions of these websites for biodiversity, community ecology and conservation are allowing comparisons of undescribed species between areas. For taxonomy, they can expedite revisionary work by helping researchers to identify target taxa and request the specimens. It is important to mention that approaches that promote taking the publication of images from databases and photo-based descriptions as a replacement to species descriptions based on type specimen(s) are misguided (Marshall & Evenhuis 2015). Such approaches functionally dismiss the most important biological empirical evidence, the specimen(s) itself (Amorim *et al*. 2016; Krell & Wheeler 2014). Biodiversity inventory websites must assist in the publication of taxonomic descriptive work abiding the International Code of Zoological Nomenclature rules (ICZN 1999).

Biodiversity inventories have been concentrated in describing community parameters, but their taxonomic counterpart never goes beyond the species list with their relative abundances in the best cases (Appendix 1). The taxonomic section presented in Appendix 2 has the objective to facilitate revisionary work by: discussing nomenclatural issues, pointing out possible new species, and indicating species groups where taxonomic work is required. We recommend including such a section in all future inventories. It is important to mention that since 2014 all taxonomic works are shared in the World Spider Catalog facilitating species identification; we would like to encourage the arachnological community to use these resources while documenting inventories.

The optimization of samples for Los Tuxtlas was similar to the obtained for other tropical forests. It is recommended to apply looking up (night), beating (day), cryptic searching (day) and looking down (night) as the preferred methods; in addition to beating at night and sweeping vegetation as recommended elsewhere (Malumbres-Olarte *et al.* 2017). The most productive field trip was after the rainy season; however, it is recommended an additional expedition either during the dry time of the year or at the beginning of the rainy season considering their optimal protocols. The optimization for 36 samples would provide data to accurately estimate and compare community parameters as implemented recently in Tanzania (Malumbres-Olarte *et al.* 2018). Furthermore, this optimization would allow splitting the sampled hectare in at least eight separated 2,500 m$^2$ plots evaluating also the beta diversity of Los Tuxtlas Station. However, we recommend the 96 samples optimization if the inventory online documentation is preferred. From a taxonomic perspective, this increase would collect approximately 90% of the observed species per expedition. This would require a team of four persons, working six hours for four days or the relevant time divisions depending on resources and logistics.

The time invested for the present inventory demonstrates that in less than two years one hectare of tropical wet forest can be: thoroughly sampled, documented online, and these data published. Los Tuxtlas spider community was sampled four times throughout the year to cover the area of study seasonality. The similarity analyses recommended sampling at least two times a year and at different seasons, contrary to only one expedition after the rainy season. This extra expedition will contribute to capture more adult spiders and achieve a better inventory completeness.

Finally, human resources in terms of number of collectors have been a limiting factor for other biodiversity inventories. It is recommended that a minimum of four people (each sampling six hours per day) are needed to achieve the proposed completeness in four days of field work for online documentation. This must be considered at the early stages of inventory planning, budget allocation, and coordinated with universities considering that undergraduate and graduate students are always eager to participate in field expeditions.

## Acknowledgments

We would like to thank Jagoba Malumbres-Olarte and Jeremy Miller for their valuable comments and thorough reviews of an earlier version of this work. The authors would like to thank Rosamond I. Coates-Lutes and Estación de Biología Los Tuxtlas IB-UNAM for allowing the conduction of this inventory. Special thanks to the following arachnologists for their help with the morphospecies identifications: Wayne Maddison, Uriel Garcilazo-Cruz, J. Prószyński, J. T. Lapp, A. Santos and A. Rivera-Quiroz. Also, to the students and researchers that helped collecting specimens: Ali Zeltzin Lira-Olguin, Dulce F. Piedra-Jimenez, Uriel Garcilazo-Cruz, Ricardo Raña-Mendoza, Hugo A. Valdes, Josue Lopez-Granados, Jair Rojas-Castillo, Maira Montejo-Cruz, Edmundo Santillan-Gonzalez. In addition, we thank Robert J. Kallal and Gustavo Hormiga for revising the English and for their valuable comments on the manuscript. Thanks to Diana L. Batista-Perales for her comments in the User Manual and Oscar F. Bejarano-Mendoza for testing the software to make spider biodiversity websites. This project was funded by UNAM-DGAPA PAPIIT IN214916.

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**Appendix 1.** Species and Morphospecies list and adult abundances. Species indicated with (*) were present throughout the year (106). Species marked with (+) were present on all methods (5).

| FAMILY | SPECIES | Abund. | FAMILY | SPECIES | Abund. |
|---|---|---|---|---|---|
| Agelenidae | *Rualena cavata* | 3 | Ctenidae | *Acanthoctenus spiniger* | 3 |
| Anapidae | *Anapisona kethleyi* * | 33 | | *Ctenus convexus* * | 38 |
| Anyphaenidae | *Anyphaena sp02* * | 46 | | *Ctenus peregrinus* | 5 |
| | *Anyphaena sp03* | 1 | | *Ctenus sp01* | 29 |
| | *Wulfila diversus* * | 139 | | *Kiekie curvipes* | 49 |
| | *Wulfila modestus* | 1 | Deinopidae | *Deinopis aurita* | 1 |
| | *Wulfila pulverulentus* * | 12 | | *Deinopis longipes* | 6 |
| | *Wulfila sp02* * | 42 | Dictynidae | *Dictyna incredula* | 7 |
| Araneidae | *Acacesia tenella* | 1 | | *Lathys dixiana* | 1 |
| | *Araniella nr. displicata* | 1 | | *Mallos sp01* | 6 |
| | *Cyclosa caroli* * | 74 | Gnaphosidae | *Eilica bicolor* | 1 |
| | *Cyclosa conigera* | 1 | | *Litopyllus sp01* | 9 |
| | *Eriophora nephiloides* * | 13 | | *Zimiromus iotus* | 3 |
| | *Eriophora ravilla* | 1 | Hahniidae | *Neoantistea unifistula* * | 115 |
| | *Eustala bifida* | 1 | Hersiliidae | *Neotama mexicana* * | 46 |
| | *Eustala cepina* * | 11 | Linyphiidae | *Agyneta sp01* | 4 |
| | *Eustala guttata* * | 26 | | *Agyneta sp05* | 1 |
| | *Mangora goodnightorum* * | 25 | | *Erigone sp01* * | 7 |
| | *Mangora melanocephala* * | 25 | | Erigoninae sp02 * | 270 |
| | *Mangora passiva* * | 14 | | Erigoninae sp03 | 1 |
| | *Mastophora alvareztoroi* | 4 | | Erigoninae sp04 | 1 |
| | *Mecynogea ocosingo* | 2 | | *Frontinella potosia* * | 138 |
| | *Metazygia nigrocincta* | 1 | | *Grammonota nr. teresta* | 11 |
| | *Micrathena mitrata* | 1 | | *Grammonota sp01* * | 135 |
| | *Micrathena sagittata* | 1 | | *Pocobletus coroniger* | 4 |
| | *Nephila clavipes* | 22 | | *Pocobletus sp02* | 4 |
| | *Parawixia destricta* | 3 | | *Primerigonina sp01* * | 49 |
| | *Parawixia guatemalensis* | 1 | | *Walckenaeria sp01* * | 18 |
| | *Verrucosa arenata* * | 113 | Lycosidae | *Hogna longitarsis* * | 383 |
| | *Wagneriana tauricornis* * | 356 | | *Lycosa transversa* | 10 |
| | *Witica crassicauda* * | 21 | | *Pirata sp01* | 14 |
| Caponiidae | *Nops sp01* | 4 | | *Rabidosa carrana* | 9 |
| Cheiracanthiidae | *Strotarchus nr. piscatorius* | 4 | Mimetidae | *Gelanor latus* | 12 |
| Clubionidae | *Elaver sp01* | 9 | | *Mimetus nr. bigibbosus* | 178 |
| | *Elaver sp02* * | 22 | | *Mimetus sp03* | 3 |
| Corinnidae | *Castianeira rica* *+ | 11 | | *Mimetus variegatus* | 19 |
| | *Corinna sp01* * | 13 | | *Mimetus verecundus* | 2 |
| | *Creugas sp05* | 1 | Mysmenidae | *Maymena mayana* | 1 |
| | *Megalostrata depicta* * | 10 | | *Maymena sp01* * | 99 |
| | *Myrmecotypus pilosus* | 2 | | *Mysmena sp01* * | 42 |

*...Continued next page*

**Appendix 1. (Continued)**

| FAMILY | SPECIES | Abund. |
|---|---|---|
| | *Mysmena sp02* * | 8 |
| | *Mysmena sp03* | 17 |
| Nesticidae | *Eidmannella pallida* | 3 |
| | *Gaucelmus augustinus* | 5 |
| Ochyroceratidae | *Ochyrocera jarocha* * | 28 |
| Oonopidae | *Costarina plena* * | 51 |
| | *Costarina subplena* * | 15 |
| | Oonopidae sp01 * | 14 |
| | Oonopidae sp02 | 4 |
| | *Orchestina guatemala* * | 10 |
| | *Pescennina murphyorum* | 9 |
| | *Ponsoonops hamus* | 8 |
| | *Scaphiella tuxtla* | 6 |
| Oxyopidae | *Hamataliwa sp01* | 1 |
| Pholcidae | *Anopsicus bolivari* *+ | 218 |
| | *Anopsicus iviei* * | 79 |
| | *Metagonia delicata* | 9 |
| | *Metagonia nr. caudata* | 45 |
| | *Metagonia sp01* * | 383 |
| | *Modisimus sp01* + | 303 |
| | *Modisimus sp02* * | 326 |
| | *Modisimus sp03* * | 34 |
| | *Modisimus sp04* * | 10 |
| | *Modisimus sp05* | 1 |
| Phrurolithidae | *Phonotimpus sp01* * | 103 |
| | *Phonotimpus sp02* * | 73 |
| | *Phonotimpus sp03* | 6 |
| Pisauridae | *Tinus palictlus* | 15 |
| | *Tinus tibialis* | 3 |
| Salticidae | *Acragas hieroglyphicus* | 1 |
| | *Amphidraus sp01* | 6 |
| | *Attidops cinctipes* | 2 |
| | *Beata sp28* | 1 |
| | *Chapoda recondita* * | 11 |
| | *Chapoda sp02* | 3 |
| | *Chapoda sp26* | 1 |
| | *Corythalia sp01* * | 70 |
| | *Corythalia sp03* | 13 |
| | *Corythalia sp04* | 6 |
| | *Corythalia sp05* * | 32 |
| | *Corythalia sp06* * | 335 |
| | *Corythalia sp07* | 1 |
| | *Corythalia sp21* | 4 |
| Salticidae | *Corythalia sp27* | 1 |
| | *Cylistella sp01* * | 91 |
| | Euophryini sp01 * | 55 |
| | *Euophrys sp02* | 9 |
| | *Hypaeus benignus* * | 112 |
| | *Lyssomanes maddisoni* * | 62 |
| | *Pensacola sp20* | 2 |
| | *Pensacola sylvestris* * | 24 |
| | *Sarinda sp01* | 4 |
| | *Synemosyna decipiens* * | 33 |
| | *Xanthofreya rustica* * | 10 |
| | *Zygoballus sp01* * | 5 |
| Scytodidae | *Scytodes itzana* * | 97 |
| | *Scytodes sp01* * | 50 |
| | *Scytodes sp02* * | 12 |
| Selenopidae | *Selenops sp01* * | 21 |
| Senoculidae | *Senoculus prolatus* | 2 |
| | *Senoculus tigrinus* | 1 |
| Sparassidae | *Decaphora nr. variabilis* | 7 |
| Symphytognathidae | *Globignatha sedgwicki* | 2 |
| Tetrablemmidae | *Caraimatta cambridgei* * | 66 |
| Tetragnathidae | *Azilia guatemalensis* * | 107 |
| | *Chrysometa alboguttata* | 4 |
| | *Chrysometa flava* * | 60 |
| | *Chrysometa nuboso* | 1 |
| | *Cyrtognatha petila* * | 105 |
| | *Dolichognatha pentagona* * | 10 |
| | *Glenognatha sp01* | 1 |
| | *Glenognatha spherella* | 10 |
| | *Leucauge idonea* * | 101 |
| | *Mecynometa globosa* | 2 |
| Theridiidae | *Ameridion armouri* | 2 |
| | *Ameridion malkini* * | 51 |
| | *Ameridion moctezuma* * | 31 |
| | *Ameridion quantum* | 2 |
| | *Ameridion ruinum* * | 205 |
| | *Ameridion sp01* * | 23 |
| | *Anelosimus dubiosus* | 1 |
| | *Chrosiothes iviei* *+ | 84 |
| | *Chrosiothes silvaticus* * | 65 |
| | *Chrysso albomaculata* | 1 |
| | *Chrysso cambridgei* * | 23 |
| | *Cryptachaea rostrata* | 2 |

*...Continued next page*

**Appendix 1. (Continued)**

| FAMILY | SPECIES | Abund. | FAMILY | SPECIES | Abund. |
|---|---|---|---|---|---|
| Theridiidae | *Dipoena anas* | 7 | Theridiidae | *Thymoites sp03* * | 48 |
| | *Dipoena insulana* | 6 | | *Thymoites sp05* | 15 |
| | *Dipoena mertoni* | 2 | | *Thymoites verus* * | 9 |
| | *Dipoena nr. atlantica* | 1 | | *Tidarren haemorrhoidale* | 1 |
| | *Dipoena sp01* * | 8 | | *Tidarren sp01* | 5 |
| | *Dipoena sp04* | 4 | | *Tidarren sp02* | 1 |
| | *Dipoena sp06* | 1 | | *Wamba crispulus* * | 21 |
| | *Emertonella taczanowskii* | 3 | | *Wamba panamensis* | 1 |
| | *Exalbidion dotanum* | 6 | | *Wirada mexicana* | 1 |
| | *Exalbidion sexmaculatum* | 1 | Theridiosomatidae | *Epilineutes sp01* * | 157 |
| | *Faiditus caudatus* | 1 | | *Epilineutes sp02* * | 148 |
| | *Faiditus dracus* * | 42 | | *Theridiosoma chiripa* * | 21 |
| | *Faiditus leonensis* | 21 | | *Theridiosoma sp01* * | 168 |
| | *Faiditus maculosus* * | 42 | Thomisidae | *Epicadus trituberculatus* | 4 |
| | *Faiditus sp01* * | 17 | | *Onocolus eloaeus* | 2 |
| | *Faiditus sp02* * | 40 | | *Strophius hirsutus* * | 33 |
| | *Faiditus sp03* | 5 | | *Tmarus innotus* * | 32 |
| | *Hentziectypus florens* * | 131 | Trachelidae | *Trachelas cambridgei* | 2 |
| | *Lasaeola sp01* | 1 | | *Trachelas sp01* | 8 |
| | *Lasaeola sp02* | 2 | | *Trachelas sp02* * | 9 |
| | *Neopisinus cognatus* | 5 | Trechaleidae | *Cupiennius remedius* | 13 |
| | *Neospintharus concisus* * | 25 | | *Cupiennius salei* * | 14 |
| | *Parasteatoda nigrovittata* * | 76 | | *Enna sp01* | 1 |
| | *Phoroncidia sp01* | 1 | Uloboridae | *Ariston aglasices* * | 64 |
| | *Phycosoma sp02* | 3 | | *Miagrammopes simus* | 3 |
| | *Phycosoma sp03* | 3 | | *Miagrammopes sp02* | 6 |
| | *Rhomphaea sp01* | 14 | | *Miagrammopes sp03* | 1 |
| | *Rhomphaea sp02* | 1 | | *Philoponella divisa* | 11 |
| | *Stemmops ornatus* | 2 | | *Philoponella republicana* * | 88 |
| | *Theridion evexum* | 5 | | *Philoponella tingens* * | 33 |
| | *Theridion hispidum* | 2 | | *Uloborus campestratus* * | 7 |
| | *Theridion omiltemi* * | 39 | Zodariidae | *Ishania aztek* | 1 |
| | *Theridion sp01* | 15 | | *Ishania mixtek* * | 64 |
| | *Theridion stannardi* * | 68 | | *Ishania olmek* | 3 |
| | *Thymoites sp01* + | 296 | | *Ishania sp01* | 41 |
| | *Thymoites sp02* * | 88 | **Total** | | **8655** |

**Appendix 2.** Species identification and taxonomic comments per family.

This appendix provides a discussion of the taxonomic challenges, possible nomenclatural issues and new taxa found while identifying this inventory species. Families with similar taxonomic cases are clustered together and organized from the taxa that have comprehensive taxonomic revisions, to the taxonomically more complicated groups. The families discussed on these paragraphs are listed at the beginning of each section. In addition, the taxonomic representation obtained by method and separated by season is available as supplementary material at www.unamf-caracnolab.com/WPGS_TUXV/tuxv.html.

Araneidae and Oonopidae. Araneidae was the easiest family for identification and all specimens were associated to a described species. The generic key and taxonomic revisions by Dr. H. Levi for all American genera were crucial (Levi 2002). The male of *Mangora goodnightorum* Levi is new to science and undescribed (Levi 2005). Oonopidae was also relatively simple because it has received recent taxonomic attention regarding generic revisions (Bolzern 2014; Platnick & Dupérré 2011, 2012). Two oonopid species were only identified to family rank and belong to those soft body oonopids known as "moles" were most of the remaining work still in progress. The male for *Orchestina guatemala* Izquierdo was previously unknown and is undescribed (Izquierdo & Ramírez 2017).

Theridiidae was easy to identify thanks to Levi's taxonomic revisions and generic keys for the Neotropical taxa (Levi 1953, 1954, 1959, 1962, 1963; Levi & Levi 1962). The diagnoses for *Dipoena* and *Phycosoma* O. Pickard-Cambridge are ambiguous. Some species of *Phycosoma* may belong to *Dipoena* based on the pedipalp sclerites, while some *Dipoena* species could belong to *Phycosoma* considering the cephalothorax shape (Levi 1953, 1963). Most of these identified species probably are new taxa. *Dipoena mertoni* Levi based on its diagnosis probably belong to *Euryopis* Menge (Levi 1954b, 1963). *Chrysso albomaculata* O. Pickard-Cambridge and *Theridion hispidum* O. Pickard-Cambridge could represent two species groups because of considerable variation on their genitalia features (Levi 1959, 1962). The male of *Theridion stannardi* Levi and the female of *Ameridion malkini* (Levi) could represent a synonymy; both species had the same size, abundances and were collected on all expeditions. The correct female of *Theridion omiltemi* Levi was identified as a variation of *Theridion evexum* Keyserling (Levi 1959), but cleared genitalia allowed to correctly identify the females of these species. The females of *Ameridion ruinum* (Levi), *Thymoites verus* (Levi) and the male of *Chrosiothes iviei* Levi are new and undescribed.

Mimetidae, Pholcidae, Tetragnathidae, Uloboridae and Pisauridae presented the following taxonomic issues. Tetragnathidae generic delimitations (Álvarez-Padilla & Hormiga 2011) and revisions for several genera (Cabra-García & Brescovit 2016; Dimitrov & Hormiga 2009; Levi 1986) facilitated these identifications, but considerably work is needed particularly within Neotropical leucauginaes that were identified with studies more than a century old (Pickard-Cambridge F. O. 1900; Pickard-Cambridge O. 1891). *Glenognatha* sp01 is probably a new species (Cabra-García & Brescovit 2016). Mimetid generic determination was trivial due to recent contributions (Benavides & Hormiga 2016) except for *Mimetus*, where taxonomic work is need and the species identifications are ambiguous. The female of *Mimetus* nr. *bigibbosus* O. Pickard-Cambridge, if indeed belongs to this species, is new to science and undescribed (Pickard-Cambridge F. O. 1902). Pholcidae presented a similar case with easy generic assignments (Gertsch 1986; Huber 2000) with probably new species of *Metagonia* Simon and *Modisimus* in need of revision. Uloboridae generic determinations were done with relatively ease and for some species (Opell 1979, 1981; Salvatierra *et al*. 2014) with *Miagrammopes* O. Pickard-Cambridge in need for taxonomic revision. *Philoponella divisa* Opell and *Philoponella tingens* (Chamberlin & Ivie) were represented by only one sex each, specimen ratios of 1/3 and same phenology suggesting synonymy. The male of *Ariston aglasices* Salvatierra, Tourinho & Brescovit is new to science and undescribed. Finally, for Pisauridae, the species *Tinus palictlus* Carico and *T. tibialis* F. O. Pickard-Cambridge were represented by only one sex each; however, the presence of an embolus part of *T. tibialis* inserted in the copulatory opening of *T. palictlus* gives evidence for a possible synonymy between both species.

Mysmenidae, Theridiosomatidae and Symphytognathidae generic rank identification were relatively easy thanks to contributions to the taxonomy of these families (Coddington 1986; Gertsch 1960, 1971; Lopardo & Hormiga 2015; Forster & Platnick 1977); however, their knowledge in Neotropical regions have presented slow progress where only species descriptions for specific areas are available (Brescovit *et al*. 2004; Dupérré & Tapia 2017; Rheims & Brescovit 2003; Rodrigues & Ott 2005a, 2005b). For Theridiosomatidae, both *Epilineutes* Coddington, 1986 species and one *Theridiosoma* O. Pickard-Cambridge are probably new taxa. For Mysmenidae, *Maymena* sp01 probably is a new species. The comparison of the three *Mysmena* Simon species with described taxa was difficult and it is ambiguous whether are new or not (Gertsch 1960). For Symphytognathidae, the standard views of the cleared epigynum were necessary to identify *Globignatha sedgwicki* Forster & Platnick.

Caponiidae, Clubionidae, Cheiracanthiidae, Selenopidae and Sparassidae identification was relatively easy due to recent taxonomic revisions focused on fauna distributed in the Americas (Crews 2011; Bonaldo *et al*. 2012; Sánchez-Ruiz & Brescovit 2018; Saturnino & Bonaldo 2015; Rheims & Alayón 2014). *Nops* MacLeay, *Elaver* O. Pickard-Cambridge and *Selenops* Latreille species probably represent new taxa. Cheiracanthiidae and Sparassidae both with one species were determined as *"*near*"*to a described taxon (Appendix 1).

Corinnidae, Dictynidae, Hahniidae, Hersiliidae and Oxyopidae presented a similar case where the species identifications were possible because the availability of taxonomic revisions of the American fauna (Bonaldo 2000;

Bond & Opell 1997; Brady 1970; Chamberlin & Gertsch 1958; Gertsch & Davis 1937; Opell & Beatty 1976; Reiskind 1969; Rheims & Brescovit 2004). For Corinnidae, the species *Creugas* sp05 and *Corinna* sp01 probably represent new taxa, as well the species *Mallos* sp01 for Dictynidae and *Hamataliwa* sp01 for Oxyopidae. The female *Megalostrata depicta* (O. Pickard-Cambridge) (Corinnidae), and the males for *Lathys dixiana* Ivie & Barrows (Dictynidae) and *Neoantistea unifistula* Opell & Beatty (Hahniidae) were previously unknown and are undescribed.

Anapidae, Gnaphosidae, Trachelidae, Scytodidae and Zodariidae generic and species identifications were relatively easy (Platnick 1975; Platnick & Shadab 1974a, 1974c, 1976, 1979, 1980; Jocqué & Baert 2002; Rheims, Brescovit & Durán-Barrón 2007). For Gnaphosidae, *Litopyllus* sp01 probably represent a new taxon as well as *Trachelas* sp01and sp02 for Trachelidae. Scytodidae and Zodariidae were represented by *Scytodes* Latreille and *Ishania* respectively and both genera have taxonomic revisions that facilitated the species identification. Two *Scytodes* species are probably new. *Ishania* sp01 was represented only by males and could be either the unidentified male of some already described female or a new species of Zodariidae. New taxonomic revisions are needed because of the great diversity of these taxa in the Neotropics.

Agelenidae, Ochyroceratidae, Tetrablemmidae, Deinopidae, Nesticidae and Senoculidae were represented by one or two species each and their identification to species level was relatively easy; however, the genera *Ochyrocera* Simon and *Rualena* Chamberlin & Ivie (Agelenidae) need taxonomic revisions (Bryant 1940; Chickering 1941, 1963; Gertsch 1984; Maya-Morales & Jiménez 2016; Pickard-Cambridge, F. O. 1902; Pickard-Cambridge, O. 1896; Valdez-Mondragón2017). The female of *Senoculus tigrinus* Chickering is new and undescribed.

Anyphaenidae and Ctenidae identification to genus and species ranks encompassed the review of several works (Chickering 1937, 1940; Pickard-Cambridge F. O. 1897, 1900, 1902; Pickard-Cambridge O. 1895, 1896). Recent progress on the taxonomy of Neotropical ctenids has been made, revisiting and proposing new taxa to allocate species not related with the genera in which they were placed such the case of *Kiekie curvipes* (Keyserling) (Polotow & Brescovit 2018). *Ctenus* sp01 is probably a new species and the males of *Ctenus convexus* F. O. Pickard-Cambridge and *Ctenus peregrinus* F. O. Pickard-Cambridge were previously unknown and still undescribed. For anyphenids, several contributions to the New World faunas have been made (Brescovit 1997; Platnick 1974; Platnick & Lau 1975); however, generic delimitations and taxonomic revisions focused on Neotropical groups are needed considering the elevated number of species that inhabit these areas (Labarque *et al*. 2015; World Spider Catalog 2019). Regarding to this inventory *Wulfila* sp02 and two *Anyphaena* Sundevall species are probably new. The male of *Wulfila diversus* O. Pickard-Cambridge is new to science and undescribed.

Lycosidae generic separation was difficult for *Hogna* Simon and *Lycosa* Latreille. They are sorted by only one feature regarding the coloration between the anterior median eyes, and a better generic delimitation is needed. Lycosid species for temperate areas of the Northern hemisphere have received more taxonomic attention and are easier to identify (Brady & McKinley 1994; Wallace & Exline 1978), but Neotropical taxa are in a dire state. *Lycosa transversa* F. O. Pickard-Cambridge and *Rabidosa carrana* (Bryant) were represented by only one sex each, but have similar size, abundances and were collected during the same season suggesting a possible synonymy. *Pirata* sp01 could be new, but this genus needs taxonomic revision for the Neotropical species. *Hogna longitarsis* epigynum presented considerable variation in the shape of the septum and genital openings grooves. These differences were considered as intraspecific variation because the male palp sclerites shape and proportions were constant among these specimens. The male of this species was previously unknown and needs to be described.

Phrurolithidae was represented by *Phonotimpus* Gertsch & Davis, but these species were difficult to identify because of the lack of taxonomic revisions on this genus. Considerable ambiguity exists in the diagnoses of *Scotinella* Banks mainly with a Nearctic distribution (Gertsch 1941; Dondale & Redner 1982) and *Phonotimpus* endemic of Mexico (Gertsch & Davis 1940; Chamé-Vázquez *et al*. 2018; Chamé-Vázquez & Ibarra-Núñez 2019) regarding the position of the copulatory openings and the RTA details. These species were identified as *Phonotimpus* based on the shape and distribution of the eyes, the presence of a conductor an embolic basal process, plus the retrolateral and dorsal tibial apophyses not joined at the base. Nevertheless, these species also shared the following features with some *Scotinella* species such as the number of prolateral macrosetae in the femur I, the position of the copulatory openings and the shape of the RTA.

Linyphiidae species identification was difficult because of the unexplored diversity that this family presents in the Neotropics and the paucity of taxonomic revisions. To date, the most recent and complete work focused on the neotropical fauna corresponds to the review of erigonine spider genera (Miller 2007). This paper was the most useful taxonomic reference to determinate the morphospecies of this group at genus level. A few contributions to the taxonomy of non-erigonine genera in the Neotropical regions were made during the 20th century (Chickering 1969; Gertsch & Davis 1946; Millidge 1985, 1991). These works allowed the identifications of *Pocobletus coroniger* Simon and *Frontinella potosia* Gertsch & Davis to species level. However, the neotropical species for these genera and many others like *Agyneta* Hull need attention to resolve their taxonomic problems. The species identified to genus rank probably represent new taxa including the ones determined only to subfamily level.

Salticidae generic identification was the most difficult in this inventory and represented the best example of the biodiversity inventory website functionality. The collaboration of J. Prószyński, W. Maddison and U. Garcilazo-Cruz to identify the morphospecies was vital. For species identifications, the online resources available for this family were also important and easy to use (Jerzy Prószyński, https://www.peckhamia.com/salticidae/salticidae.php?adres=permision.php; Wayne Maddison, http://salticidae.org/salticidImages/). Phylogenetic studies show that many genera are monophyletic, but their alpha taxonomy is in disarray (Zhang & Maddison 2015). Eight species were identified to species rank; most of them described in taxonomic works of the Central and South American fauna (Cutler 1985; Edwards 2015; Galiano 1963; Logunov 2014; Peckham & Peckham 1885, 1896). *Corythalia* eight species could be new, but the available descriptions and illustrations were ambiguous to draw definitive conclusions. The contrary case was found in *Amphidraus* Simon that has recent taxonomic revisions from Brazil and Colombia (Galvis 2017; Salgado & Ruiz 2017, 2019) that helped to identify *Amphidraus* sp01 as a new species. The species identified to genera *Beata* Peckham & Peckham, *Chapoda* Peckham & Peckham, *Cylistella* Simon, *Euoprhys* C. L. Koch, *Pensacola* Peckham & Peckham, *Sarinda* Peckham & Peckham and *Zygoballus* Peckham & Peckham possibly represent new taxa. The female of *Pensacola sylvestris* (Peckham & Peckham) was previously unknown and needs to be described.

Thomisidae and Trechaleidae presented similar changes. The species *Tmarus innotus* Chickering, *Onocolus eloaeus* Lise and *Enna* sp01 were identified using the website standard views in collaboration with J. T. Lapp and A. Santos. Only the species *Strophius hirsutus* O. Pickard-Cambridge and *Epicadus trituberculatus* (Taczanowski) were identified using recent taxonomic revisions (Machado *et al*. 2018; Silva-Moreira & Machado 2016) and *Biologia Centrali-Americana* (Pickard-Cambridge F. O. 1900; Pickard-Cambridge O. 1891). The trechaleid specie *Enna* sp01 could either new or correspond to one of the 60% of the species represented only by females (World Spider Catalog 2019). The genus *Cuppienius* Simon was recently transferred from Ctenidae to Trechaleidae (Piacentini & Ramírez 2019) and was represented by two described species in this inventory. The male of *Strophius hirsutus* O. Pickard-Cambridge (Thomisidae) was previously unknown and needs to be described.

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