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A taxonomist's nightmare – Cryptic diversity in Caribbean intertidal arthropods (Arachnida, Acari, Oribatida)

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ABSTRACT

There has been a long controversy about what defines a species and how to delimitate them which resulted in the existence of more than two dozen different species concepts. Recent research on so-called "cryptic species" heated up this debate as some scientists argue that these cryptic species are only a result of incompatible species concepts. While this may be true, we should keep in mind that all concepts are nothing more than human constructs and that the phenomenon of high phenotypic similarity despite reproductive isolation is real. To investigate and understand this phenomenon it is important to classify and name cryptic species as it allows to communicate them with other fields of science that use Linnaean binomials. To provide a common framework for the description of cryptic species, we propose a possible protocol of how to formally name and describe these taxa in practice. The most important point of this protocol is to explain which species concept was used to delimitate the cryptic taxon. As a model, we present the case of the allegedly widespread Caribbean intertidal mite *Thalassozetes barbara*, which in fact consists of seven phenotypically very similar but genetically distinct species. All species are island or short-range endemics with poor dispersal abilities that have evolved in geographic isolation. Stabilizing selection caused by the extreme conditions of the intertidal environment is suggested to be responsible for the morphological stasis of this cryptic species complex.

1. Introduction

Species are one of the fundamental units of biology making organisms comparable in various biological aspects (e.g. Mayr, 1982). In this sense, species are nothing more than a human construct allowing biologists to classify and compare organisms. For that purpose, it is vital to define exactly what a 'species' is, but different groups of biologists advocate different definitions (e.g. Harrison, 1998) and so more than two dozen of sometimes incompatible species concepts exist (de Queiroz, 2007). This resulted in seemingly endless debates between advocates of the different concepts whereas de Queiroz (2007) proposed a unified species concept to end this debate. But his concept, which defines a species as a separately evolving metapopulation lineage, has remained widely neglected. The most commonly used concept is still the biological species concept, that defines a species as a group of organisms with natural reproduction resulting in viable and fertile offspring (e.g. Mayr, 1940). The problem with this concept is that it only applies to sexually reproducing organisms and it needs proof of successful reproduction which is difficult if animals are not collected alive and bred in the laboratory. Most faunistic studies collecting specimens in the field and investigating afterwards the preserved specimens in the laboratory will not be able to apply the biological species concept and must rely on other concepts. Applied taxonomy mostly uses the morphological species concept which defines a species as a group of individuals that show the same morphological characteristics and thus can be delimitated from other groups on the base of morphological differences (e.g. Ax, 1984). However, there are certain cases in which morphological characters alone do not allow reliable determination and hence challenge taxonomists. The phenomenon of morphological conformity in genetically, ecologically or otherwise recognizable lineages is known as cryptic diversity. Cryptic species are defined as species that are difficult to distinguish using traditional morphology-based taxonomic methods (Knowlton, 1993), or species that are classified as a single nominal species because they are at least superficially morphologically identical (Bickford et al., 2007). The biological species or other concepts may still apply to these taxa but not the morphospecies concept, which brings us back to the discussion about the definition of a species. Among biologists, there are varying opinions as to the existence of cryptic species.

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Some scientists claim that cryptic species are nothing more than an incompatibility of species concepts (e.g. Heethoff, 2018) because the term 'cryptic' only refers to morphology and some concepts do not include the morphological aspect at all. Indeed, prioritizing one species concept over the other may result in cryptic diversity, i.e. according to the one concept there is only one species while according to another concept there may be more (Heethoff, 2018). There are many other biological features of an organism that may be responsible for reproductive isolation, as for example ecological, biochemical, acoustic characters, and thus may be used for species delimitation. The relative importance of each biological character for a species concept is again a product of the human mind and therefore, it is the focus on morphology that creates 'cryptic species' as defined above by Knowlton (1993) or Bickford et al. (2007). Nevertheless, the phenomenon of high phenotypic similarity despite restricted or even a complete lack of gene flow is real and could be well demonstrated in various cases (e.g. Fennessy et al., 2016; Struck et al., 2018b; Schäffer et al., 2019). Even though cryptic species may be an artificial construct, the evolutionary processes leading to phenotypic similarity are not and should be further investigated. Studying these cases may allow us to better understand evolutionary processes like parallelism, convergence and stasis (Struck et al., 2018a, Struck and Cerca, 2019). To investigate these cases in a proper way, it is necessary to establish these taxa in our classification system, which means they should be named and classified because otherwise, biological data will lose value as it is linked to unnamed vague biological groups and scientists will not be sure if they are talking about the same taxon (Pante et al., 2015). Moreover, taxa need to be named for being included in conservation programs (Delić et al., 2017) and no matter if reproductively isolated species show diverging morphologies or not, they need the same attention in terms of conservation.

Research on cryptic diversity has intensified over the last two decades and the existence of cryptic species was demonstrated in various animal groups, as for example in hydrozoans (Holland et al., 2004), arachnids (Crews and Gillespie, 2010; Knee et al., 2012; McHugh et al., 2014; Pfingstl et al., 2014; Dziki et al., 2015; Schäffer and Koblmüller, 2020), insects (Hebert et al., 2004; Williams et al., 2006; Zangl et al., 2021), crustaceans (Lee, 2000; Lefébure et al., 2006; Belyaeva and Taylor, 2009), amphibians (Stuart et al., 2006), reptiles (Smith et al., 2011) and fish (Colborn et al., 2001; Wagner et al., 2019). Due to the increasing use of integrative approaches and advanced methods, the number is continuously growing (Knee et al., 2012). Nevertheless, many authors refrain from giving cryptic taxa species names, others do, but fail to explain what species concept was used to erect the new taxon. The latter further fuels the conflict about cryptic species being valid species, therefore, describing a cryptic species should always be based on a specific species concept, so that it can be treated like any other species. In this way, research on the evolutionary processes causing hidden diversity is performed in a common system of reference that allows better comparison.

Despite the recent increase of detected cryptic species, the underlying evolutionary mechanisms are still poorly understood. In theory, several processes could lead to the evolution of cryptic species. First, recent speciation may result in phenotypic similarity as detectable morphological traits have yet to appear (Holland et al., 2004). Second, extreme or homogeneous habitat conditions might impose stabilizing selection on morphology, resulting in highly conserved morphological traits (Colborn et al., 2001; Lefébure et al., 2006; Bickford et al., 2007). And third, evolutionary convergence and parallelism may also result in similar morphotypes across distantly related lineages (Holland et al., 2004; Belyaeva and Taylor, 2009; Struck and Cerca, 2019).

Regardless of biological definition and evolutionary processes, cryptic diversity concerns specialists in a broad range of scientific fields and is supposed to be responsible for gross underestimates of biodiversity in various taxa (Bickford et al., 2007; Pfenninger and Schwenk, 2007; Skoracka et al., 2015). Cryptic species are almost evenly distributed among major metazoan taxa and biogeographic areas when corrected for study intensity, and thus the phenomenon of hidden diversity is even thought to represent an evolutionary constant (Pfenninger and Schwenk, 2007).

Although cryptic speciation (meaning speciation without morphological diversification) is supposed to occur in all biogeographic regions (Pfenninger and Schwenk, 2007), some authors argue that tropical rainforests and marine habitats represent key targets for investigating this phenomenon, because they are the most species rich habitats on the globe and thus the probability of finding cryptic species is also higher (Holland et al., 2004; Bickford et al., 2007). The Caribbean, which offers both, lush tropical rainforest and pristine marine habitats, is known to harbor several cryptic species complexes. Examples are the orb-weaver spider *Micrathena*, in which six nominal species consist of eight divergent genetic lineages that are probably all single island endemics (McHugh et al., 2014), the skipper butterfly *Astraptes fulgerator*, which has long been regarded as a single species but then was suggested to contain ten separate species (Hebert et al., 2004), or the cobweb spider *Spintharus flavidus*, previously presumed to be a single widespread species that turned out to represent a complex of at least 16 different species (Dziki et al., 2015) and the intertidal mite *Carinozetes mangrovi*, that shows a *trans*-Caribbean distribution but consists of three distinct genetic lineages (Pfingstl et al., 2019a). This diversity is due to the complex geological history of the Caribbean area, characterized by continental islands, which broke off from the mainland, land-bridge islands, which were connected to the continent, uplifted limestone islands and volcanic islands (Iturralde-Vinent, 2006). In general, Caribbean biota show high levels of endemism and only a relatively small percentage of the Caribbean biodiversity is represented by widespread species, presumably taxa with excellent dispersal abilities (Dziki et al., 2015). Accordingly, allegedly widespread Caribbean taxa that are poor dispersers are likely to contain hidden species complexes as shown in the examples mentioned above.

Thalassozetes barbara Pfingstl, 2013, a small intertidal sexually reproducing mite, may in fact represent such a case and thus may serve as excellent case study to investigate the causes of cryptic diversity and to demonstrate how to classify such taxa to make them available and comparable with other non-cryptic taxa. This species is a member of the intertidal oribatid mite family Selenoribatidae, which has successfully colonized the extreme intertidal environment of subtropical and tropical shorelines, where it feeds on marine associated algae (Pfingstl, 2017). These animals are still air breathing but use elaborate plastron respiration to withstand daily tidal flooding (Pfingstl and Krisper, 2014). *Thalassozetes barbara* was the first officially described intertidal mite species of the whole Caribbean and was found on the coast of Barbados (Pfingstl, 2013a). Over the last decade subsequent records were made at various Caribbean locations resulting in a theoretical distribution pattern ranging from the Bahamas to the Greater Antilles and finally to the Lesser Antilles close to the coast of South America (Pfingstl, 2021). Given the very small size (approx. 0.3 mm), the wingless body and the complete lack of active dispersal behavior of these intertidal arthropods, this wide distribution is puzzling and difficult to explain.

However, littoral oribatids are able to survive submerged in seawater for even more than a month (Pfingstl, 2013b). Accordingly, long distance transport is suggested to happen mainly by hydrochory (Schatz, 1991; Pfingstl, 2013b; 2017), i.e. drifting along ocean currents. In fact, *Thalassozetes balboa* Pfingstl, Lienhard & Baumann, 2019, a recently discovered species, was found in Panama as well as in Florida and hence may show a vast distribution range in the western Caribbean. Additionally, it was suggested that transport along the Gulf Stream could have facilitated dispersal along the Central American shoreline (Pfingstl et al., 2019b). The allegedly wide distribution of the Eastern Caribbean *T. barbara* could also be the result of hydrochory, but the frequency of drifting events as well as the real probability of effective gene-flow between populations of distant islands is unknown and hence the status of *T. barbara* as a single nominal species remains questionable.

Here, we present a comprehensive morphometric and molecular

genetic investigation of Caribbean *T. barbara* populations to clarify and assess the amount of diversity hidden within this group and to provide new distribution patterns and first extensive phylo- as well as biogeographic insights into this group of intertidal Caribbean arthropods. Additionally, we emphasize the importance of naming cryptic taxa and propose a possible way of how to describe such species in practice.

2. Material and methods

2.1. Sample collection and preparation

In February 2016 and February 2017, animals were collected on two different field trips to selected Caribbean areas. Samples of intertidal algae (patches of approx. 10 cm^2) were scraped off the substrate (e.g. rock, mud, mangrove roots etc.) with a knife during low tide and put in a Berlese-Tullgren funnel to extract the mites. Extracted specimens were stored in > 99% ethanol for transport and further investigation. A complete list of sample locations is given in the Appendix A.

2.2. PCR and sequencing

In total, 117 specimens of Caribbean *Thalassozetes* spp. were analysed (see Appendix A). Total genomic DNA was extracted from single individuals preserved in > 99% ethanol. Extraction was carried out using a Chelex-based method (Casquet et al., 2012) with some adjustments for small arthropods (Lienhard and Schäffer, 2019). Samples were extracted for 3–4 hr at 56 °C. Three gene fragments were sequenced for this study: the mitochondrial *cytochrome c oxidase subunit 1* gene (*COI*; N = 117), the nuclear *elongation factor 1 alpha* gene (*EF-1α*; N = 55), and the nuclear *18S* rRNA gene (*18S*; N = 42). Even though PCR amplification of the nuclear markers failed in part of the samples, all main lineages/islands are also represented in the nuclear data. A 567 bp fragment of the *COI* gene was amplified using the primer pairs Mite COI 2F and Mite COI 2R (Otto and Wilson, 2001), and for amplifying 513 bp of the *EF-1α* gene, the primers 40.71F and 52.RC (Regier and Shultz, 1997) were used. The complete *18S* (~1.8 kb) was amplified in two overlapping fragments according to the PCR protocol of (Dabert et al., 2010), using the recommended primers (Skoracka and Dabert, 2010). PCR conditions for the *COI* gene fragment are given in (Pfingstl et al., 2014) and those for the *EF-1α* gene fragment in (Lienhard et al., 2014). DNA purification with ExoSAP-IT (Affymetrix), cycle sequencing using the BigDye Sequence Terminator v3.1 Cycle Sequencing chemistry (Applied Biosystems) and cycle sequencing product purification with Sephadex G 50 (FE Healthcare) were conducted following (Schäffer et al., 2008). Sequencing was performed in both directions and sequences were visualized on an automated capillary sequencer (ABI PRISM 3130xl, Applied Biosystems). All sequences obtained from this study were deposited in GenBank (www.ncbi.nlm.nih.gov/genbank; accession numbers for *COI*: MZ169923-MZ170038, *EF-1α*: MZ220224-MZ220278, and *18S*: MZ220279-MZ220318; more details are given in the Appendix A).

2.3. Phylogenetic analysis

Electropherograms were checked by eye and sequences were aligned using MUSCLE (Edgar, 2004) as implemented in MEGA6 (Tamura et al., 2013), employing the default settings. Gene fragments were analyzed individually and as a concatenated dataset comprising mtDNA and nucDNA (*COI*, *EF-1α* and *18S*, 2882 bp). The best fitting models of molecular evolution were selected based on the Bayesian Information Criterion (BIC) in Modelfinder (Kalyaanamoorthy et al., 2017) (*COI*, HKY + I; *EF-1α*, HKY + I; *18S*, HKY + I). For all gene fragments and the concatenated dataset (partitioned by gene), both a Bayesian inference (BI) and Maximum Likelihood (ML) tree were inferred in MrBAYES 3.1.2 (Ronquist and Huelsenbeck, 2003) and RAxML (Stamatakis, 2014) via raxmlGUI 2.0.0 (Endler et al., in press), respectively. MrBayes analyses applied a MC^3 simulation with 10 million generations (2 independent runs, 6 chains, 25% burn-in, best fitting substitution model for each gene). The average standard deviation of split frequencies (<0.01 in all analyses) was used to assess whether runs were run long enough. In addition, the resulting log-files were analysed in Tracer 1.6 (Rambaut and Drummond, 2007) to check for convergence and to ensure stationarity of all parameters. RAxML was run using the ML + rapid bootstrap setting with the GTRGAMMA substitution model (for all genes) 10,000 bootstrap replicates.

2.4. Species delimitation

Single-locus species delimitation was performed by applying different approaches on the full *COI* dataset (117 specimens): (1) the distance based Automatic Barcode Gap Discovery method (ABGD; Puillandre et al., 2012), and the tree-based (2) Bayesian Poisson Tree Processes model (bPTP; Zhang et al., 2013), (3) single threshold general mixed Yule coalescent model (sGMYC; Pons et al., 2006), and (4) the Bayesian general mixed Yule coalescent model (bGMYC; Reid and Carstens, 2012). In addition, we employed a Bayesian multilocus species delimitation method, based on all our three loci, as implemented in Bayesian Phylogenetics and Phylogeography (BPP 4.1; Rannala and Yang, 2003; Yang and Rannala, 2010)

The ABGD analysis was conducted via the ABGD web server (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html). We used simple distances and ran ABGD under following parameter settings: pmin = 0.005, pmax = 0.1, X (relative gap width) = 1.0, no. of steps = 20. We recorded the hypothetical species assignments over 20 recursions.

The bPTP analysis was conducted via the bPTP web server (https://species.h-its.org/ptp/), applying 500,000 MCMC generations and using the BI tree as input tree.

The sGMYC analysis was conducted on the GMYC web server (https://species.h-its.org/gmyc/). GMYC requires and ultrametric input tree. Therefore, the BEAST 2.5.1 package (Bouckaert et al., 2014) was used to infer an ultrametric tree. We ran two MC^3 simulation with 200 million generations, sampling every 1000th tree (of which 10% were discarded as burn-in), applying the best-fitting substitution model, a birth–death tree prior, and a strict clock (a clock-like evolution could not be rejected at the 0.05 significance level by means of likelihood ratio tests in TREE-PUZZLE 5.3; Schmidt et al., 2002). Tracer 1.6 was used to verify the chains had reached stationarity. Treefiles were combined using LogCombiner (implemented in the BEAST2 package) and TreeAnnotator (implemented in the BEAST2 package) was used to calculate a maximum clade credibility (MCC) tree from the post burn-in tree sample. A single-threshold was employed for GMYC analysis due to its generally better performance in delimitating species as compared to the multi-threshold GMYC approach (Fujisawa and Barraclough, 2013).

The bGMYC analysis was conducted on 500 posterior trees from the BEAST analysis and run (MCMC = 50,000; burnin = 40,000; thinning = 100) in R v3.6.0 (R Core Team, 2013) using the package bGMYC v.1.0.2 (Reid & Carstens 2012).

For the BPP analysis, the heredity scalar was set to 0.25 and 1.0 for the mitochondrial and nuclear loci, respectively. Multiple unguided (i.e. without a guide tree; Rannala and Yang, 2017) analyses were run with varying prior combinations for ancestral population size (θ) and divergence time (τ) (Leache and Fujita, 2010), to account for the effects of prior settings on the number of inferred species (different configurations assume different levels of gene tree discordance): i) large ancestral population size and deep divergence ($\theta \sim$ IG (3, 0.4), $\tau \sim$ IG (3, 0.4)); ii) large ancestral population size and intermediate divergence ($\theta \sim$ IG (3, 0.4), $\tau \sim$ IG (3, 0.04)); iii) large ancestral population size and shallow divergence ($\theta \sim$ IG (3, 0.4), $\tau \sim$ IG (3, 0.004)); iv) intermediate ancestral population size and deep divergence ($\theta \sim$ IG (3, 0.04), $\tau \sim$ IG (3, 0.4)); v) intermediate ancestral population size and intermediate divergence ($\theta \sim$ IG (3, 0.04), $\tau \sim$ IG (3, 0.04)); vi) intermediate ancestral population size and shallow divergence ($\theta \sim$ IG (3, 0.04), $\tau \sim$ IG (3, 0.004)); vii)

small ancestral population size and deep divergence ($\theta \sim$ IG (3, 0.004), $\tau \sim$ IG (3, 0.4)); viii) small ancestral population size and intermediate divergence ($\theta \sim$ IG (3, 0.004), $\tau \sim$ IG (3, 0.04)); and ix) small ancestral population size and shallow divergence ($\theta \sim$ IG (3, 0.004), $\tau \sim$ IG (3, 0.004)). The prior assignment of individuals to a maximum number of hypothesized species was based on the identified mitochondrial lineages / maximum number of species delimited based on the *COI* data. The analyses were run twice for 50,000 generation (following a burn-in of 10,000 steps), sampled every 10 steps.

For all markers, maximum intraspecific and minimum interspecific distances, based on uncorrected p-distances, were calculated using SPIDER 1.5.0 (Brown et al., 2012) in R v.3.6.0. SPIDER was also used to identify diagnostic nucleotides in the *COI* gene.

2.5. Species tree

A multispecies coalescent analysis based on all three loci was conducted in StarBEAST2, implemented in BEAST2 version 2.5.1 (Bouckaert et al., 2014). Eight putative species (see discussion for why we assigned the samples to these eight putative species) were predefined and data was partitioned by gene (best-fitting substitution models *COI*: HKY + I; *EF-1α*: HKY + I; *18S*: HKY + I). As likelihood ratio tests in TREE-PUZZLE 5.3.rc16 (Schmidt et al., 2002) did not reject a clock-like evolution for any of the three loci, we applied the strict-clock model for all three genes. The Birth-Death model was selected as tree prior. Three independent replicates were run with random starting seeds and $2x10^8$ generations, sampled every 20,000 generations, and discarding the first 10% as burn-in. The effective sample sizes (ESS) of parameters were checked in Tracer, runs were combined using LogCombiner (part of the BEAST2 package), and the species tree was visualized as a cloudogram in DensiTree2 (part of the BEAST2 package).

2.6. Intra-island diversity

To infer the genetic structure within main lineages, statistical parsimony haplotype networks based on the *COI* data were inferred using the program PopART (Leigh and Bryant, 2015), applying the default settings.

2.7. Morphometric investigations

Specimens were embedded in lactic acid for temporary slides and measurements were performed using a compound light microscope (Olympus BH-2) and ocular micrometer. Twenty continuous variables (Supplementary Fig. S1) were measured in 91 presumed *Thalassozetes barbara* individuals from six Caribbean islands (Barbados, Curaçao, Grenada, Martinique, Guadeloupe and New Providence Island/ Bahamas) and in 46 *T. balboa* specimens from Central America (Panama). As specimens were destroyed for DNA-extraction, these specimens, used here for morphometry, were different individuals but originated from the exact same samples (10 cm^2 patch of algae), meaning they belonged to the same population. There were not enough specimens from the Dominican Republic available for morphometric investigation, therefore this material was only used for molecular genetic studies.

Non-Metric Multidimensional Scaling (NMDS, based on Euclidian distances, two-dimensional) and Linear Discriminant Analysis (LDA) was performed on log_{10}-transformed raw and size-corrected data to reveal possible differences between the populations and to determine the most important differentiating variables. Size correction was done by dividing each variable through the geometric mean of the respective specimen. For testing the equality of means of all populations, Multivariate Analysis of Variance (MANOVA) was used and for pairwise comparisons, Hotelling's T^2-tests were conducted.

For the variables contributing most to differentiation between the supposedly cryptic *Thalassozetes* (excluding *T. balboa* which is morphologically significantly different) as detected by LDA, univariate statistics were performed. Mean, minimum, maximum, standard deviation and coefficient of variation (cv) were calculated, and Kruskal-Wallis and Mann-Whitney *U* test were used for comparing the means of variables between all populations and for pairwise comparisons, respectively. All analyses were performed with PAST 3.11 (Hammer et al., 2001).

2.8. Morphological investigations

For microscopic investigation in transmitted light, preserved animals were embedded in Berlese mountant. Depictions were made with an Olympus BH-2 Microscope equipped with a drawing attachment. These drawings were first scanned, then processed and digitized with the free and open-source vector graphics editor Inkscape (freeware available under www.inkscape.org).

For photographic documentation, specimens were air-dried and photographed with a Keyence VHX-5000 digital microscope in reflected light.

Due to the low number of specimens (n = 3, 2 adults, 1 juvenile) we were not able to provide any depiction of the cryptic *Thalassozetes* species from the Samaná Peninsula (Dominican Republic). The two adults were used for molecular genetic analyses and hence partly destroyed but microscopic investigations of the remains confirmed the identical morphology with the other members of the cryptic species complex.

3. Results

3.1. Phylogenetic analyses

For all datasets, Bayesian Inference (BI) and Maximum Likelihood (ML) analyses produced congruent phylogenies. Resolution differed among the datasets. Most nodes connecting the main lineages were well resolved in the trees based on *COI* and the concatenated dataset (Figs. 1 & 2). Ten main lineages, largely corresponding to distinct geographic regions, were recovered in these trees: i) Panama and Florida, ii) Bahamas, iii) Curaçao and southern coast of Dominican Republic, iv) northern coast of Dominican Republic, v) Martinique, vi) Guadeloupe, vii) Barbados, viii – x) three lineages from Grenada, including two samples from Barbados. In contrast, the two nuclear markers produced only poorly resolved trees, with only some of the mitochondrial lineages recovered as distinct lineages also in these trees (Supplementary Fig. S2). In both nuclear datasets, however, both samples from Panama and the Bahamas were resolved as quite divergent from the rest. In addition, Curaçao plus southern Dominican Republic, Barbados and northern Dominican Republic plus Guadeloupe, resulted as somewhat distinct in the *EF-1α* tree (Supplementary Fig. S2A).

3.2. Species delimitation

Results from single locus species delimitation methods were incongruent, especially between distance- and tree-based approaches, but all methods identified ≥ 7 putative species (Fig. 1). ABGD recognized seven distinct *Thalassozetes* species at an initial partition with intraspecific divergence $< 5\%$: i) Panama and Florida (*T. balboa*), and with the *T. barbara* complex ii) Bahamas, iii) Curaçao and southern coast of Dominican Republic, iv) northern coast of Dominican Republic, v) Martinique and Guadeloupe, vi) Barbados, viii) Grenada. At an initial partition with intraspecific divergence $< 2.5\%$, ABGD identified 11 species, by splitting samples from Martinique and Guadeloupe into two distinct species, and further assigning samples from Grenada to three distinct entities. GMYC detected 15 species with high support values (>80). Also, the bPTP analysis resulted in a best supported partition of 15 (11–21) species, as well as bGMYC, when assuming a rather conservative probability threshold (posterior probability: $0.5 < P < 0.9$) for

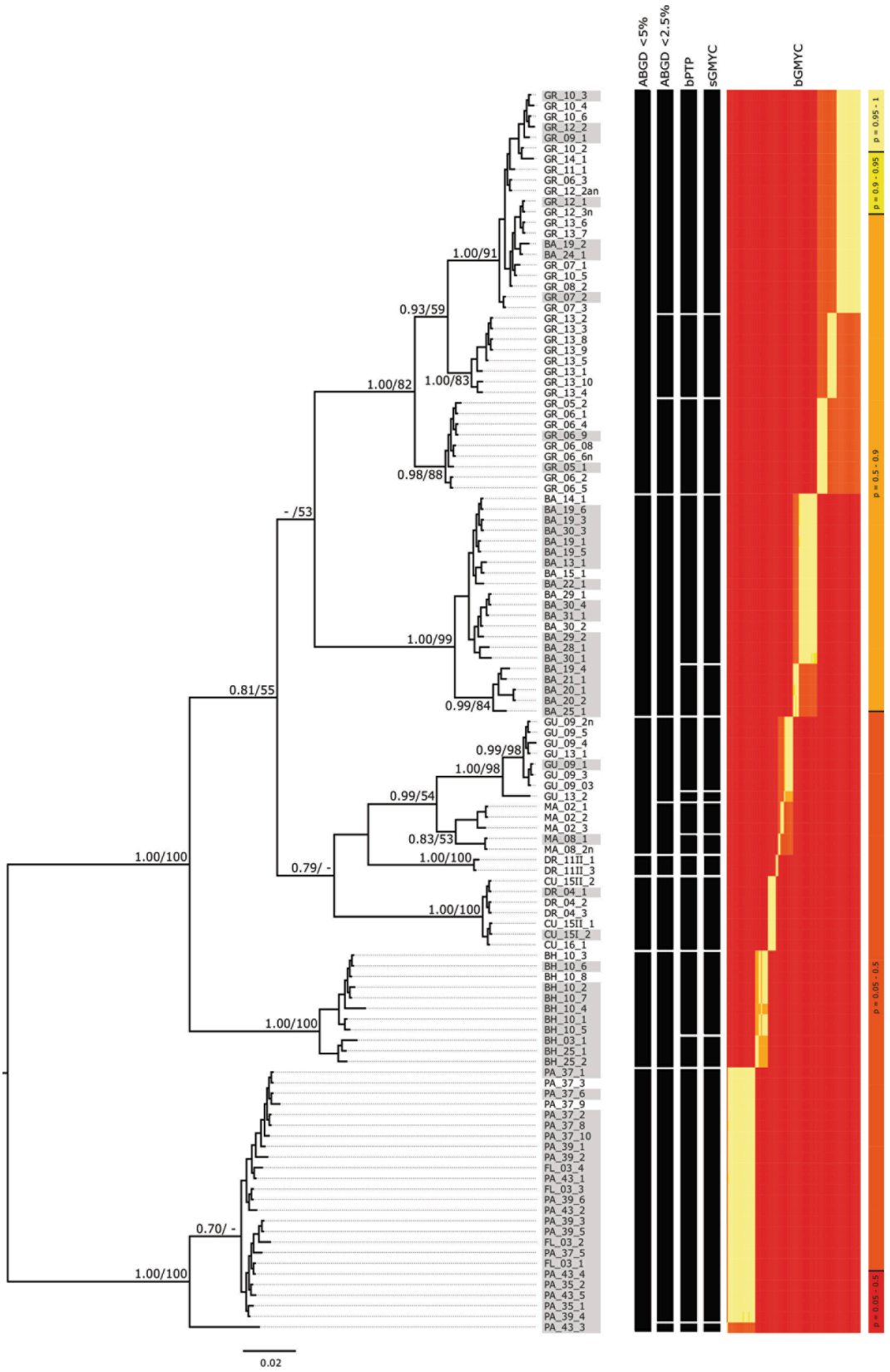

Fig. 1. BI tree of *Thalassozetes* spp. based on the *COI* data. As measures of nodal support, posterior probabilities (from BI; only values > 0.7) and bootstrap support values (from ML tree inference; only values > 50) are shown. For samples highlighted in grey, only *COI* data are available. Bars and heatmap to the right indicate the number of putative species inferred by different single-locus species delimitation methods.

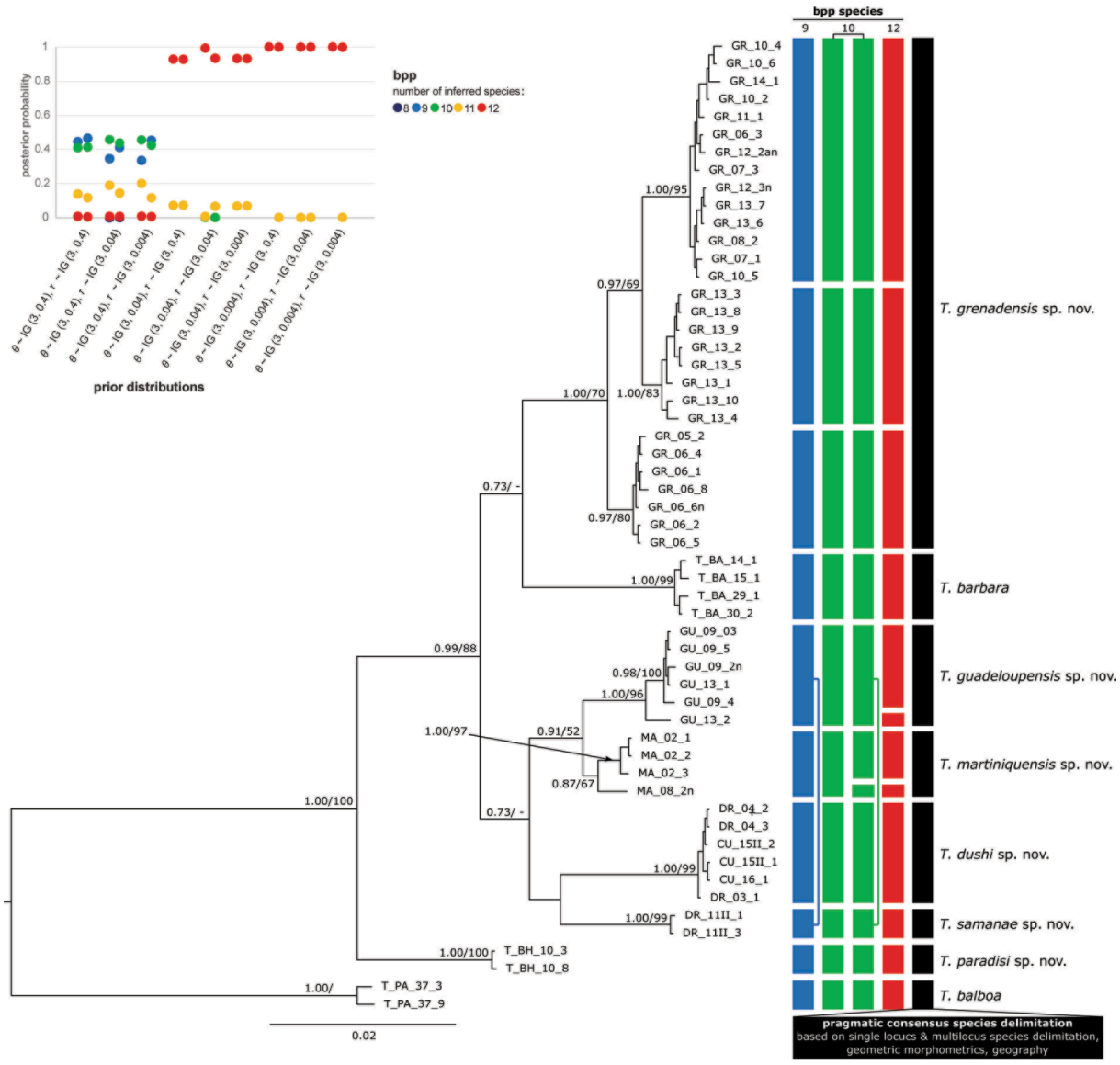

Fig. 2. Multi-locus species delimitation in the genus *Thalassozetes*. (a) BI tree showing the phylogenetic relationships among *Thalassozetes* spp. based on the concatenated datasets. As measures of nodal support posterior probabilities (from BI; only values > 0.7) and bootstrap support values (from ML tree inference; only values > 50) are shown. Bars to the right indicate the best supported species hypotheses inferred by BPP under various prior settings as shown in (b) and the final conservative species delimitation considering not only molecular, but also morphological and geographic evidence.

identifying putative species, compared to higher thresholds that could overestimate the species number (Kornilios et al. 2020). These three tree-based methods identified additional species in Panama, the Bahamas, Martinique, Guadeloupe and Barbados. Some of these putative species comprise only a single sample, characterized by a rather long branch length. Indeed, long branch lengths might be indicative for alignment errors or pseudogenes, but a careful double check (including translation into an amino acid sequence) suggested these sequences were okay and not pseudogenes.

Multilocus species delimitation using BPP favored, depending on the prior settings, a different number of putative species (Fig. 2). All prior combinations with small and intermediate ancestral population sizes strongly favored the existence of 12 putative species, corresponding to the main clades in the phylogenetic tree and two singletons (from Martinique and Guadeloupe) with slightly longer branch lengths. Prior combinations that included large ancestral population sizes, on the other hand, yielded roughly equal support for 9 or 10 putative species. However, the 10 species scenario mainly consisted of two equally well supported models (posterior probability of ~ 0.2 for each of these two models), while a single model contributed most to the 9 species scenario. The inferred putative species again largely corresponded to the main clades in the phylogenetic tree, but the 9 species model grouped northern Dominican Republic and Guadeloupe together as one species, which was also the case for the second 10 species model that also identified a singleton from Martinique as distinct species.

In general, the various molecular species delimitation methods suggested a minimum of seven and a maximum of 15 distinct species. Based on these results and on morphometric and distribution data (see below), we postulate eight Caribbean *Thalassozetes* species including six yet undescribed taxa. The latter will be referred to in the following text with their names: *Thalassozetes grenadensis* sp. n., *Thalassozetes dushi* sp. n., *Thalassozetes guadeloupensis* sp. n., *Thalassozetes martiniquensis* sp. n., *Thalassozetes paradisi* sp. n. and *Thalassozetes samanae* sp. n.

Minimum interspecific *COI* distances among the proposed eight species range from 5.4 to 16.3% (maximum of 12.4% among the island taxa), and always exceeded maximum intraspecific distances in these species, indicating a clear barcoding gap (Fig. 3a,d). In the nuclear data, no barcoding gap was observed (Fig. 3b,c), due to the much lower

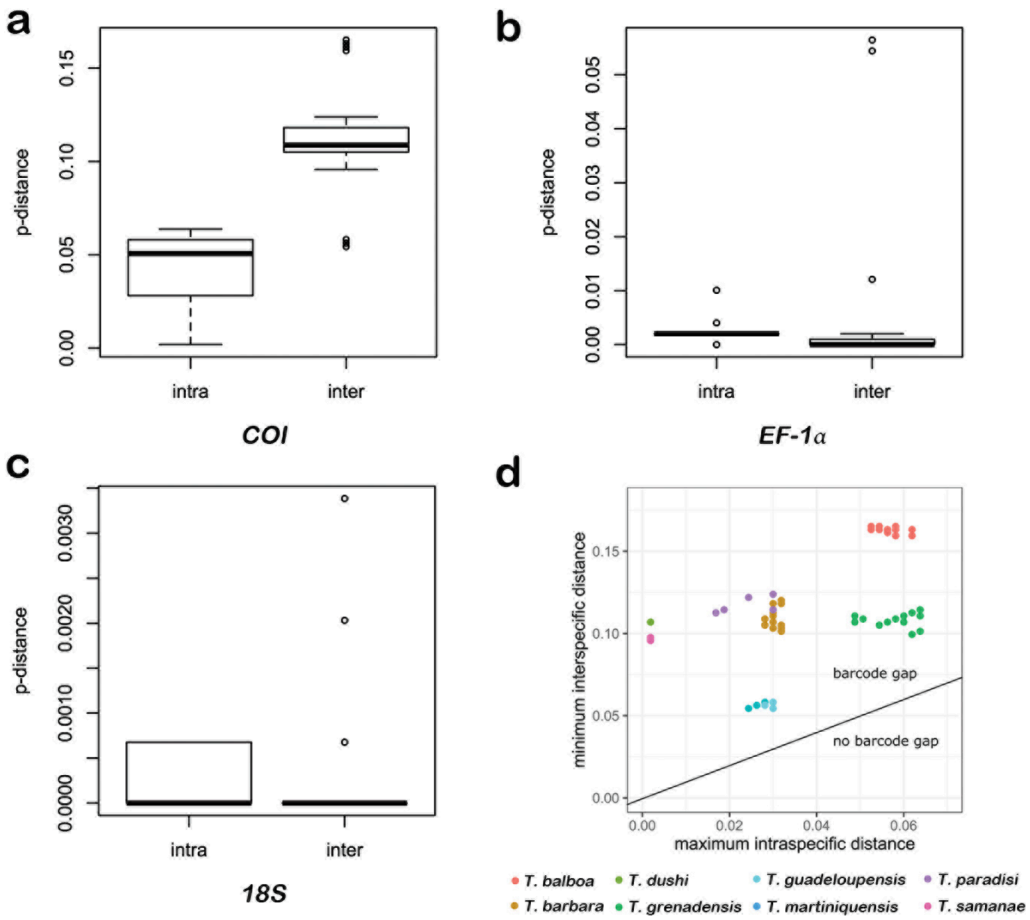

Fig. 3. Comparison of maximum intraspecific and minimum interspecific distances among *Thalassozetes* spp. (a) COI. (b) EF-1α. (c) 18S. (d) Barcode gap plot showing the minimum interspecific vs. the maximum intraspecific p-distance based on the *COI* data. Dots above the 1:1 line indicate the presence of a barcode gap. None of the species exhibits higher intraspecific than interspecific divergence.

substitution rates of these markers as compared to the mitochondrial *COI* gene.

3.3. Species tree

The topology of the species tree (Fig. 4) based on all three loci (*COI*, *EF-1α*, *18S*) is different from the phylogenetic tree inferred from the concatenated dataset (Fig. 2), which is to be expected as the tree topology from the concatenated dataset is heavily driven by the *COI* data, which shows much more variation than the two nuclear markers. Again, the mainland species *T. balboa* resulted as sister species of the Caribbean taxa. Within this species complex, *T. paradisi* sp. n. (Bahamas) was resolved as sister taxon of the remaining Caribbean species. Phylogenetic relationships among these were largely poorly supported, with the exception of a monophylum comprising *T. martiniquensis* sp. n. (Martinique) and *T. guadeloupensis* sp. n. (Guadeloupe) and *T. grenadensis* sp. n. (Grenada), which were resolved as sister species with high statistical support.

3.4. Phylogeographic structure and distribution patterns on islands

The haplotype network for the mainland species *T. balboa* shows no clear phylogeographic structure with a generally high haplotype diversity and haplotype sharing between the sampling sites in Florida and Panama (Fig. 5a). In contrast, the Caribbean island species do show, sometimes pronounced, phylogeographic structure, even on comparatively small islands (Fig. 5b, 6). Low levels of haplotype sharing suggest occasional gene flow between geographically distant localities on these

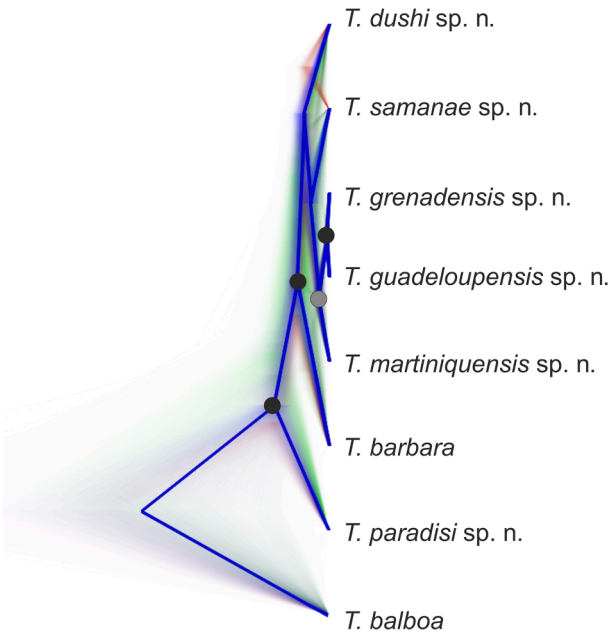

Fig. 4. Multispecies coalescent tree of the genus *Thalassozetes*. The consensus phylogeny is superimposed on a DensiTree cloudogram of alternative sampled trees, with contrasting topologies highlighted by different colors. Nodal support in form of posterior probabilities of $\geq 0.90 \geq 0.99$ is indicated by grey and black circles, respectively (only values > 0.7 are shown).

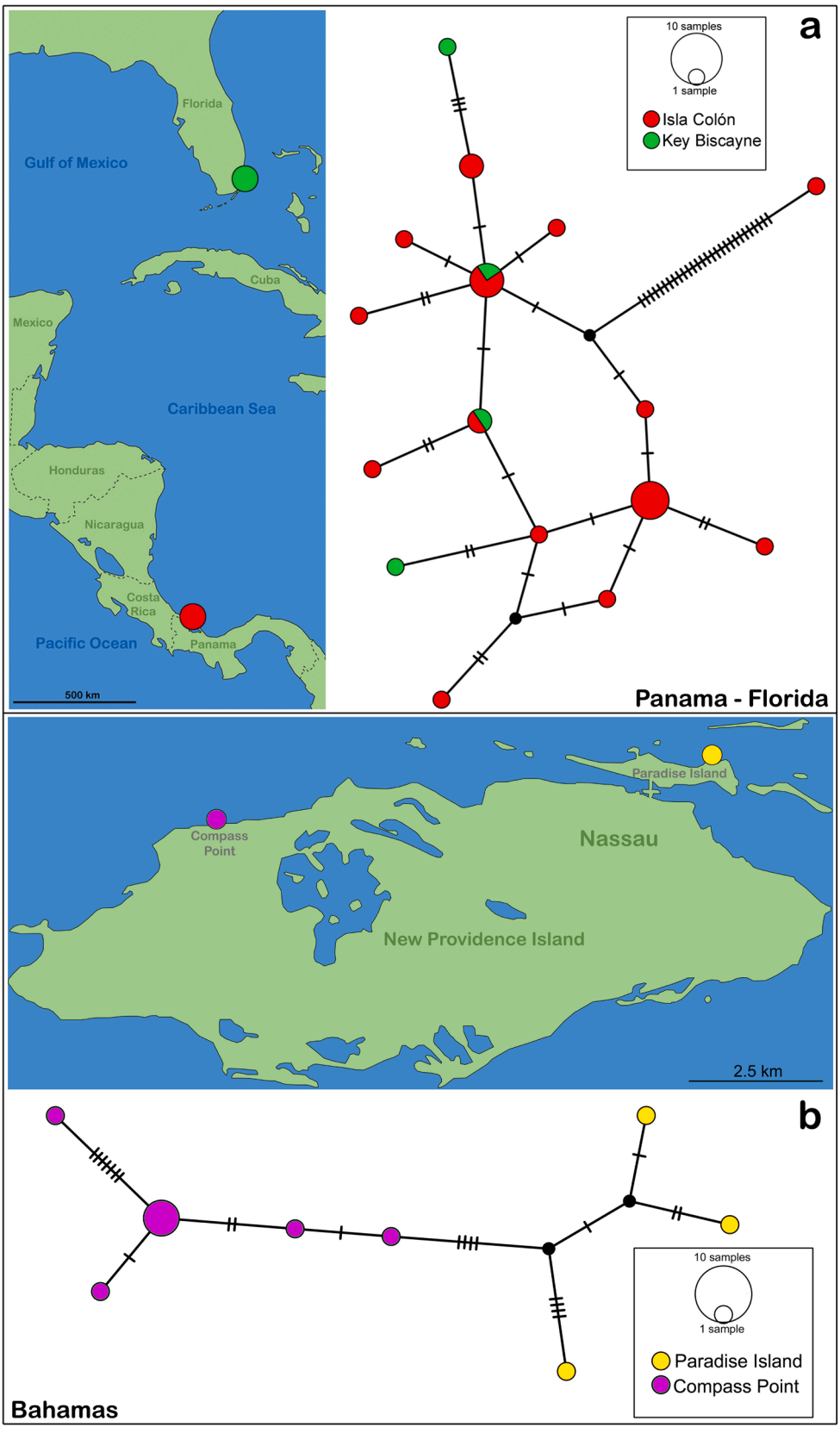

Fig. 5. Statistical parsimony haplotype networks based on *COI* sequences. Each circle corresponds to one haplotype and its size is proportional to its frequency. The number of mutations is indicated as hatch marks. Small black circles represent intermediate haplotypes not present in the dataset. Colors refer to different locations/islands as indicated on the respective map. (a) *Thalassozetes balboa* from Panama and Florida. (b) *Thalassozetes paradisi* sp. n. haplotypes from New Providence Island, Bahamas.

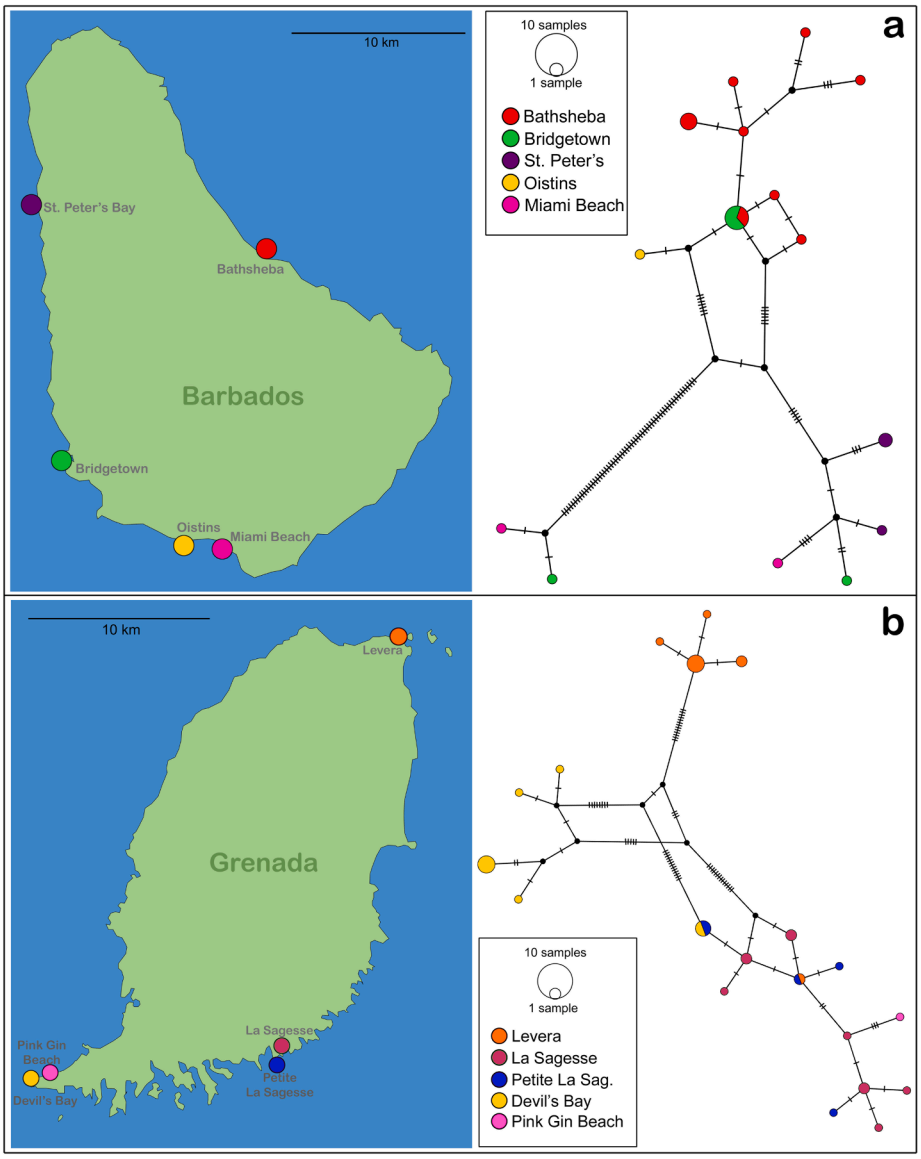

Fig. 6. Statistical parsimony haplotype networks based on COI sequences. Each circle corresponds to one haplotype and its size is proportional to its frequency. The number of mutations is indicated as hatch marks. Small black circles represent intermediate haplotypes not present in the dataset. Colors refer to different locations/islands as indicated on the respective map. (a) *Thalassozetes barbara* populations from Barbados. (b) *Thalassozetes grenadensis* sp. n. populations from Grenada.

islands (e.g. Barbados, Fig. 6a, Grenada, Fig. 6b). The two quite divergent haplotypes on Barbados belong to a different species, *T. grenadensis* sp. n., otherwise only found on Grenada.

3.5. Morphometry

Excluding the present cryptic taxa, the genus *Thalassozetes* comprises five morphospecies and is mainly characterized by the presence of lamellar ridges, a clavate sensillum and 13–14 notogastral setae (Pfingstl 2013a). These five species can be distinguished by the pattern of notogastral cuticular structure, number of notogastral ridges, shape of epimeral cavity and the presence of proximoventral teeth on claws. The present Caribbean island taxa, on the other hand, show complete conformity in all these diagnostic characters with *T. barbara* and lack additional distinctive features allowing to distinguish between them. Although these taxa cannot be distinguished based on distinct morphological characters, they do differ in morphometric characteristics. In accordance with the results of the molecular genetic analyses, the populations of each island will subsequently be labelled with their respective new species names.

LDA conducted on both raw and size-corrected data revealed that mites from each island, show diverging clusters, whereas some of the cluster still exhibit overlaps (Fig. 7). The populations from Curaçao (*T. dushi* sp. n.), Guadeloupe (*T. guadeloupensis* sp. n.) and Martinique (*T. martiniquensis* sp. n.) are largely overlapping, indicating few morphometric differences between them. The population from Barbados (*T. barbara*) shows fewer overlaps, and the populations from Bahamas (*T. paradisi* sp. n.), Grenada (*T. grenadensis* sp. n.) and Panama (*T. balboa*) are clearly separated from all other populations. MANOVA showed highly significant differences ($p < 0.001$) between all populations in raw as well as size-corrected data, and pairwise Hotelling's T^2-tests between the populations always revealed significant differences ($p < 0.05$). All-samples LDA correctly classified 96.35% (Jackknifed 84.67%) of all specimens in raw and 94.89% (Jackknifed 69.34%) in size-corrected data. The most important variables responsible for separation, gained by LDA, were *db*, *ll*, *dnr*, *efw1*, *efw2* and *gl*, which means the posterior prodorsal, the anterior notogastral area and the epimeral foveae are mainly affected (Supplementary Table S1).

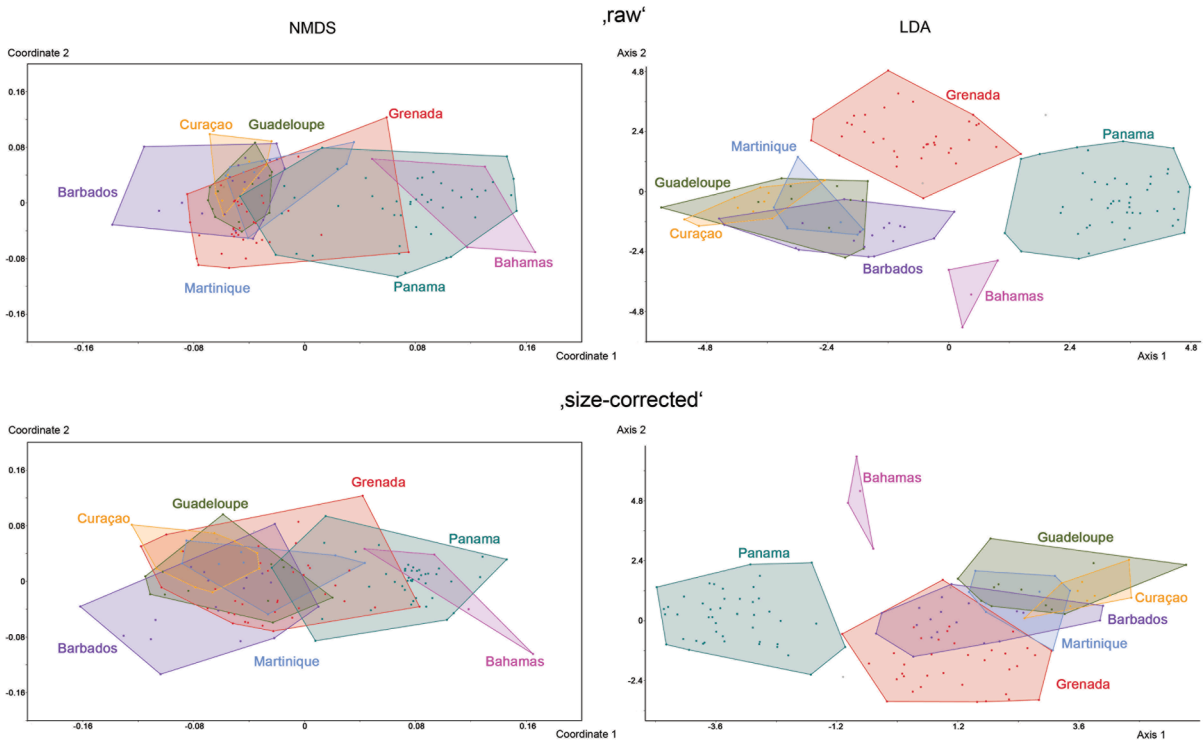

Fig. 7. Scatter plots gained from Non-Metric Multidimensional Scaling (left side) and Linear Discriminant Analysis (right side) on raw data (upper row) and size-corrected data (lower row). Overlapping clusters indicate morphological similarity and displaced clusters reflect diverging body shapes.

These variables, as well as body length (*bl*) and width (defined by nw_{dm}), were subsequently analyzed by univariate statistics and showed significant differences between all populations, cryptic species respectively, as well as in several pairwise comparisons (Table 1). However, no reliable variables for species delimitation could be defined as almost all variables overlap when their whole range is considered. The only exception can be found in the population from Bahamas (*T. paradisi* sp. n.), as its values for *db* (distance between bothridia) are always smaller than in the other populations and its values for *ll* (lenticulus length) are always higher. Still, these differences are minute and can thus not be considered as reliable for species delimitation, either.

3.6. Taxonomy and morphology

As all species show conformity in their morphology with *Thalassozetes barbara*, the original detailed description and the diagnosis given by Pfingstl (2013a) are valid for all members of this cryptic species complex. A slightly updated version of this diagnosis is available in the supplementary material (Supplementary Text file S1). Herein, we only provide depictions of each species and information about slightly diverging non-diagnostic characters, if present (e.g. body size, surface structure).

Family Selenoribatidae Schuster, 1963

Genus *Thalassozetes* Schuster, 1963

Type species: *Thalassozetes barbara* Pfingstl, 2013

Thalassozetes dushi sp. n.

Types: Holotype - Curaçao, Boca Ascención, from *Bostrychia* growing on intertidal rock, 5 Feb. 2016; preserved in ethanol, deposited at the Naturhistorisches Museum Wien (Vienna). Paratypes - four specimens, same location as holotype, preserved in ethanol, deposited at the US National Museum collection (USDA-Beltsville, MD).

Type locality: Curaçao, Boca Ascención; Lesser Antilles

GenBank accession numbers: *COI*: MZ169923–MZ169929, *EF-1α*: MZ220224–MZ220229, *18S*: MZ220313–MZ220318

Molecular diagnosis: In our *COI* alignment, position 3 is occupied by base C, position 27 by base C, position 123 by base G, position 135 by base G, position 360 by base C, position 411 by base G, position 516 by base C, and position 549 by base A (Supplementary Table S2).

ZooBank registration: urn:lsid:zoobank.org:act:60368C76-E98A-42F6-B128-BC099B808B46

Etymology: The specific epithet is the Papiamentu (Creole language) word *dushi*, which means charming or cute. As this eight-legged species may not look cute or charming to most of us, the word *dushi* rather refers to Curaçao, the type locality, where people often use this adjective to describe the island. Here it is given as noun in apposition.

Distribution: Curaçao, Hispaniola (Dominican Republic) (see Fig. 8).

Morphological remarks: Body length 277–297 μm, body width 135–160 μm (n = 10) (Fig. 9, Supplementary Fig. S3).

Thalassozetes grenadensis sp. n.

Types: Holotype - Grenada, La Sagesse Bay, from *Bostrychia* growing on intertidal rock, 27 Feb. 2016; preserved in ethanol, deposited at the Naturhistorisches Museum Wien (Vienna). Paratypes - four specimens, Grenada, Devil's Bay, from green intertidal algae on limestone rock, 28 Feb. 2016; preserved in ethanol, deposited at the US National Museum collection (USDA-Beltsville, MD).

Type locality: Grenada, La Sagesse Bay; Lesser Antilles

GenBank accession numbers: *COI*: MZ169932–MZ169967, *EF-1α*: MZ220232–MZ220260, *18S*: MZ220395–MZ220311

Molecular diagnosis: In our *COI* alignment, position 15 is occupied by base A or G, position 51 by base C, position 426 by base T, and position 528 by base C (Supplementary Table S2).

ZooBank registration: urn:lsid:zoobank.org:act:342ED980-049B-415C-9204-1C0385D8202A

Etymology: This species is named after the Lesser Antillean island Grenada, the type locality of this species.

Distribution: Grenada, Barbados western coast (see Fig. 8).

Morphological remarks: Body length 280–317 μm, body width 166–191 μm (n = 36). Prodorsal ridges not as prominent as in *T. barbara* (Fig. 9, Supplementary Fig. S3). Notogastral ridges variable in height and shape.

Table 1
Univariate statistics for the cryptic Caribbean *Thalassozetes* species and comparison of the eight most important morphological variables. Min-max = minimum–maximum values in µm; sd = standard deviation and cv = coefficient of variation (marked light grey if equal or higher than 0.10). Results of Kruskal-Wallis Test (KW) and Mann–Whitney-*U* test are given; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. a - Grenada vs. Barbados, b - Grenada vs. Bahamas, c - Grenada vs. Martinique, d - Grenada vs. Curacao, e - Grenada vs. Guadeloupe, f - Barbados vs. Curacao, g - Bahamas vs. Barbados, h - Bahamas vs. Curacao, i - Barbados vs. Guadeloupe, j - Bahamas vs. Guadeloupe.

	Barbados (*T. barbara*) n = 19			Bahamas (*T. paradisi*) n = 4			Curacao (*T. dushi*) n = 10			Guadeloupe (*T. guadeloupensis*) n = 12			Grenada (*T. grenadensis*) n = 36			Martinique (*T. martiniquensis*) n = 8			KW	Mann-Whitney-U
	Min-max	Mean	sd	cv	Min-max	Mean	sd	cv	Min-max	Mean	sd	cv	Min-max	Mean	sd	cv	Min-max	Mean	sd	cv		
bl	271–302	**284.7**	8.63	0.03	280–295	**285.3**	6.65	0.02	277–297	**284.4**	6.87	0.02	280–317	**297.6**	11.16	0.04	280–317	**302.3**	8.04	0.03	277–308	**283.5**	10.16	0.04	***	a.d ***; d **; i *
nw_{dm}	163–182	**170.3**	5.62	0.03	160–172	**164.5**	5.20	0.03	157–175	**165.0**	4.94	0.03	166–175	**171.5**	3.09	0.02	166–191	**179.8**	6.69	0.04	160–182	**165.5**	6.87	0.04	***	a. d ***; c.e **; b *
ll	28–34	**30.5**	2.29	0.08	40–46	**42.3**	2.87	0.07	34–37	**34.6**	1.26	0.04	31–40	**33.3**	2.60	0.08	31–40	**34.7**	2.04	0.06	31–34	**33.1**	1.25	0.04	***	a ***; f **; b.g.h *
dnr	31–43	**36.6**	2.65	0.07	31–34	**31.8**	1.50	0.05	37–43	**41.2**	2.10	0.05	34–43	**38.2**	3.10	0.08	35–49	**40.6**	3.48	0.09	37–43	**40.0**	2.27	0.06	***	a ***; f **;b *
efw_1	22–31	**25.5**	2.29	0.09	25–26	**25.3**	0.50	0.02	22–31	**27.3**	2.50	0.09	25–31	**27.3**	2.19	0.08	22–28	**25.0**	1.76	0.07	25–26	**25.1**	0.35	0.01	**	e *
efw_2	19–25	**21.1**	2.28	0.11	15–19	**16.0**	2.00	0.13	19–22	**20.5**	1.58	0.08	19–22	**20.3**	1.54	0.08	15–25	**20.2**	2.13	0.11	16–22	**19.4**	2.50	0.13	*	
db	49–53	**51.3**	1.33	0.03	46–48	**47.0**	1.15	0.02	49–55	**51.8**	2.10	0.04	49–55	**52.5**	1.93	0.04	51–62	**55.5**	2.90	0.05	49–55	**51.3**	2.12	0.04	***	a ***; b.c.d.e.g.j *
gl	34–45	**38.3**	3.77	0.10	37–43	**40.0**	3.46	0.09	34–40	**36.1**	2.85	0.08	34–43	**37.7**	3.58	0.09	34–46	**40.9**	3.79	0.09	34–45	**35.5**	3.85	0.11	***	c.d *

Thalassozetes guadeloupensis sp. n.

Types: Holotype - Guadeloupe, Capesterre-Belle-Eau (Basse-Terre), from *Bostrychia* on intertidal rock, 19 Feb. 2016; preserved in ethanol, deposited at the Naturhistorisches Museum Wien (Vienna). Paratypes - four specimens, same location as holotype, preserved in ethanol, deposited at the US National Museum collection (USDA-Beltsville, MD).

Type locality: Guadeloupe, Capesterre-Belle-Eau (Basse-Terre); Lesser Antilles

GenBank accession numbers: *COI*: MZ169968–MZ169975, *EF-1α*: MZ220261–MZ220266, *18S*: MZ220289–MZ220294

Molecular diagnosis: In our *COI* alignment, position 213 is occupied by base G, and position 408 by base A (Supplementary Table S2).

ZooBank registration: urn:lsid:zoobank.org:act:D53F8B4B-E32A-4ADB-8DB6-5C117CB2C654

Etymology: The species name refers to the type locality, the Antillean island of Guadeloupe.

Distribution: Guadeloupe - endemic (see Fig. 8).

Morphological remarks: Body length 280–317 µm, body width 166–175 µm (n = 12). Notogastral ridges less prominent than in *T. barbara* but stronger than in *T. paradisi* sp. n. (Fig. 9, Supplementary Fig. S3).

Thalassozetes martiniquensis sp. n.

Types: Holotype - Martinique, Trinité, from *Bostrychia* growing on intertidal rock, 24 Feb. 2016; preserved in ethanol, deposited at the Naturhistorisches Museum Wien (Vienna). Paratypes - four specimens, Martinique, Pointe du Bout, from *Bostrychia* on conglomerate rock, 22 Feb. 2016; preserved in ethanol, deposited at the US National Museum collection (USDA-Beltsville, MD).

Type locality: Martinique, Trinité; Lesser Antilles

GenBank accession numbers: *COI*: MZ169976–MZ169980, *EF-1α*: MZ220267–MZ220270, *18S*: MZ220285–MZ220288

Molecular diagnosis: In our *COI* alignment, position 96 is occupied by base T (Supplementary Table S2).

ZooBank registration: urn:lsid:zoobank.org:act:0122B1D1-43A3-4111-A9AB-6954F548F5C1

Etymology: The specific epithet refers to the type locality, the Antillean island of Martinique.

Distribution: Martinique – endemic (see Fig. 8).

Morphological remarks: Body length 277–308 µm, body width 160–182 µm (n = 8) (Fig. 9, Supplementary Fig. S3).

Thalassozetes paradisi sp. n.

Types: Holotype - Bahamas, Paradise Island, from *Bostrychia* growing on littoral rock, 18 Feb. 2017; preserved in ethanol, deposited at the Naturhistorisches Museum Wien (Vienna). Paratypes - four specimens, Bahamas, New Providence Island, Compass Point, from *Bostrychia* growing in rock crevice, 19 Feb. 2017; preserved in ethanol, deposited at the US National Museum collection (USDA-Beltsville, MD).

Type locality: Bahamas, Paradise Island, New Providence.

GenBank accession numbers: *COI*: MZ170003–MZ170013, *EF-1α*: MZ220275–MZ220276, *18S*: MZ220280–MZ220281

Molecular diagnosis: In our *COI* alignment, position 279 is occupied by base C, position 342 by base T, position 363 by base G, position 543 by base C, and position 552 by base C (Supplementary Table S2).

ZooBank registration: urn:lsid:zoobank.org:act:06AEFC6A-082C-4C06-8CEF-7B844FA00D34

Etymology: This species is named after Paradise Island, a small island and part of New Providence Bahamas, where it was originally discovered; the Latin name for paradise is given in the genitive case.

Distribution: Bahamas – endemic (see Fig. 8).

Morphological remarks: Body length 280–295 µm, body width 160–172 µm (n = 4). Anterior notogastral ridges weakly developed and less protruding than in *T. barbara* and all other cryptic species. Cerotegumental layer showing basically stronger and finer granulation than in all other species (Fig. 9, Supplementary Fig. S3).

Thalassozetes samanae sp. n.

Types: Holotype – Dominican Republic, El Portillo, from *Bostrychia*

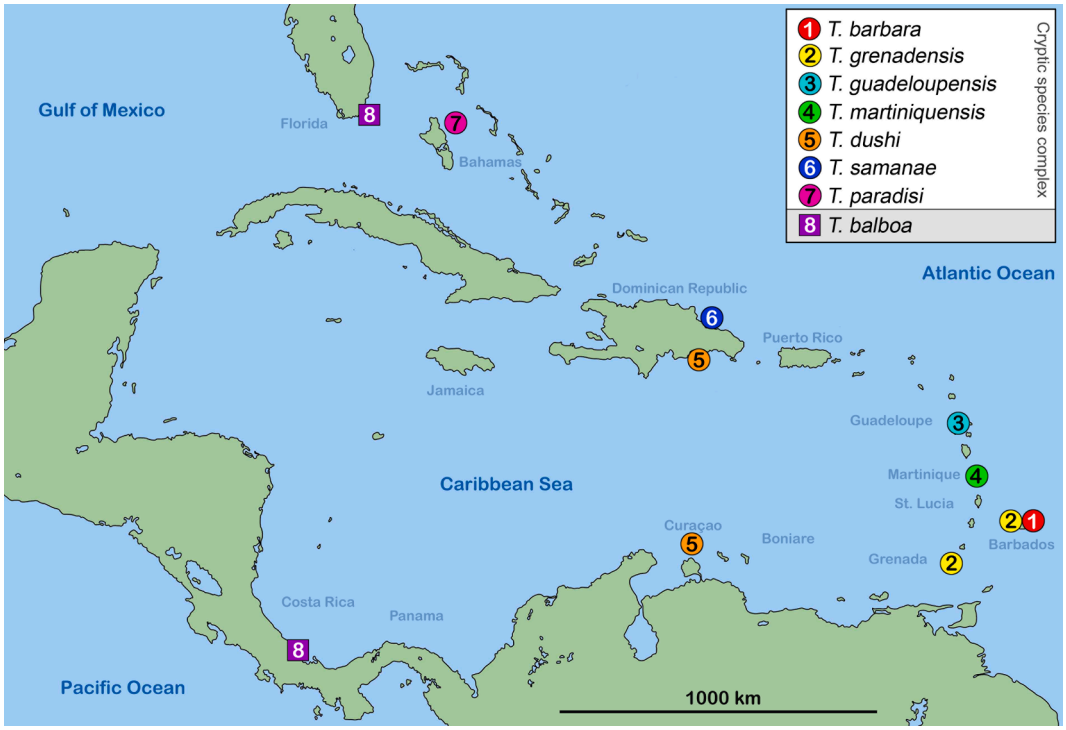

Fig. 8. Map showing the geographic distribution of all Caribbean *Thalassozetes* species. Different colors and numbers refer to different species. Circles represent members of the cryptic *Thalassozetes* species complex, squares indicate non-cryptic species.

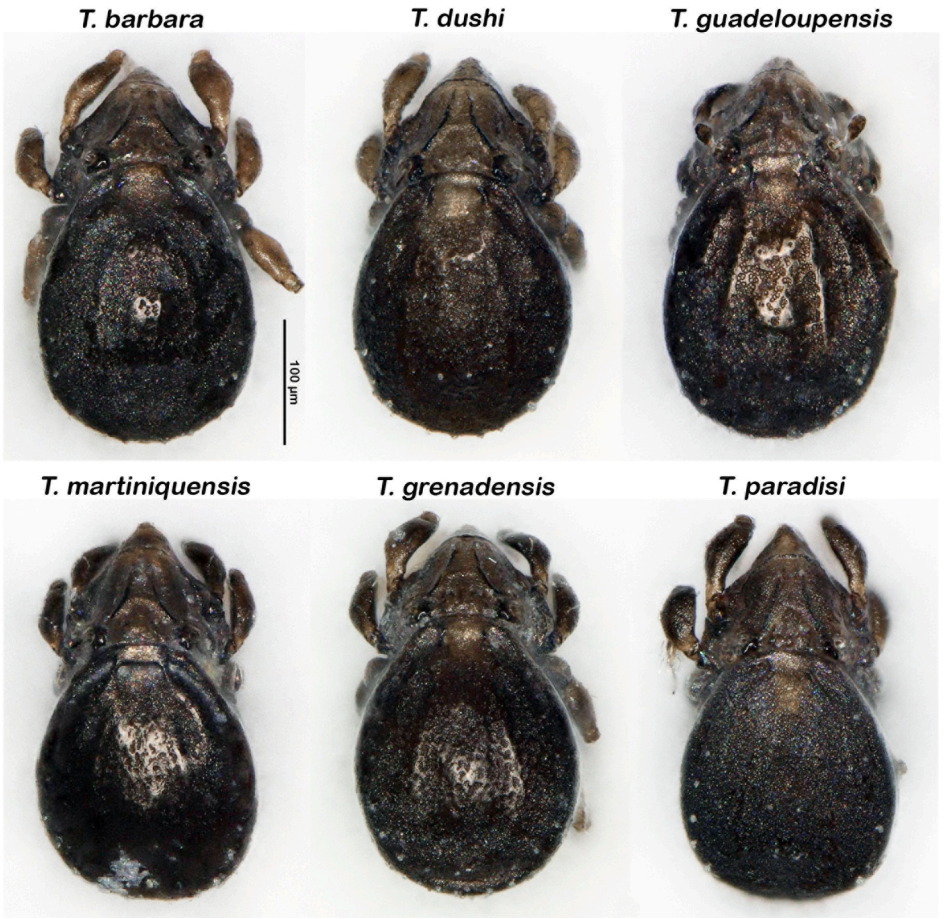

Fig. 9. Photographic comparison (stacked stereomicroscopic images) of cryptic *Thalassozetes* species in dorsal view. Scale bar is valid for all photographs. Photographs show some of the specimens used in the morphometric analysis.

growing on mangrove root (*Rhizophora mangle*), 11 Feb. 2016; preserved in ethanol, deposited at the Naturhistorisches Museum Wien (Vienna). Paratypes - two specimens, same location as holotype, preserved in ethanol, deposited at the Museo Nacional de Historia Natural "Prof. Eugenio de Jesús Marcano", Dominican Republic.

Type locality: Dominican Republic, El Portillo, Samaná; Hispaniola, Greater Antilles

GenBank accession numbers: *COI*: MZ169930–MZ169931, *EF-1α*: MZ220230–MZ220231, *18S*: MZ220312

Molecular diagnosis: In our *COI* alignment, position 288 is occupied by base G, position 432 by base C, position 453 by base G, position 486 by base T, and position 513 by base C (Supplementary Table S2).

ZooBank registration: urn:lsid:zoobank.org:act:DFE98ECD-E8EA-4471-815B-53D6B6C3CDAD

Etymology: This species was only found on coasts of Samaná, a peninsula and province of the Dominican Republic, therefore the specific epithet refers to this location and is given as noun in the genitive case.

Distribution: endemic to Hispaniola (Dominican Republic) (see Fig. 8).

Morphological remarks: Body length 278–289 μm, body width 163–169 μm (n = 2).

Remarks: The species of this cryptic complex can be distinguished from the Caribbean *T. balboa* by the presence of three adanal setae (instead of two), by having only one ventral tooth on each leg claw (instead of two) and by the cuticular notogastral pattern with unevenly distributed circular depressions resulting in an irregular reticulate-foveate pattern (vs. evenly distributed depressions resulting in regular reticulate-foveate pattern).

4. Discussion

4.1. Cryptic diversity and its causes

Widespread species are improbable taxonomic hypotheses for lineages with poor dispersal abilities, as for example flightless arthropods (Dziki et al., 2015), and this is particularly true for the Caribbean mite *Thalassozetes barbara*. Our results demonstrate that this supposedly widespread species actually represents a complex that includes at least six additional cryptic groups, nearly all of which are endemic to single islands. Minimum interspecific *COI* distances among the proposed eight species range from 5.4 to 16.3% (maximum of 12.4% within the island taxa), and were always larger than the maximum intraspecific distances. In arthropods, a ten percent divergence in *COI* exceeds known species delimitation thresholds (Cosgrove et al., 2016) and the values of most *Thalassozetes* groups are in accordance with this suggested benchmark. Moreover, recent studies on cryptic diversity in tree-living oribatid mites found uncorrected p-distances ranging from 16 to 24.8% (Schäffer et al., 2019) and 12.7–19.6% (Schäffer and Koblmüller, 2020) in the *COI* gene between eight and six putative species, respectively. Only some of these species were classified as clear (but morphologically very similar) morphospecies. However, congruent clustering of individual specimens in mitochondrial and nuclear single gene trees and syntopic occurrence of two or more genetic clusters at several locations indicated reproductive isolation and the existence of previously unknown true biological species even when morphological differentiation was lacking (at least in the morphological characters looked at; Schäffer et al., 2019; Schäffer and Koblmüller, 2020). Reproductive isolation among the *Thalassozetes* groups cannot be verified directly, because they do not occur syntopically and cross-breeding experiments are more or less unfeasible as these would last for years due to low reproductive rates and difficulties in simulating the intertidal environment in the lab. Interspecific sequence divergences in the *COI* lie (with the exception of *T. guadeloupensis* sp. n. and *T. martiniquensis* sp. n.) within the range (though at the lower edge) previously inferred for other oribatid species (e.g. Pfingstl et al., 2019b; Schäffer et al., 2019; Seniczak et al., 2019; Schäffer and Koblmüller, 2020). The lack of resolution we see in our two nuclear markers, that does not permit us to separate all *Thalassozetes* species with confidence with these markers, is due the more recent divergence of *Thalassozetes* as compared to other previously studied oribatid mite taxa, as indicated by the observed levels of *COI* divergence.

In morphologically cryptic taxa, molecular approaches have been widely used to delineate species, but, where a range of methods are employed on the same dataset, species delimitation results are often incongruent. Several factors have been shown to affect molecular species delimitation analyses, e.g. population size and divergence time (and the ratio thereof), gene flow, number of species involved, speciation rate, sample size and geographic coverage per species, or number of loci (e.g. Dellicour and Flot, 2015; 2018;; Ahrens et al., 2016; Eberle et al., 2018; Luo et al., 2018). Among the single-locus species delimitation methods, distance-based methods like ABGD tend to underestimate species numbers, while tree-based approaches like GMYC, bGMYC and PTP often oversplit species (e.g. Dellicour and Flot, 2018; Luo et al., 2018). We observed the same tendencies in our data, with ABGD finding fewer putative species than GMYC, PTP and bGMYC, mainly because the latter approaches often identified somewhat divergent singletons as distinct species. The multilocus-method BPP shows lower rates of species overestimation and underestimation, and should be generally more robust to various potential confounding factors (Luo et al., 2018). BPP, as a method that employs the multispecies coalescent, however, diagnoses genetic structure and not necessarily species, and importantly, it does not statistically distinguish between structure associated with population isolation and species boundaries (Sukumaran and Knowles, 2017). Therefore, it is important to interpret the results of molecular species delimitation, be it based on single-locus approaches or the multispecies coalescent, together with other lines of evidence, e.g., from morphology, ecology, geography, or population genetics (Solis-Lemus et al., 2015). As our nuclear data contain only very limited variation, the BPP analysis was probably heavily influenced by the highly variable mitochondrial data. All scenarios with low to intermediate ancestral population size priors identified a larger number of species (some singletons were identified as distinct species) than scenarios assuming large ancestral population sizes. Notably, most methods, except for ABGD at the < 5% threshold, identified three species on Grenada. The haplotype network, showed some clear phylogeographic structure with the three divergent lineages predominant in distinct parts of the island. But, since we also found low levels of haplotype sharing among these regions, no difference in the nuclear markers, and no obvious morphometric clusters that might correspond to the mitochondrial clades, we refrain from considering these as different species.

Despite several overlaps, morphometric results and clusters coincide very well with *COI* data. Considering that the morphologically distinct *T. balboa* from Panama is not conspicuously clearer contrasted in morphospace than some of the cryptic taxa, morphometric data provides additional evidence for the distinctness of each *Thalassozetes* lineage. However, morphometric data does neither provide distinct separation of all cryptic taxa nor does it clearly conform with any of the species delimitation analyses, therefore establishing exactly six new species might appear to be based on weak reasoning. By adding the geographic component, however, the six new species are well justified. All these species are poor dispersers, as clearly indicated by genetic data, and thus can be considered as island endemics. Even though restricted recent (potentially human-induced) migration between islands was found in two of the species, it is highly unlikely that strong hybridization or intermingling events between populations of different islands occur.

Nevertheless, formally designating cryptic species necessitates using a species concept, other than the morphospecies concept, to determine species boundaries. However, this may lead to problems with incompatible concepts resulting in grouping artifacts (Heethoff, 2018) as mentioned in the introduction. De Queiroz (2007) argued that, despite their various differences, there is a common element in all species concepts and therefore he proposed a unified concept that defined

species as separately evolving metapopulation lineages. He suggested that the presence of any property used in different species concepts, e.g. reproductive isolation used in the biological concept or deficits of genetic intermediates used in the genetic concept, constitutes evidence for lineage separation and hence for a separate species. We interpret our results in the sense of this concept and define the species herein. Despite the lack of distinct separating morphological traits and evidence of reproductive isolation, molecular genetic data as well as the geographic setting with large oceanic barriers between the islands renders the *Thalassozetes* taxa as separately evolving metapopulation lineages and thus confirms the species in the sense of De Queiroz (2007).

From a practical point of view, this means that studies including any of the Caribbean *Thalassozetes* species should ideally use molecular genetic data to identify the respective cryptic taxon. However, faunistic investigations may not always be able to apply these methods due to high costs or lacking infrastructure. For these cases we recommend to determine the species based on their geographic origin, i.e. if the species was found on Grenada it should be identified as *T. grenadensis* or if it was found on Guadeloupe it should be identified as *T. guadeloupensis*. If a *Thalassozetes* specimen is found on any other Caribbean location than investigated in the present study and shows the phenotype of *T. barbara*, it should be classified as *Thalassozetes* cf. *barbara* until molecular genetic data allows a more precise identification. The latter may also be applied when specimens are found on coasts of Hispaniola because at least two species are present on this Greater Antillean island which prevents using geographic origin as classification tool.

4.2. Phylogeography and dispersal

Considering the geographic distribution of these cryptic Caribbean *Thalassozetes*, with nearly all species being single island endemics, geographic isolation and associated genetic drift are most likely the primary cause for speciation. However, the question arises, what caused the identical phenotypic appearance of this cryptic *Thalassozetes* species complex. There are three evolutionary processes that could lead to phenotypic similarity in historically isolated lineages, namely recent speciation, evolutionary convergence and stabilizing selection (Colborn et al., 2001; Lefébure et al., 2006; Bickford et al., 2007; Fišer et al., 2017). Considering the large sequence divergence among the distinct *Thalassozetes* species, recent speciation can be excluded. Moreover, DNA sequence data renders all Caribbean *Thalassozetes* species a monophyletic group, which clearly contradicts convergence being responsible for superficially identical morphologies. The observed morphological stasis is therefore most likely a product of stabilizing selection, imposed by extreme and homogeneous environments, reducing or eliminating morphological change that usually accompanies speciation (Bickford et al., 2007; Lefébure et al., 2006). All of the cryptic *Thalassozetes* species dwell in the intertidal zone, which is an extreme environment because parameters change constantly and animals have to cope with terrestrial and marine conditions at the same time. Although the species occur on distant islands, selective constraints are the same in each littoral zone and therefore each species is subject to the same selective regime preserving the bauplan.

However, different microhabitats may be present in the intertidal zone and cryptic species may use different ecological niches, as for example shown in two Bermudian cryptic intertidal mite species of the genus *Carinozetes*, where one species dwells predominantly on rocky shores while the other occurs exclusively in mangrove forests (Pfingstl et al., 2014). This is not the case in *Thalassozetes*, as all populations were collected from algae growing on littoral rocks, with only one single specimen of *T. samanae* sp. n. found on algae growing on mangrove roots. The vast majority of populations were extracted from the red alga *Bostrychia tenella*, which is used as substrate and food source by the mites. Though a recent study also demonstrated this alga to represent a complex of at least three cryptic and closely resembling species (Zuccarello et al, 2015), a correlation with cryptic *Thalassozetes* species may be excluded because distribution patterns are not in agreement at all.

Mitochondrial *COI* sequence data, and to a lesser extent also the nuclear data, suggest that after the radiation of the Caribbean *Thalassozetes* group nearly all species have evolved in isolation without any considerable gene flow between the islands. Moreover, the haplotype networks show strong diversification and phylogeographic structure on single islands, which indicates restricted gene flow even on a local scale. Accordingly, these *Thalassozetes* species are poor dispersers that probably rely on rare and stochastic hydrochorous transport, i.e. drifting along ocean currents (e.g. Pfingstl, 2017). Other small arthropods, as for example the cobweb spider *Spintharus* or the orb-weaver *Micrathena* also show high levels of single island or short range endemics in the Caribbean (McHugh et al., 2014; Dziki et al., 2015) indicating that limited overwater dispersal and vicariance is one of the main factors shaping the evolutionary history of these small organisms. Nevertheless, haplotype data show that at least two recent dispersal events have happened: first, *T. dushi* sp. n. has successfully crossed the Caribbean Sea between Curaçao and the Dominican Republic and second, a few *T. grenadensis* sp. n. specimens have reached the coasts of Barbados. The former is quite unusual as the Caribbean Sea stretches over 600 km between these two locations and thus should represent a large barrier. How gene flow has nevertheless happened is presently only a matter of conjecture but large eddies, bird mediated transport or even recent anthropogenic dispersal could be responsible.

The non-cryptic Western Caribbean *Thalassozetes* species, *T. balboa*, shows a completely different pattern with ongoing gene flow and a possible wide distribution from Panama to Florida. The occurrence of this species seems to range across the whole Caribbean Central American coastline (Pfingstl et al., 2019b). Therefore, in this species, dispersal and exchange between populations may occur along the shore without any oceanic barriers. Moreover, the Gulf Stream may facilitate dispersal along the coastline at least in one direction and this together may result in the obviously diverging biogeographic pattern.

As the open ocean clearly represents a barrier for the species of the cryptic *Thalassozetes* complex, the common ancestor of this group supposedly occupied former large Caribbean landmasses and could disperse along its continuous coast. A continuous land bridge, so called GAARlandia (Greater Antilles-Aves Ridge), connecting the South American continent with the Greater Antilles and dating to ca. 33–35 mya is thought to have existed (Iturralde-Vinent, 2006). Unfortunately, there is no reliable substitution rate available for the *COI* gene of mites. Previous attempts (Salomone et al., 2002; Heethoff et al., 2007) to infer divergence times in oribatid mites used a general arthropod substitution rate of 1–1.15%/MY (DeSalle et al., 1987). However, to unambiguously link geological events to particular divergence events in *Thalassozetes* a reliable substitution rate for oribatid mites is required, as rates might differ considerably among taxa. Hence, we refrained from applying a standard arthropod substitution rate to our data and thus cannot relate the radiation of the cryptic species to any known geological event. But given the species tree based on all gene fragments and the observed large interspecific pairwise distances in the *COI* gene, we can at least state that a common ancestor split into the Western Caribbean *T. balboa* that persisted on the coasts of Central America and into the ancestor of the cryptic species complex that radiated subsequently in the Eastern Caribbean, and that the radiation of Caribbean *Thalassozetes* is not something very recent. This scenario supports the GAARlandia hypothesis (Iturralde-Vinent, 2006) because this land bridge may have provided an avenue for the *Thalassozetes* ancestral species to colonize the Greater Antilles from South America. The breakup of GAARlandia resulted in the split between the ancestor of the mainland Caribbean *T. balboa* and the island taxa, which further diversified due to the subsequent submergence and emergence of Antillean islands.

4.3. Implications for other taxa and geographic regions

The present case of cryptic intertidal arthropods confirms

morphological stasis as an important evolutionary process induced by the extreme intertidal environment. Therefore, intertidal organisms can be expected to contain further cryptic species. Especially taxa with poor dispersal abilities may harbor many cryptic species complexes. In intertidal mites, there are several cases of poor dispersers with unusually wide distribution areas spanning a few thousand kilometers. For example, *Schusteria melanomerus* occurring from coasts of Kenya to shores of South Africa (Pfingstl, 2016), *Fortuynia smiti* with records from New Caledonia and from Singapore (Pfingstl, 2015), *Fortuynia rotunda* with occurrences in southern Africa and in Japan, or *Fortuynia elamellata* being reported from southern Africa, Japan and New Zealand (Pfingstl and Schuster, 2014). These are just a few cases of potential cryptic mite species and the list is surely longer. The same may apply to other taxa dwelling in the marine littoral, as for example non-winged/flightless insects. Moreover, geographic areas with many archipelagos and oceanic islands separated by vast stretches of open Ocean, such as the Caribbean or the Southeast Asian Sunda Region, are most likely hiding large numbers of cryptic species across diverse littoral taxa.

4.4. The problematic nature of dealing with cryptic species

For taxonomists performing faunistic or taxonomic investigations, cryptic taxa may become an issue, as they will probably remain undetected and the researcher will be left scratching his head about an intangible 'intraspecific' variation. Detecting cryptic species usually requires integrative approaches including multivariate morphometrics, molecular tools, chemical assays, intensive sampling, crossing etc. (Skoracka et al., 2015). DNA barcoding initiatives have revealed a considerably large number of cryptic species in the last few years (e.g. Hebert et al., 2004; Smith et al., 2006; Vasconcelos et al., 2016; Lavinia et al., 2017), but only those collaborating with taxonomic specialists unraveled the complex nature of these cases (e.g. Hebert et al., 2004; Van Ginneken et al., 2017; Wagner et al., 2021).

However, detecting cryptic species is not enough, formally naming them is even more crucial for a number of reasons. First, it is the only way to ensure that scientists are talking about the same taxon, second, biological data linked to an unnamed species loses value because other authors cannot easily build on these data, and third, taxa need to be named for being included in conservation programs (Pante et al., 2015; Delić et al., 2017). The latter is of major importance especially for cryptic species that are endemics occurring on very small islands, as for example most of the Caribbean *Thalassozetes* species complex. Slight changes in these locally restricted environments can have tremendous impacts on the species (Bickford et al., 2007). Apart from conservation, unnamed species are also unavailable to biological control and pest management and the failure to recognize pathogenic cryptic species might have serious negative consequences (Bickford et al., 2007). Thus, naming cryptic species is important as it allows to communicate them with other fields of science that use Linnaean binomials in their research (Fišer et al. 2017).

Despite these important reasons, many cryptic species remain unnamed (Pante et al., 2015) and somehow get lost in literature as 'species B', 'species 3′ etc. Researchers usually refrain from formally naming a species because of a lack of support of species justification, the lack of knowledge about diagnosing new species using non-morphological characters, the unwillingness to perform a formal description, the difficulties of publishing species descriptions in high impact factor journals (Pante et al., 2015) and the ongoing controversy about species concepts and their proper application. But most of these problems can be easily overcome as shown by the following examples: several authors (Cook et al., 2010; Jörger and Schrödl, 2013) provided specific guidelines for how to describe and name a cryptic species based on diagnostic DNA sequence characters only. Others (Wang et al., 2016; Delić et al., 2017) published exemplary descriptions of cryptic species in high impact factor journals and hence provided excellent standard works.

In accordance with the above mentioned authors, we propose to take the following actions when naming a cryptic species: (I) state which species concept was used to clarify the reasoning of species delimitation, (II) provide diagnostic characters from different types of data (molecular genetic markers, morphometric variables, ecological traits, geographic distributions etc.), (III) provide a depiction of the species and/or of important morphological features (photograph, drawing, electron micrograph etc.), (IV) register and upload data to online repositories (GenBank, ZooBank), (V) deposit holotypes and paratypes in a museum, (VI) if valid for all cryptic species, provide a clear reference to the original description of the nominal species or provide own descriptions as supplementary files and (VII) if possible, provide distribution areas as allopatric endemics may be identified based on their geographic origin. However, all these recommendations should not just be seen as a standard for describing cryptic species, they should apply more generally for all species descriptions. In this way, species, no matter if cryptic or not, are treated the same way and named under the same conditions.

To sum up, an integrative taxonomic approach is vital to detect and understand the phenomenon of cryptic diversity and the association with a formal species description makes it available for further important research, biodiversity estimates and conservation management. A recent study (Kuroshunova et al., 2019) argued that the cryptic species concept needs to be reconsidered because with progressing methodology distinguishing characters will be found rendering the formerly cryptic species as 'non-cryptic' species. While this may be true, we think we should not spend too much time discussing about an eternally valid definition of cryptic species, we rather should focus on finding and classifying these diverging taxa and on understanding the evolutionary mechanisms responsible for the similar phenotypes.

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Author Contributions

T.P. performed the sampling, all morphometric measurements and wrote large parts of the paper, A.L. assisted in the sampling and performed molecular genetic laboratory work, J.B. performed all analyses based on morphometric data and S.K. analyzed and interpreted molecular genetic results.

Appendix A

Information on sampling location and GenBank accession numbers for *COI*, *EF-1α* and *18S* sequences comprising all specimens included in genetic analyses.

					GenBank accession nr.		
Country	Location	Sample ID	species	coordinates	COI	efa	18S
Curaçao	Boca Ascención	CU_15I_2	*T. dushi*	12.273242, −69.052882	MZ169923		
		CU_15II_1			MZ169924	MZ220224	MZ220318
		CU_15II_2			MZ169925	MZ220225	MZ220317
		CU_16_1		12.273342, −69.052667	MZ169926	MZ220226	MZ220316
Dominican Republic	Boca Chica	DR_04_1	*T. dushi*	18.447819–69.620430	MZ169927	MZ220227	MZ220315
		DR_04_2			MZ169928	MZ220228	MZ220314
		DR_04_3				MZ220229	MZ220313
Dominican Republic	El Limón	DR_11II_1	*T. samanae*	19.324285–69.482856	MZ169930	MZ220230	
		DR_11II_3			MZ169931	MZ220231	MZ220312
Guadeloupe	Capesterre-belle-Eau	GU_09_03	*T. guadeloupensis*	16.034611, −61.564938	MZ169968	MZ220261	MZ220294
		GU_09_1			MZ169969		
		GU_09_2n			MZ169970	MZ220262	MZ220293
		GU_09_3			MZ169971		
		GU_09_4			MZ169972	MZ220263	MZ220292
		GU_09_5			MZ169973	MZ220264	MZ220291
Guadeloupe	Sainte-Anne	GU_13_1	*T. guadeloupensis*	16.234413, −61.363318	MZ169974	MZ220265	MZ220290
		GU_13_2			MZ169975	MZ220266	MZ220289
Martinique	Pointe du Bout	MA_02_1	*T. martiniquensis*	14.558542–61.053438	MZ169976	MZ220267	MZ220288
		MA_02_2			MZ169977	MZ220268	MZ220287
		MA_02_3			MZ169978	MZ220269	MZ220286
Martinique	La Trinité	MA_08_1	*T. martiniquensis*	14.740511–60.953304	MZ169979		
		MA_08_2n			MZ169980	MZ220270	MZ220285
Grenada	Levera Beach	GR_05_1	*T. grenadensis*	12.228186–61.613385	MZ169932		
		GR_05_2			MZ169933	MZ220232	MZ220311
Grenada	Levera Beach	GR_06_08	*T. grenadensis*	12.22888–61.614432	MZ169934	MZ220233	
		GR_06_1			MZ169935	MZ220234	
		GR_06_2			MZ169936	MZ220235	MZ220310
		GR_06_3			MZ169937	MZ220236	
		GR_06_4			MZ169938	MZ220237	
		GR_06_5			MZ169939	MZ220238	MZ220309
		GR_06_6n			MZ169940	MZ220239	
		GR_06_9			MZ169941		
Grenada	La Sagesse	GR_07_1	*T. grenadensis*	12.023456–61.669976	MZ169942	MZ220240	
		GR_07_2			MZ169943		
		GR_07_3			MZ169944	MZ220241	MZ220308
Grenada	La Sagesse	GR_08_2	*T. grenadensis*	12.023512–61.670203	MZ169945	MZ220242	MZ220307
Grenada	La Sagesse	GR_09_1	*T. grenadensis*	12.023967, −61.671536	MZ169946		
Grenada	La Sagesse	GR_10_2	*T. grenadensis*	12.023512–61.670203	MZ169947	MZ220243	MZ220306
		GR_10_3			MZ169948		
		GR_10_4			MZ169949	MZ220244	
		GR_10_5			MZ169950	MZ220245	
		GR_10_6			MZ169951	MZ220246	
Grenada	Petite La Sagesse	GR_11_1	*T. grenadensis*	12.017501–61.675096	MZ169952	MZ220247	MZ220305
Grenada	Petite La Sagesse	GR_12_1	*T. grenadensis*	12.018659–61.673232	MZ169953		
		GR_12_2			MZ169954		
		GR_12_2a			MZ169955	MZ220248	MZ220304
		GR_12_3			MZ169956	MZ220249	MZ220303
Grenada	Devil's Bay	GR_13_1	*T. grenadensis*	12.006653–61.796438	MZ169957	MZ220250	MZ220302
		GR_13_10			MZ169958	MZ220251	MZ220301
		GR_13_2			MZ169959	MZ220252	
		GR_13_3			MZ169960	MZ220253	MZ220300
		GR_13_4			MZ169961	MZ220254	MZ220299
		GR_13_5			MZ169962	MZ220255	MZ220298
		GR_13_6			MZ169963	MZ220256	MZ220297
		GR_13_7			MZ169964	MZ220257	MZ220296
		GR_13_8			MZ169965	MZ220258	MZ220295
		GR_13_9			MZ169966	MZ220259	
Grenada	Pink Gin Beach	GR_14_1	*T. grenadensis*	12.008939, −61.791091	MZ169967	MZ220260	
Panamá	Isla Colón	T_PA_35_1	*T. balboa*	9.362898–82.239319	MZ170018		
		T_PA_35_2			MZ170019		
Panamá	Isla Colón	T_PA_37_1	*T. balboa*	9.370821–82.239908	MZ170020		
		T_PA_37_10				MZ170021	
		T_PA_37_2			MZ170022		
		T_PA_37_3			MZ170023	MZ220277	MK035018[2]
		T_PA_37_5			MZ170024		
		T_PA_37_6			MZ170025		

(*continued on next page*)

(*continued*)

Country	Location	Sample ID	species	coordinates	GenBank accession nr. COI	efa	18S
		T_PA_37_8			MZ170026		
		T_PA_37_9			MZ170027	MZ220278	MZ220279
Panamá	Isla Colón	T_PA_39_1	*T. balboa*	9.385454–82.23524	MZ170028		
		T_PA_39_2			MZ170029		
		T_PA_39_3			MZ170030		
		T_PA_39_4			MZ170031		
		T_PA_39_5			MZ170032		
		T_PA_39_6			MZ170033		
Panamá	Isla Colón	T_PA_43_1	*T. balboa*	9.415057–82.330787	MZ170034		
		T_PA_43_2			MZ170035		
		T_PA_43_3			MZ170036		MK035019[2]
		T_PA_43_4			MZ170037		
		T_PA_43_5			MZ170038		
Florida	Key Biscayne	T_FL_03_1	*T. balboa*	25.677296–80.164818	MZ170014		
		T_FL_03_2			MZ170015		
		T_FL_03_3			MZ170016		
		T_FL_03_4			MZ170017		
Bahamas	Paradise Island	T_BH_03_1	*T. paradisi*	25.085983, −77.29966	MZ170003		
Bahamas	Compass Point	T_BH_10_1	*T. paradisi*	25.065252, −77.470981	MZ170004		
		T_BH_10_2			MZ170005		
		T_BH_10_3			MZ170006	MZ220275	MZ220281
		T_BH_10_4			MZ170007		
		T_BH_10_5			MZ170008		
		T_BH_10_6			MZ170009		
		T_BH_10_7			MZ170010		
		T_BH_10_8			MZ170011	MZ220276	MZ220280
Bahamas	Paradise Island	T_BH_25_1	*T. paradisi*	25.086345, −77.301111	MZ170012		
		T_BH_25_2			MZ170013		
Barbados	Bathsheba	T_BA_13_1	*T. barbara*	13.213011, −59.520318	MZ169981		
Barbados	Bathsheba	T_BA_14_1	*T. barbara*	13.213834, −59.521433	MZ169982	MZ220271	MZ220284
Barbados	Bathsheba	T_BA_15_1	*T. barbara*	13.21393, −59.521825	MZ169983	MZ220272	MZ220283
Barbados	Bridgetown	T_BA_19_1	*T. barbara*	13.078423, −59.612556	MZ169984		
		T_BA_19_2	*T. grenadensis*		MZ169985		
		T_BA_19_3				MZ169986	
		T_BA_19_4				MZ169987	
		T_BA_19_5				MZ169988	
		T_BA_19_6				MZ169989	
Barbados	St. Peters Bay	T_BA_20_1	*T. barbara*	13.240601, −59.645153	MZ169990		
		T_BA_20_2		13.240601, −59.645153		MZ169991	
Barbados	St. Peters Bay	T_BA_21_1	*T. barbara*	13.240219, −59.645069	MZ169992		
Barbados	Oistins	T_BA_22_1	*T. barbara*	13.062537, −59.541903	MZ169993		
Barbados	Miami Beach	T_BA_24_1	*T. grenadensis*	13.060559–59.540786	MZ169994		
Barbados	Miami Beach	T_BA_25_1	*T. barbara*	13.060006–59.538893	MZ169995		
Barbados	Bathsheba	T_BA_28_1	*T. barbara*	13.212719, −59.517116	MZ169996		
Barbados	Bathsheba	T_BA_29_1	*T. barbara*	13.212719, −59.517116	MZ169997	MZ220273	MZ220282
		T_BA_29_2				MZ169998	
Barbados	Bathsheba	T_BA_30_1	*T. barbara*	13.212719, −59.517116	MZ169999		MK035020[2]
		T_BA_30_2			MW289085[1]	MZ220274	MW298484[1]
		T_BA_30_3			MZ170000		
		T_BA_30_4	*T. barbara*		MZ170001		
Barbados	Bathsheba	T_BA_31_1	*T. barbara*	13.216753, −59.526811	MZ170002		

[1]Sequence from Pfingstl et al. (2021); [2]Sequence published by Pfingstl et al. (2019b).

Appendix B. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2021.107240.

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