1 INTRODUCTION

Among the archipelagos, the Caribbean offers one of the best-researched natural arenas for addressing biogeographic hypotheses (Ricklefs & Bermingham, 2008). Caribbean islands are numerous and are of sufficiently varied ages and sizes to provide a historical context that generated interesting biogeographic histories of the organisms that inhabit...
An emerging issue that is relevant to organismal biology, lineage diversification, as well as biogeographic histories and patterns, is the degree to which variation in dispersal propensity can predict species richness of lineages (Borda-de-Água et al., 2017; Laube, Graham, & Böhning-Gaese, 2013). The Caribbean is an ideal archipelago to pose these questions (Čandek, Agnarsson, Binford, & Kuntner, 2019).

The Intermediate Dispersal Model of biogeography (henceforth IDM; Agnarsson, Cheng, & Kuntner, 2014; Claramunt, Derryberry, Remsen, & Brumfield, 2012) posits that differences among comparable lineages in dispersal potential over long distances affect their levels of gene flow over discrete units, such as islands, and that this variation is reflected in species richness patterns among these lineages. For example, if lineages contain poor dispersers, these organisms rarely colonize remote islands, leading to overall low species richness. Conversely, those lineages that are biologically capable of long-distance travel may maintain such a lively gene flow among islands, or between island and continent, as to severely restrict speciation. Finally, those organisms with intermediate dispersal potential get to be carried to remote islands rarely enough so that their founding populations may start to speciate, a hypothetical scenario that may result in the highest species richness. What this model implies is that biological attributes that define higher taxa, say genera, may link to the overall potential how these organisms disperse, and therefore, affect their species richness, and biogeography.

The IDM, therefore, predicts variation in species richness among lineages to be a consequence of varying dispersal potential. However, in order to test the general validity of the model, one needs to identify appropriate test lineages. Ideally, these would be co-distributed in an archipelago, be of comparable taxonomic ranks, and would furthermore exhibit a measurable variation in phenotypes that pertain to dispersal. This has rarely been done, as studies testing the IDM have mainly focused on either only excellent dispersers (Claramunt et al., 2012) or poor dispersers (Pabijan, Wollenberg, & Vences, 2012), on laboratory-reared organisms (Venail et al., 2008), or they included multiple lineages of incomparable taxonomic ranks (Agnarsson et al., 2014). In this vein, our prior work has compared a tetragnathid spider lineage with a hypothetical low dispersal potential (Cyrtognatha; Čandek et al., 2019) with its close relative (Tetragnatha; Čandek, Agnarsson, Binford, & Kuntner, 2018) over the Caribbean and the mainland. We found Tetragnatha to be extremely species-rich in the Caribbean and attributed this richness to a biology that has elements of excellent dispersal, mixed with repeated secondary loss of dispersal ability, all this resulting in a mixed pattern of cosmopolitan, as well as narrowly endemic lineages. In comparison, Cyrtognatha was relatively species-poor, with exclusively single island endemic species. We here explore the biogeographic pattern of another lineage for which we a priori expect to show excellent dispersal ability that is co-distributed over the Caribbean.

Our present study thus focuses on the nephilid genus Trichonephila Dahl in the Caribbean. Trichonephila is a global genus of golden orbweavers (Nephilidae) that contains species known to readily cross long, overwater...
distances (Kuntner et al., 2019). Only two species are currently known in the New World, with *T. clavipes* distributed widely from North to South America (Kuntner, 2017). We here examine the population genetic structure of *T. clavipes* in Americas through a Caribbean transect, and with numerous terminals from other parts of the New World. We predict that in accordance with the IDM, *Trichonephila* will be species-poor compared with the above tetragnathids and will show the least structured genetic pattern over the archipelago. If so, this would implicate a lively gene flow over all the islands.

2 | RESULTS AND DISCUSSION

Our numerous species delimitation analyses (Figure 1; Figures S1–S15, Table S1), a haplotype network analysis (Figure 2), and population genetic analyses (Table S2, S3, Note S1) reveal a phylogenetic and population genetic structure of *T. clavipes* that may be inconsistent with a single species throughout its range. While *T. clavipes* always forms a clade, species delimitation analyses detect potential cryptic diversity and thus more than a single species over the entire New World. What this pattern is consistent with is that one species inhabits the focal area of this paper (Caribbean and North America), suggesting high levels of gene flow in the region (Figure 3). Using the best fit model, GMYC delineates two putative species (marked as A and B in Figure 1). The vertical bar in Figure 1 indicates the threshold where GMYC model shifts from phylogenetic Yule processes to coalescent population processes. “Species” (a) contains a dichotomy with a subclade containing the Caribbean and mainland North American populations, and a subclade with Colombian and Costa Rican populations. “Species” (b) is native to South and Central America. While species delimitations using some GMYC and a ABGD agree with the two species, alternatives exist (see Figures S1–S14). More precisely, some GMYC, mPTP and some ABGD suggest more than two species over the entire *T. clavipes* range. While *T. clavipes* may in fact contain more than a single, Panamerican species, consistent with a population genetic study in South America (Bartoleti, Peres, Fontes, da Silva, & Solferini, 2018), this result contradicts the classical morphological taxonomy (Kuntner, 2017).

The results from DNA barcoding analyses (Figure S15) are inconclusive and do not reveal a clear barcoding gap that would enable us to confidently resolve the status of *T. clavipes*. DNA barcoding gap is defined as at least 10 times greater average interspecific distance over the average intraspecific genetic distance (Hebert, Stoeckle, Zemlak, & Francis, 2004). In *Trichonephila*, the difference between the average intraspecific distance and the average interspecific genetic distance is less than fivefold (0.027 vs. 0.11). Moreover, we do not recover a barcoding gap even if we treat the most divergent *T. clavipes* populations as separate species (0.011 vs. 0.084 average genetic distance, Figure S15C). Pairwise distances of the highly divergent *T. clavipes* populations (South America vs. North America) range from 0.055 to 0.065, and these values fill the area on the barcoding gap plot where, in theory, the gap would be present between intraspecific and interspecific genetic distances (Figure S15A). If these DNA barcoding results reflect reality, this might indicate a speciation in the process.

However, caution is needed when interpreting species delimitation results based on a mitochondrial marker. It may be erroneous to imply cryptic diversity in *T. clavipes* (over its entire range) if mitochondrial heterogeneity resulted from population or mitochondrial bottlenecks (Rubinoff, Cameron, & Will, 2006; Toews & Brelsford, 2012). Because
mitochondria are maternally inherited, male-biased dispersal (Dávalos & Russell, 2014; Rubinoff et al., 2006), selective sweeps or inherited symbionts (Hurst & Jiggins, 2005) can also lead to misinterpretations of genetic structure based solely on mitochondrial DNA.

The haplotype network (Figure 2) depicts a single, well-represented haplotype present on the sampled Caribbean islands, as well as in continental North America. A few point mutations separate this highly frequent haplotype with those present on Jamaica, Mexico, Puerto Rico, and Turks and Caicos. Other haplotypes are more distant, and form two distinct groups, one in Colombia and Costa Rica (putatively conspecific with the Caribbean), and another in Brazil, Panama and French Guiana that corresponds to the putative species (b). This haplotype network is consistent with potential cryptic diversity consisting of two or three species of T. clavipes. It also strongly suggests that the Caribbean populations maintain gene flow with the North American mainland.

While the haplotype network allows for a graphical interpretation of the genetic structure, the results from population genetic analyses (Table S2, S3, Note S1) provide statistical measures for the observed pattern. Analysis of molecular variance (AMOVA) reveals that 81.2% of the genetic variation in T. clavipes (across its entire range) is explained by the between-population variation (South America vs. North America + the Caribbean), while the within-population variation contributes only 18.8% of the total genetic variation ($F_{st} = 0.81$, $p < .001$, Table S2). On the other hand, only a fraction of genetic heterogeneity is explained by between-population variation (9.5%) when we focus solely on the T. clavipes from the continental North America versus T. clavipes from the Caribbean. In the latter case, 90.5% of the total variability is explained by the within-population variation ($F_{st} = 0.09$, $p < .001$, Table S2), again suggesting a high gene flow between populations on continental North America and on the Caribbean. We present other measures of gene flow and genetic differentiation estimates in Table S3 and Note S1.
All these results lend support for our hypothesis that predicted a lively gene flow and a homogenous genetic structure of *T. clavipes* among the Caribbean islands and North America.

Genetic distances among *T. clavipes* individuals show a strong correlation with geographic distances when all specimens are considered (Figure 4; all coloured data points, Mantel correlation $r = 0.83$). However, this correlation is much less pronounced when considering only South American populations (Figure 4; grey coloured data points, Mantel correlation $r = 0.31$) and is absent when considering only North American and Caribbean populations (Figure 4; blue coloured data points, Mantel correlation $r = 0.12$). While the all American *T. clavipes* range (considering *T. clavipes* as one species) might be an area large enough for isolation by distance to take effect, the combined continental North America and the Caribbean region is not. *T. clavipes* spiders likely balloon among the Caribbean islands and North America and maintain a homogeneous genetic structure across at least 3,000 km.

These data reinforce the known biology of *Trichonephila* species as excellent dispersers. Although ballooning has not been directly observed in *Trichonephila*, it has been shown in the closely related and similar *Nephila* (V. M. J. Lee, Kuntner, & Li, 2015). Ballooning is an effective means of overcoming oceanic and continental barriers to gene flow, as several studies on Asian *Nephila pilipes* show (J. W. Lee, Jiang, Su, & Tso, 2004; V. M. J. Lee et al., 2015; Su, Chang, Lee, & Tso, 2006). More close relatives of *T. clavipes* also show extremely wide, genetically poorly structured population patterns worldwide. Good examples are *T. inaurata* that maintains gene flow between Africa and the islands of the Western Indian Ocean (Kuntner & Agnarsson, 2011), as well as *T. edulis* and *T. plumipes* reported to travel seasonally from Australia to New Zealand (Harvey, Austin, & Adams, 2007). There also seems to be a constant gene flow between the Korean and the Japanese populations of *T. clavata* (Jung, Lee, Kim, & Kim, 2006).

*Trichonephila clavipes* resembles the Caribbean pattern detected in the araneid *Argiope argentata* where island populations clearly interbreed (Agnarsson et al., 2016). At a higher taxonomic level and within the area of interest, the Caribbean *Trichonephila* contrasts the two tetragnathid lineages: *Cyrtognatha* is a relatively poor to intermediate disperser with significant species richness and high endemism, *Tetragnatha* is a dynamic disperser, with species apparently ranging from extremely good to relatively poor, and shows a high species richness and a mixed endemic to widespread mix of species (Čandek et al., 2018, 2019). As we show in this study, *Trichonephila* is an excellent disperser with a single species over the archipelago, exhibiting little genetic structure. Although *Tetragnatha* is an unusual case with apparently frequent evolutionary change in dispersal potential, this triplet of genera provides preliminary support of the IDM.

### 3 | METHODS

#### 3.1 | Data set assembly

Our total data set contains every available *T. clavipes* cytochrome c oxidase subunit 1 (COI) sequence from the combined Caribbean + continental North American region ($N = 57$), an equal number of COI sequences randomly selected from Brazilian *T. clavipes* (Bartoleti et al., 2018), and every available sequence of *T. clavipes* from other areas (5 × Colombia, 2 × Panama, 1 × French Guiana, 1 × Costa Rica). We also targeted other *Trichonephila* global exemplars (1–6 terminals per species, 8 species total), and four individuals of *Nephila pilipes* as the outgroup (Table 1). We emphasized the richness in geographic terminal coverage over that of using more genes with
| Species | Area     | COI—GenBank | COI—BOLD | Original material codes | Lat.  | Lon.   |
|---------|----------|-------------|----------|------------------------|-------|--------|
| clavipes | Brazil   | MF476261    |          | −21.59                 | −47.52|
| clavipes | Brazil   | MF476266    |          | −22.31                 | −52.19|
| clavipes | Brazil   | MF476270    |          | −22.31                 | −52.19|
| clavipes | Brazil   | MF476272    |          | −22.31                 | −52.19|
| clavipes | Brazil   | MF476278    |          | −21.47                 | −48.52|
| clavipes | Brazil   | MF476282    |          | −25.09                 | −47.55|
| clavipes | Brazil   | MF476284    |          | −25.09                 | −47.55|
| clavipes | Brazil   | MF476286    |          | −25.09                 | −47.55|
| clavipes | Brazil   | MF476296    |          | −23.52                 | −46.20|
| clavipes | Brazil   | MF476298    |          | −21.46                 | −46.34|
| clavipes | Brazil   | MF476304    |          | −21.46                 | −46.34|
| clavipes | Brazil   | MF476305    |          | −21.46                 | −46.34|
| clavipes | Brazil   | MF476306    |          | −18.57                 | −48.12|
| clavipes | Brazil   | MF476308    |          | −18.57                 | −48.12|
| clavipes | Brazil   | MF476309    |          | −18.57                 | −48.12|
| clavipes | Brazil   | MF476312    |          | −18.57                 | −48.12|
| clavipes | Brazil   | MF476315    |          | −22.43                 | −46.33|
| clavipes | Brazil   | MF476319    |          | −22.43                 | −46.33|
| clavipes | Brazil   | MF476322    |          | −22.41                 | −46.39|
| clavipes | Brazil   | MF476329    |          | −22.41                 | −46.39|
| clavipes | Brazil   | MF476330    |          | −22.42                 | −51.23|
| clavipes | Brazil   | MF476342    |          | −29.25                 | −50.51|
| clavipes | Brazil   | MF476357    |          | −12.56                 | −49.09|
| clavipes | Brazil   | MF476369    |          | −15.56                 | −47.56|
| clavipes | Brazil   | MF476370    |          | −15.56                 | −47.56|
| clavipes | Brazil   | MF476371    |          | −15.56                 | −47.56|
| clavipes | Brazil   | MF476373    |          | −22.22                 | −44.57|
| clavipes | Brazil   | MF476374    |          | −22.22                 | −44.57|
| clavipes | Brazil   | MF476376    |          | −22.22                 | −44.57|
| clavipes | Brazil   | MF476377    |          | −22.22                 | −44.57|
| clavipes | Brazil   | MF476390    |          | −3.05                  | −59.58|
| clavipes | Brazil   | MF476391    |          | −3.05                  | −59.58|
| clavipes | Brazil   | MF476400    |          | −2.31                  | −54.56|
| clavipes | Brazil   | MF476401    |          | −2.31                  | −54.56|
| clavipes | Brazil   | MF476439    |          | −19.49                 | −40.15|
| clavipes | Brazil   | MF476442    |          | −19.49                 | −40.15|
| clavipes | Brazil   | MF476451    |          | −12.34                 | −38.01|
| clavipes | Brazil   | MF476455    |          | −23.02                 | −44.34|
| clavipes | Brazil   | MF476458    |          | −23.02                 | −44.34|
| clavipes | Brazil   | MF476473    |          | −19.52                 | −43.58|
| clavipes | Brazil   | MF476474    |          | −19.52                 | −43.58|
| clavipes | Brazil   | MF476475    |          | −19.52                 | −43.58|
| clavipes | Brazil   | MF476482    |          | −20.45                 | −42.51|
| Species | Area            | COI—GenBank | COI—BOLD | Original material codes | Lat.  | Lon.  |
|---------|----------------|-------------|----------|------------------------|-------|-------|
| clavipes | Brazil         | MF476491    |          | −21.46                 | −43.22 |
| clavipes | Brazil         | MF476493    |          | −18.06                 | −47.37 |
| clavipes | Brazil         | MF476495    |          | −0.52                  | −46.39 |
| clavipes | Brazil         | MF476514    |          | −27.03                 | −49.32 |
| clavipes | Brazil         | MF476515    |          | −27.03                 | −49.32 |
| clavipes | Brazil         | MF476516    |          | −27.03                 | −49.32 |
| clavipes | Brazil         | MF476519    |          | −27.03                 | −49.32 |
| clavipes | Brazil         | MF476522    |          | −22.24                 | −47.33 |
| clavipes | Brazil         | MF476523    |          | −22.24                 | −47.33 |
| clavipes | Brazil         | MF476537    |          | −25.39                 | −54.26 |
| clavipes | Brazil         | MF476542    |          | −25.39                 | −54.26 |
| clavipes | Brazil         | MF476545    |          | −10.17                 | −67.45 |
| clavipes | Brazil         | MF476552    |          | −10.17                 | −67.45 |
| clavipes | Brazil         | MF476573    |          | −2.24                  | −59.43 |
| clavipes | Colombia       | MF476560    |          | 4.06                   | −77.29 |
| clavipes | Colombia       | MF476561    |          | 4.06                   | −77.29 |
| clavipes | Colombia       | MF476562    |          | 4.06                   | −77.29 |
| clavipes | Colombia       | MF476563    |          | 4.10                   | −76.12 |
| clavipes | Colombia       | MF476564    |          | 4.10                   | −76.12 |
| clavipes | Costa Rica     | MK161001    | GBA16660-14 | 00003178A             | 10.43 | −84.00 |
| clavipes | French Guiana  |             |          | 5.49                   | −54.02 |
| clavipes | Hispaniola     | MK161015    |          | 18.35                  | −68.61 |
| clavipes | Hispaniola     | MK161016    |          | 18.35                  | −68.61 |
| clavipes | Hispaniola     | MK161018    |          | 18.32                  | −68.81 |
| clavipes | Hispaniola     | MK161019    |          | 00785163               | 19.69 | −71.26 |
| clavipes | Hispaniola     | MK161020    |          | 007854590              | 19.06 | −69.46 |
| clavipes | Hispaniola     | MK161026    |          | 18.07                  | −71.11 |
| clavipes | Hispaniola     | MK161028    |          | 18.07                  | −71.11 |
| clavipes | Hispaniola     | MK161029    |          | 18.07                  | −71.11 |
| clavipes | Hispaniola     | MK161031    |          | 18.32                  | −71.58 |
| clavipes | Hispaniola     | MK161032    |          | 18.32                  | −71.57 |
| clavipes | Jamaica        | MK161002    |          | 18.27                  | −77.66 |
| clavipes | Jamaica        | MK161003    |          | 18.27                  | −77.66 |
| clavipes | Mexico         | MK161004    |          | 18.47                  | −95.33 |
| clavipes | Mona           | MK161017    |          | 18.08                  | −67.90 |
| clavipes | Mona           | MK161021    |          | 18.08                  | −67.90 |
| clavipes | Mona           | MK161022    |          | 18.08                  | −67.93 |
| clavipes | Mona           | MK161023    |          | 18.08                  | −67.93 |
| clavipes | Mona           | MK161024    |          | 18.08                  | −67.93 |
| clavipes | Mona           | MK161025    |          | 00786904               | 18.08 | −67.93 |
| clavipes | Mona           | MK161027    |          | 18.08                  | −67.93 |
| clavipes | Mona           | MK161030    |          | 18.08                  | −67.93 |
| clavipes | Panama         | GBCH3892-09 |          | 9.16                   | −79.83 |

(Continues)
| Species | Area           | COI—GenBank | COI—BOLD   | Original material codes | Lat.  | Lon.   |
|---------|----------------|-------------|------------|-------------------------|-------|--------|
| clavipes | Panama         | GBCH7190-13 |            |                         | 9.16  | −79.83 |
| clavipes | Puerto Rico    | MK161008    | 00392678   |                         | 17.97 | −66.88 |
| clavipes | Puerto Rico    | MK161009    | 00392719   |                         | 18.45 | −66.6  |
| clavipes | Puerto Rico    | MK161010    | 00392722   |                         | 17.97 | −66.88 |
| clavipes | Puerto Rico    | MK161011    | 00392766   |                         | 17.97 | −66.88 |
| clavipes | Puerto Rico    | MK161012    | 00392851   |                         | 17.97 | −66.88 |
| clavipes | Puerto Rico    | MK161013    | 00782148   |                         | 18.45 | −66.6  |
| clavipes | Puerto Rico    | MK161014    | 00782167   |                         | 18.45 | −66.6  |
| clavipes | Turks and Caicos | MK161005 | 00006021A  |                         | 21.74 | −71.74 |
| clavipes | Turks and Caicos | MK161006 | 00006183A  |                         | 21.74 | −71.74 |
| clavipes | Turks and Caicos | MK161007 | 00006203A  |                         | 21.74 | −71.74 |
| clavipes | USA            | BBUSE1911-12 |            |                         | 29.38 | −95.59 |
| clavipes | USA            | BBUSE1912-12 |            |                         | 29.38 | −95.59 |
| clavipes | USA            | BBUSE1913-12 |            |                         | 29.38 | −95.59 |
| clavipes | USA            | BBUSE1914-12 |            |                         | 29.38 | −95.59 |
| clavipes | USA            | BBUSE1940-12 |            |                         | 29.38 | −95.59 |
| clavipes | USA            | BBUSE2941-12 |            |                         | 29.38 | −95.59 |
| clavipes | USA            | BBUSU1034-15 |            |                         | 29.38 | −95.59 |
| clavipes | USA            | BBUSU1042-15 |            |                         | 29.38 | −95.59 |
| clavipes | USA            | BBUSU1043-15 |            |                         | 29.38 | −95.59 |
| clavipes | USA            | BBUSU1044-15 |            |                         | 29.38 | −95.59 |
| clavipes | USA            | BBUSU1045-15 |            |                         | 29.38 | −95.59 |
| clavipes | USA            | BBUSU1046-15 |            |                         | 29.38 | −95.59 |
| clavipes | USA            | BBUSU1048-15 |            |                         | 27.20 | −81.32 |
| clavipes | USA            | BBUSU1093-15 |            |                         | 27.20 | −81.32 |
| clavipes | USA            | BBUSU1094-15 |            |                         | 27.20 | −81.32 |
| clavipes | USA            | BBUSU1095-15 |            |                         | 27.20 | −81.32 |
| clavipes | USA            | BBUSU1096-15 |            |                         | 27.20 | −81.32 |
| clavipes | USA            | BBUSU1097-15 |            |                         | 27.20 | −81.32 |
| clavipes | USA            | BBUSU1099-15 |            |                         | 27.20 | −81.32 |
| clavipes | USA            | BBUSU564-15  |            |                         | 27.26 | −82.28 |
| clavipes | USA            | BBUSU603-15  |            |                         | 27.24 | −82.30 |
| clavipes | USA            | BBUSU617-15  |            |                         | 27.24 | −82.30 |
| clavipes | USA            | BBUSU662-15  |            |                         | 27.24 | −82.30 |
| clavipes | USA            | MK160998     | 00000695A  |                         | 33.04 | −79.56 |
| clavipes | USA            | MK160999     | 00001094A  |                         | 33.04 | −79.56 |
| clavipes | USA            | MK161000     | 00001139A  |                         | 29.47 | −82.55 |
| clavata  | China          | GBA16309-14  |            |                         |       |        |
| clavata  | China          | GBCH7192-13  |            |                         |       |        |
| clavata  | Japan          | GBCH7191-13  |            |                         |       |        |
| clavata  | Taiwan         | GBCH7193-13  |            |                         |       |        |
| clavata  | USA            | GBA24390-15  |            |                         |       |        |
| clavata  | USA            | GBA24391-15  |            |                         |       |        |
fewer terminals. Using COI to resolve phylogeographic questions is a common and valid approach (Bartoleti et al., 2018; Čandek & Kuntner, 2015; Su et al., 2006).

3.2 | Phylogenetic reconstructions

To identify the best priors for the reconstruction of ultrametric phylogenies, we performed stepping-stone sampling and Bayes factor test (Baele et al., 2012) within BEAST2 (Bouckaert et al., 2014). We reduced the total data set (Table 1) and constrained the topology according to a phylogenomic hypothesis (Kuntner et al., 2019). Model tests (Note S2) selected a strict clock with the rate 0.0112 (Bidegaray-Batista & Arnedo, 2011) and a coalescent constant population tree prior. We used bModelTest (Bouckaert & Drummond, 2017) as nucleotide substitution model and ran a MCMC chain for 30 million generations. We discarded 20% of the samples as burn-in.

| Species   | Area                | COI—GenBank | COI—BOLD | Original material codes | Lat. | Lon. |
|-----------|---------------------|-------------|----------|-------------------------|------|------|
| fenestrata| S. African Rep.     | AUSPI296-14 |          |                         |      |      |
| fenestrata| S. African Rep.     | GBA17451-14 |          |                         |      |      |
| fenestrata| S. African Rep.     | GBCH7186-13 |          |                         |      |      |
| inaurata  | Madagascar          | GBA17318-14 |          |                         |      |      |
| inaurata  | Madagascar          | GBCH5857-13 |          |                         |      |      |
| inaurata  | Madagascar          | RNOCF133-17 |          |                         |      |      |
| inaurata  | Mauritius           | GBCH5828-13 |          |                         |      |      |
| inaurata  | Mayotte             | GBCH5838-13 |          |                         |      |      |
| inaurata  | S. African Rep.     | GBCH7185-13N|         |                         |      |      |
| komaci    | S. African Rep.     | GBA17452-14 |          |                         |      |      |
| pilipes   | Australia           | GBCH5872-13 |          |                         |      |      |
| pilipes   | Indonesia           | GBA16765-14 |          |                         |      |      |
| pilipes   | Indonesia           | GBMIN114575-17 |      |                         |      |      |
| pilipes   | Taiwan              | GBCH7183-13 |          |                         |      |      |
| plumipes  | Australia           | GBCH7183-13 |          |                         |      |      |
| plumipes  | Australia           | GBCH7181-13 |          |                         |      |      |
| plumipes  | Australia           | GBCH7182-13 |          |                         |      |      |
| plumipes  | Australia           | GBMIN117428-17 |      |                         |      |      |
| senegalens| S. African Rep.     | AUSPI344-14 |          |                         |      |      |
| senegalens| S. African Rep.     | GBA17453-14 |          |                         |      |      |
| senegalens| S. African Rep.     | GBCH7180-13 |          |                         |      |      |
| sexpunctata| Argentina          | GBMIN116269-17 |      |                         |      |      |
| sexpunctata| Argentina          | GBMIN116272-17 |      |                         |      |      |
| sexpunctata| Brazil             | GBMIN116252-17 |      |                         |      |      |
| sexpunctata| Brazil             | GBMIN116261-17 |      |                         |      |      |
| sexpunctata| Brazil             | GBMIN116303-17 |      |                         |      |      |
| sumpituosa| Yemen               | GBA17823-14 |          |                         |      |      |

Note: Specimens written in bold were used in species delimitation analyses.
We reconstructed a Bayesian phylogeny using the reduced data set as above in MrBayes (Huelsenbeck & Ronquist, 2001), running four independent MCMC chains with 30 million generations, 25% burn-in and a sampling frequency of every 1,000. Based on jModelTest 2 (Darriba, Taboada, Doallo, & Posada, 2012), we used GTR + G + I as the nucleotide substitution model. Finally, we ran another Bayesian phylogeny for the T. clavipes ingroup only, with settings and model selection as above, except with 10 million generations.

### 3.3 Species delimitation analyses

For species delimitation analyses, we employed three methods, the Generalized Mixed Yule Coalescent (GMYC; Fujisawa & Barraclough, 2013), the Multi-rate Poisson Tree Processes (mPTP; Kapli et al., 2017) and the Automatic Barcode Gap Discovery (ABGD; Puillandre, Lambert, Brouillet, & Achaz, 2012). We ran GMYC delimitations in “splits” package of R version 3.5.1. (Ezard, Fujisawa, & Barraclough, 2009), using the ultrametric tree and testing single, as well as multiple thresholds settings. We ran mPTP delimitations online using default settings and with ultrametric as well as Bayesian trees. For ABGD delimitations, we uploaded fasta sequences to its online platform and tested all three implemented substitution models (JC69, K80 and Simple distance). Here, we present a GMYC delimitation result using the best model for the data (Figure 1), while 14 additional delimitation results (based on suboptimal models) are in the supplementary material (Figures S1–S14).

We performed DNA barcoding analyses according to Čandek & Kuntner (2015). We used MEGA X (Kumar, Stecher, Li, Knyaz, & Tamura, 2018) to calculate pairwise K2P genetic distances among all ingroup individuals of the reduced Trichonephila data set (as above; Table 1). First, we treated T. clavipes as one species and then, based on the results from our previous species delimitation analysis (Figure 1), as two separate species (Figures S15A, C). We also calculated pairwise genetic distances using the complete haplotype data set of T. clavipes to compare intraspecific distances in T. clavipes across its entire range to intraspecific distances of T. clavipes populations residing in the area of interest (continental North America + the Caribbean; Figure S15D vs. 15E).

### 3.4 Haplotype network reconstruction

We used “pegas” package in R (Paradis, 2010) to reconstruct a haplotype network of all 127 T. clavipes individuals from 12 areas. We trimmed the sequences to equal lengths, resulting in 537 remaining nucleotides. The sizes of circles in the reconstructed network correspond to the frequency of a specific haplotype.

### 3.5 Population genetics

We used DNAsp v6.12 (Rozas et al., 2017) to calculate gene flow and genetic differentiation estimates and to export haplotype information in Arlequin format for further analyses. We then used Arlequin v3.5 (Excoffier & Lischer, 2010) to perform the analyses of molecular variance (AMOVA) among selected populations (North America vs. Caribbean islands, North America + Caribbean islands vs. South + Central America) using default settings and 999 permutations to test for statistical significance.

### 3.6 Isolation by distance

We used MEGA X (Kumar et al., 2018) to calculate pairwise K2P distances among individuals from selected populations (combined/all data, North America and the Caribbean, South and Central America). We used Geographic Distance Matrix Generator v1.2.3 (Ersts, 2014) to calculate geographic distances (in km) among individuals from selected populations (as above). We then used the “vegan” package in R (Oksanen et al., 2013) to perform Mantel tests that calculate correlations among distance matrices.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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