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So different, yet so alike: North American slider turtles (*Trachemys scripta*)

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Abstract

We studied for the first time the molecular differentiation of all three currently recognized subspecies of *Trachemys scripta*, including the morphologically distinct western populations of *T. s. elegans* ('western red-eared sliders'), using mitochondrial and nuclear DNA sequences (up to 3,236 bp and 2,738 bp, respectively) and 14 microsatellite loci. We found that only the quickly evolving microsatellite loci discriminated *T. s. troostii* and the western red-eared slider from the remaining two subspecies, while *T. s. elegans* and *T. s. scripta* were not distinct in any marker system. Our findings challenge the current intraspecific systematics of *T. scripta* and suggest that the conspicuous differences in coloration and pattern reflect population-specific, rather than taxonomic, differentiation. We abstain from synonymizing any subspecies because, for traditionalists and conservationists, abandoning the well-established and morphologically distinct subspecies of *T. scripta* is not desirable. However, if subspecies of *T. scripta* continue to be recognized, the current taxonomy with three subspecies is difficult to justify. Western red-eared sliders are morphologically distinct and differ from *T. s. elegans* and *T. s. scripta*, with respect to microsatellites, as much as *T. s. troostii* does. In view of this morphological and genetic evidence, subspecies status should be considered for western red-eared sliders.

Key words

Biogeography, Emydidae, intraspecific variation, subspecies, systematics, taxonomy.

Introduction

Slider turtles, genus *Trachemys* (family Emydidae), have a wide natural distribution across the Americas, spanning from the Great Lakes region in North America, through Central America and the Antilles, to the region of the Rio de la Plata in South America (Ernst & Barbour, 1989; Ernst, 1990; Legler, 1990; Legler & Vogt, 2013; Seidel & Ernst, 2017; TTWG, 2017). Despite being distributed across such a broad area, until recently, most or all continental populations of *Trachemys* were thought to represent a single species, *Trachemys scripta* (Moll & Legler, 1971; Ernst & Barbour, 1989; Legler & Vogt, 2013). This widespread species was formerly divided into numerous subspecies, but at present, only three subspecies, all native to North America, are still retained within *T. scripta*. The remaining continental populations are now placed in up to 12 distinct species. Four additional species, restricted to the Antilles, complete the currently recognized diversity of *Trachemys* (Seidel, 2002; Fritz *et al*., 2012; Seidel & Ernst, 2017; TTWG, 2017; Vargas-Ramírez *et al*., 2017).

The three currently recognized subspecies of *T. scripta* differ significantly in coloration and pattern (Carr, 1952;

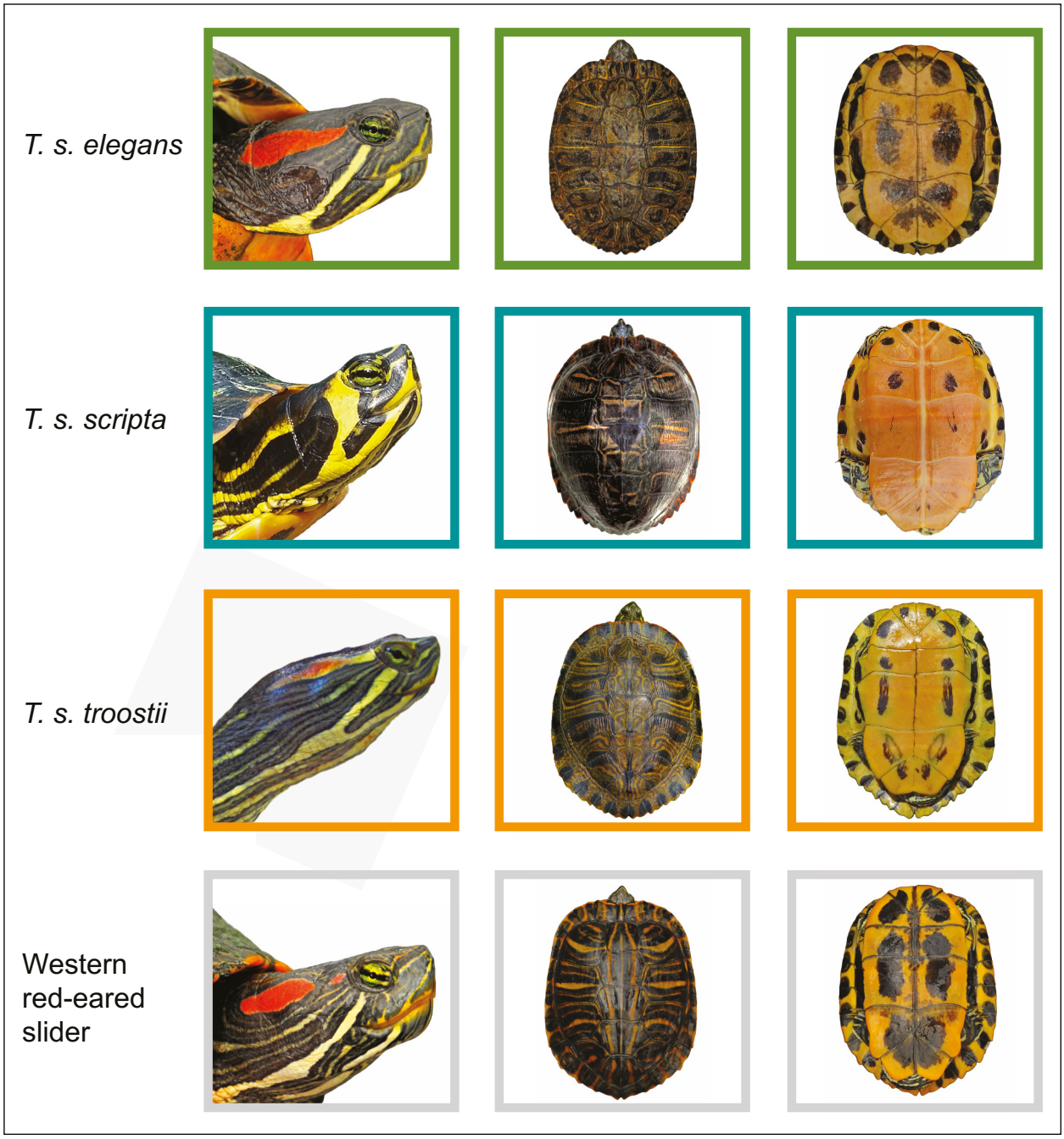

Fig. 1. Comparison of coloration and pattern of the three subspecies of *Trachemys scripta* and the western red-eared slider. Frame colors correspond to Fig. 2.

ERNST & LOVICH, 2009; Fig. 1). The nominotypical subspecies, the yellow-bellied slider *T. s. scripta* (Schoepff, 1792), further deviates from the other two subspecies by having a less elongated, but more domed, carapace with a characteristically wrinkled surface (CARR, 1952). *Trachemys scripta scripta* is distributed along the southern Atlantic coastal plain of the USA, which encompasses portions of the states of Virginia, North and South Carolina, Georgia, Florida, and Alabama (ERNST & LOVICH, 2009; TTWG, 2017).

The red-eared slider, *T. s. elegans* (Wied, 1839), has the widest native distribution of all three subspecies, occurring in the Mississippi River drainage and beyond in the USA (Alabama, Arkansas, Florida, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Mississippi, Missouri, Nebraska, eastern New Mexico, Ohio, Oklahoma, Tennessee, Texas, and West Virginia) and in the Mexican states of Nuevo León and Tamaulipas (ERNST & LOVICH, 2009; TTWG, 2017). A distinctive morph of *T. s. elegans* from Texas, New Mexico, and adjacent Mexico (LEGLER & VOGT, 2013), was highlighted by early authors as 'western *elegans*' and has been suggested to be the result of hybridization with adjacent taxa (CAGLE, 1950; CARR, 1952). In addition to its natural distribution, *T. s. elegans* has been spread by humans almost worldwide, establishing feral popula-

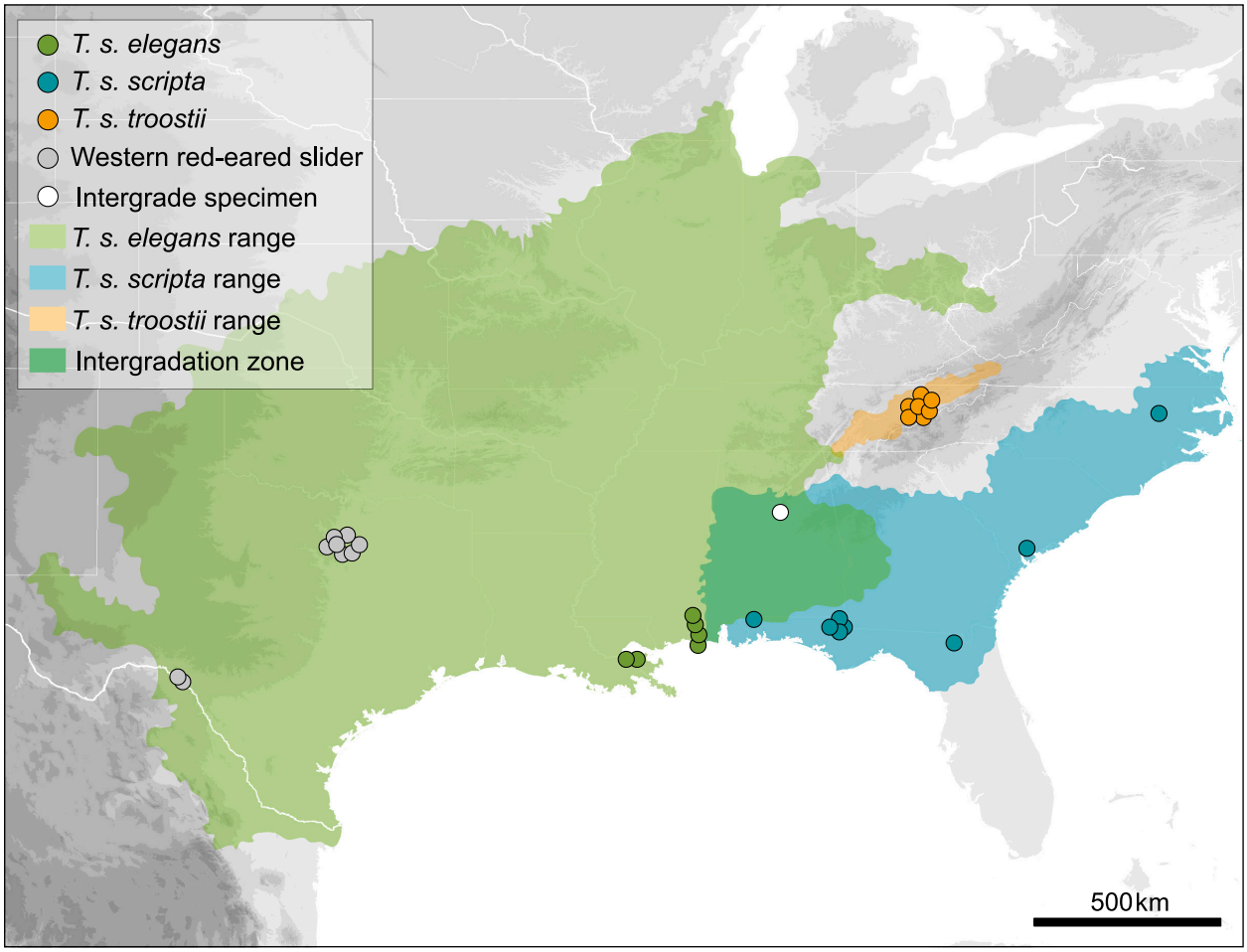

Fig. 2. Geographic distribution of the currently recognized subspecies of *Trachemys scripta* according to TTWG (2017). Circles indicate collection sites of samples used in the present study.

tions in many climatically suitable regions, and consequently, has been included among the 100 most invasive species of the world (ISSG, 2015). Populations that are morphologically intermediate between *T. s. scripta* and *T. s. elegans* occur over a large area of Alabama, western Georgia, and the Florida panhandle (Mount, 1975; TTWG, 2017).

The final subspecies, the Cumberland slider, *T. s. troostii* (Holbrook, 1836), has the most restricted range of the three taxa. It occurs in the upper reaches of the Cumberland and Tennessee rivers and has been reported from southeastern Virginia and Kentucky, through eastern Tennessee, to northeastern Alabama (Ernst & Lovich, 2009). However, reports of *T. s. troostii* in Alabama may be based upon *T. s. scripta* × *T. s. elegans* intergrades (Mount, 1975). To the south and east, the distribution of *T. s. troostii* is separated from that of *T. s. scripta* by a gap coinciding with the Appalachian Mountains (Pope, 1946). Based on morphologically intermediate characters, intergradation between *T. s. elegans* and *T. s. troostii* has been reported in Madison County, Kentucky (Ernst & Jett, 1969) but is otherwise unknown. To the north, a large distribution gap lies between *T. s. troostii* and the closest records of *T. s. elegans* in the Ohio River drainage (Fig. 2).

Trachemys scripta is one of the most studied turtle species of the world (Lovich & Ennen, 2013). Yet, surprisingly, no detailed phylogeographic studies have been conducted on all three North American subspecies, even though two were included in the pioneering investigations by Avise *et al*. (1992) and Walker & Avise (1998). Avise *et al*. (1992) and Walker & Avise (1998) found *T. s. scripta* to differ in mitochondrial DNA (mtDNA) from *T. s. elegans*, with haplotypes corresponding to *T. s. scripta* being largely confined to river drainages flowing into the Atlantic Ocean. In contrast, haplotypes matching with *T. s. elegans* occurred in drainages of the Gulf Coast, including the intergrade region in Alabama, Georgia, and the Florida panhandle.

In the present study, we use mitochondrial and nuclear DNA sequences (up to 3,236 bp and 2,738 bp, respectively) and 14 microsatellite loci for characterizing intraspecific variation of *T. scripta*. Our sampling included representatives of all three currently recognized subspecies (*T. s. scripta*, *T. s. elegans, T. s. troostii*), along with one individual from the putative intergradation zone of *T. s. elegans* and *T. s. scripta*. Our samples of *T. s. elegans* include representatives of the distinctive western morph, which we hereafter refer to as the 'western red-eared slider.' *Trachemys scripta troostii* has never been studied genetically before.

Materials and Methods

Sampling, selected loci, and data evaluation strategy

Forty-two samples of slider turtles (*Trachemys scripta* spp.) from Alabama, Florida, Louisiana, Mississippi, North Carolina, South Carolina, Tennessee, and Texas were studied, including samples from captive individuals in the Turtle Island collection, Graz. Seven samples were sequenced for previous studies (FRITZ *et al*., 2011, 2012). The remaining 35 samples were processed for the present study (Table S1). New samples were either collected during fieldwork, originated from the Turtle Island stock, or were donated by other researchers. Live wild turtles were caught either by hand, dip net, or baited funnel traps. Following collection of up to 1.0 ml blood, all live turtles were immediately released at the capture site. From dead turtles that were opportunistically encountered (i.e., road-killed individuals), a small piece of tissue was salvaged. Samples were preserved in ethanol or using FTA classic cards (Whatman, GE Healthcare, Munich, Germany). Ethanol-preserved samples were stored at −80°C until processing; FTA classic cards, at room temperature.

The same mitochondrial and nuclear genomic markers were targeted as in our previous studies on *Trachemys* (FRITZ *et al*., 2012; STANDFUSS *et al*., 2016; VARGAS-RAMÍREZ *et al*., 2017). Three mitochondrial DNA fragments (12S, cyt *b* + 29 bp DNA coding for tRNA-Thr, ND4L/ND4), three protein-coding nuclear genes (Cmos, Rag1, Rag2), and the intron 1 of the nuclear R35 gene were sequenced. In addition, all samples of sufficient DNA quality were genotyped at 14 previously characterized microsatellite loci (SIMISON *et al*., 2013).

Total DNA was isolated using the InnuPrep DNA Mini Kit or the InnuPrep Blood DNA Mini Kit (Analytik Jena AG, Jena, Germany). DNA from blood preserved on FTA cards was extracted using the illustra tissue & cells genomicPrep Mini Spin Kit (GE Healthcare) following the protocol for genomic DNA from animal tissue. Details of PCR and sequencing are described in FRITZ *et al*. (2012) and genotyping in STANDFUSS *et al*. (2016). The obtained 12S fragments were up to 395 bp long (with gaps); the complete cyt *b* gene plus adjacent DNA coding for tRNA-Thr, up to 1,169 bp; and an mtDNA fragment comprising the ND4L and ND4 genes, up to 1,672 bp. All nuclear DNA blocks could be sequenced directly. Cmos sequences had a length of up to 563 bp; R35 sequences, up to 958 bp; Rag1 sequences, up to 614 bp; and Rag2 sequences, up to 603 bp.

Mitochondrial DNA is maternally inherited, whereas nuclear loci are inherited biparentally. Moreover, mtDNA is prone to introgression, also across species borders, often leading to conflicting results for the two marker systems (CURRAT *et al*., 2009; FUNK & OMLAND, 2003; SLOAN *et al*., 2017; TOEWS & BRELSFORD, 2012; see also for an extreme case in turtles: KEHLMAIER *et al*., 2019). To avoid the danger of such distortion, mitochondrial and nuclear sequence data were examined separately.

Phylogenetic analyses of mtDNA, uncorrected *p* distances

Sequences were aligned and inspected using BIOEDIT 7.0.5.2 (HALL, 1999). All sequences aligned perfectly and gaps occurred only in sequence blocks not coding for proteins. Individual mtDNA fragments were concatenated for phylogenetic analyses, resulting in an alignment of 3,236 bp length. It included data for 41 *Trachemys scripta* and one *Pseudemys texana* that served as outgroup. The *P. texana* was sequenced for the present study using the above-described approaches. European Nucleotide Archive (ENA) accession numbers and collection sites are given in Table S1. The best partitioning scheme for phylogenetic analyses was assessed using PARTITIONFINDER (LANFEAR *et al*., 2012) and the Bayesian Information Criterion (BIC). Three partitioning schemes were tested: (1) unpartitioned, (2) partitioned by mtDNA fragment, and (3) for protein-coding genes partitioned by gene and codon position, with the 12S gene and the DNA coding for tRNAs assigned to one additional partition each. According to the results of PARTITIONFINDER, scheme (3) was used.

Phylogenetic relationships were inferred using Bayesian and Maximum Likelihood (ML) approaches. Bayesian trees were obtained with MRBAYES 3.2.3 (RONQUIST *et al*., 2012) using the partition schemes and evolutionary models of Table S2 and default parameters. Two parallel runs, each with four chains, were conducted. The chains ran for 10 million generations with every 500th generation sampled. Calculation parameters were analyzed using the software TRACER 1.7 (RAMBAUT, *et al*., 2018) and a burn-in of 2.5 million generations to assure that both runs converged. Subsequently, only the plateau of the remaining trees was sampled, and a 50% majority rule consensus tree was generated. In addition, we inferred phylogenetic relationships under ML using RAxML 7.2.8 (STAMATAKIS, 2006) and the GTR+G substitution model across all partitions. We performed five independent ML searches using different starting conditions and the fast bootstrap algorithm to explore the robustness of the results by comparing the best trees. Then, we calculated 1,000 non-parametric thorough bootstrap replicates and plotted the values against the best tree.

For the concatenated mtDNA sequences and the cyt *b* gene (1,040 bp) alone, uncorrected *p* distances were calculated using MEGA 7.0.21 (KUMAR *et al*., 2016) and the pairwise deletion option.

Parsimony networks

For each nuclear DNA fragment, a parsimony network was constructed using POPART (http://popart.otago.ac.nz). Heterozygous sequences of Cmos, R35, Rag1, and Rag2

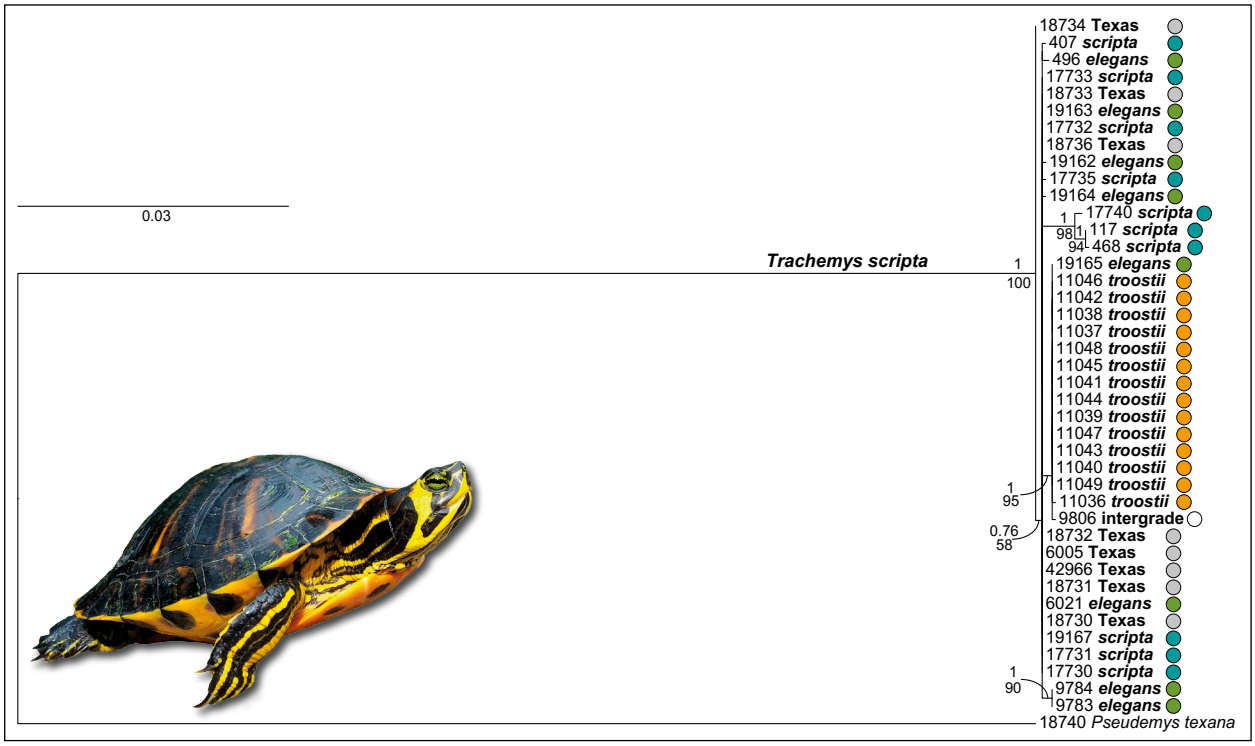

Fig. 3. Maximum Likelihood tree for 41 *Trachemys scripta* based on 3,236 bp mtDNA, rooted with *Pseudemys texana*. Numbers above nodes represent posterior probabilities; below nodes, thorough ML bootstrap values. 'Texas' refers to samples of the western red-eared slider. Note the weak resolution within *T. scripta*. Colors correspond to Fig. 2. Inset: Male *T. s. scripta*, Marianna Co., Florida. Photo: Flora Ihlow.

were phased using the Phase algorithm in DNASP 5.10 (LIBRADO & ROZAS, 2009). Nuclear DNA sequences for the networks had the same lengths as given above.

Principal Component Analyses

Principal Component Analyses (PCAs) were run to examine population structuring using microsatellite data and the package ADEGENET (JOMBART, 2008) for CRAN R 3.2.3. Compared to software based on population genetic presumptions, PCAs are less sensitive to sample size bias and not dependent on population genetic presumptions (PUECHMAILLE, 2016). Altogether, 32 individuals representing all subspecies and morphological groups were included.

Results

Based on a 3,236-bp-long alignment of mtDNA, the two tree-building methods revealed a decidedly weak resolution, both with respect to the currently recognized subspecies of *Trachemys scripta* and the morphologically distinct western red-eared slider (Fig. 3). The identical topologies of the Bayesian and ML trees corresponded to only one well supported comb-like clade that included all ingroup sequences. Neither the three subspecies nor the western red-eared slider constituted reciprocally monophyletic clades. Within this single clade, three sequences of *T. s. scripta* (117, 468, 17740) grouped together with high support; however, seven other sequences of the same subspecies were interspersed among sequences of the other subspecies and the western red-eared slider. Two sequences of *T. s. elegans* (9783, 9784) also clustered together, as did another sequence of the same subspecies (19165), the sequence of a *T. s. elegans* × *T. s. scripta* intergrade (9806), plus all sequences of *T. s. troostii*. The weak mitochondrial divergence within *T. scripta* is also reflected by the uncorrected *p* distances. For the cyt *b* gene alone (1,040 bp), the overall mean divergence across the entire data set amounted to 0.15%, with a range from 0–0.79%. For the concatenated mtDNA (3,236 bp), the respective values were 0.13% and 0–0.58%.

Also, the networks for the four nuclear loci showed no taxon-specific haplotype clusters (Fig. 4). In contrast, using 14 microsatellite loci, the PCA revealed distinct clusters for *T. s. troostii* and the western red-eared slider, whereas the nominotypical subspecies and *T. s. elegans* were not differentiated (Fig. 5).

Discussion

For decades, the taxonomy and systematics of slider turtles of the genus *Trachemys* have been in flux, and even now, there is no consensus about the numbers of species and subspecies. Yet, most authors now agree that the northernmost representatives of *Trachemys*, distributed

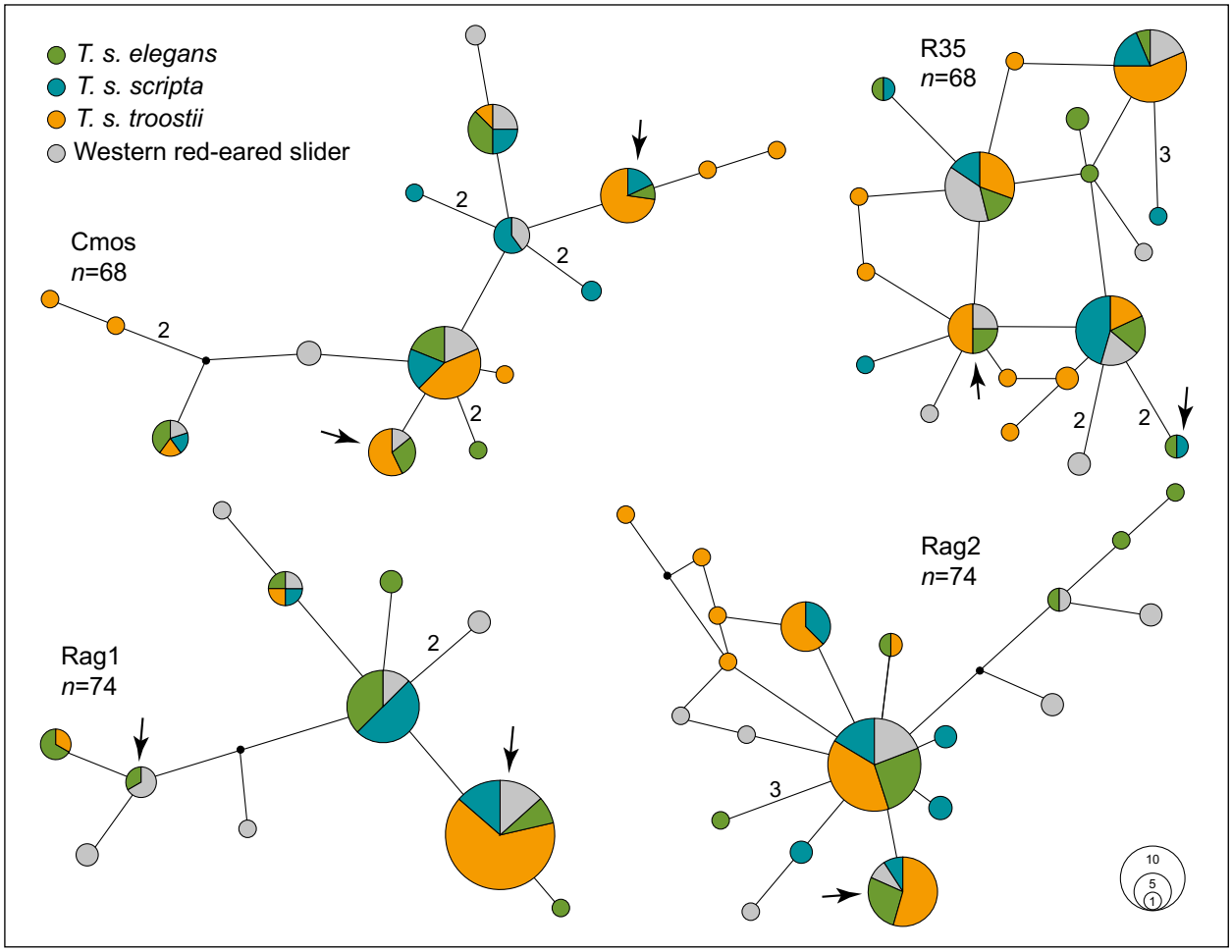

Fig. 4. Parsimony networks for the nuclear loci. Symbol size indicates haplotype frequency; slices represent percentages of shared haplotypes. Lines connecting two haplotypes represent one mutation step, if not otherwise indicated by numbers next to lines. Small black circles stand for missing node haplotypes. Note that phased sequences were used, i.e., two sequences each correspond to one individual. Colors correspond to Fig. 2. Arrows highlight the placement of the sequences from the *T. s. elegans* × *T. s. scripta* intergrade (9806).

in the USA and adjacent northeastern Mexico, represent the polytypic species *T. scripta*, while other continental slider turtles belong to other species (SEIDEL, 2002; ERNST & LOVICH, 2009; FRITZ *et al.*, 2012; SEIDEL & ERNST, 2017; TTWG, 2017; VARGAS-RAMÍREZ *et al.*, 2017; but see LEGLER & VOGT, 2013).

Three subspecies of *T. scripta* are currently recognized, *T. s. elegans*, *T. s. scripta*, and *T. s. troostii*. These taxa differ considerably in coloration and pattern, and *T. s. scripta* also differs in shell shape (CARR, 1952; ERNST & LOVICH, 2009). Western red-eared sliders also differ significantly in coloration and pattern from *T. s. elegans* found in the central and eastern parts of the distribution range (LEGLER & VOGT, 2013). Western red-eared sliders, in particular those from the Rio Grande drainage, typically show wide yellow carapace markings with reduced green coloration and a divided postorbital mark that does not extend to the orbit (CAGLE, 1950; CARR, 1952; SEIDEL *et al.*, 1999; VETTER, 2004; LEGLER & VOGT, 2013; FORSTNER *et al.*, 2014). In view of the conspicuous morphological differences between the three subspecies and the western red-eared slider, the negligible genetic differentiation is unexpected.

Using 768 bp mtDNA of two samples each of *T. s. scripta* and *T. s. elegans*, JACKSON *et al.* (2008) found no consistent differences between the two subspecies. Among the three marker systems applied in our study, only the quickly evolving microsatellite loci discriminated *T. s. troostii* and the western red-eared slider from the remaining two subspecies, while *T. s. elegans* and *T. s. scripta* were not distinct. With respect to the latter two subspecies, we cannot explain the conflict of our results with those of AVISE *et al.* (1992) and WALKER & AVISE (1998). Based on fingerprints using mtDNA digested with restriction endonucleases, these authors found a phylogeographic split in *T. scripta* largely in agreement with the distribution ranges of *T. s. scripta* and *T. s. elegans*. Further research is required for elucidating this discordance. Also, FRITZ *et al.* (2012) reported differences between *T. s. scripta* and *T. s. elegans* with respect to mtDNA (four mitochondrial genes, up to 3,242 bp), but their study was based on only a few specimens that have also been included in the present investigation and proved to be not representative for the variation in each subspecies.

A recent study found weak genetic divergence similar to *T. scripta* in another widely distributed North American

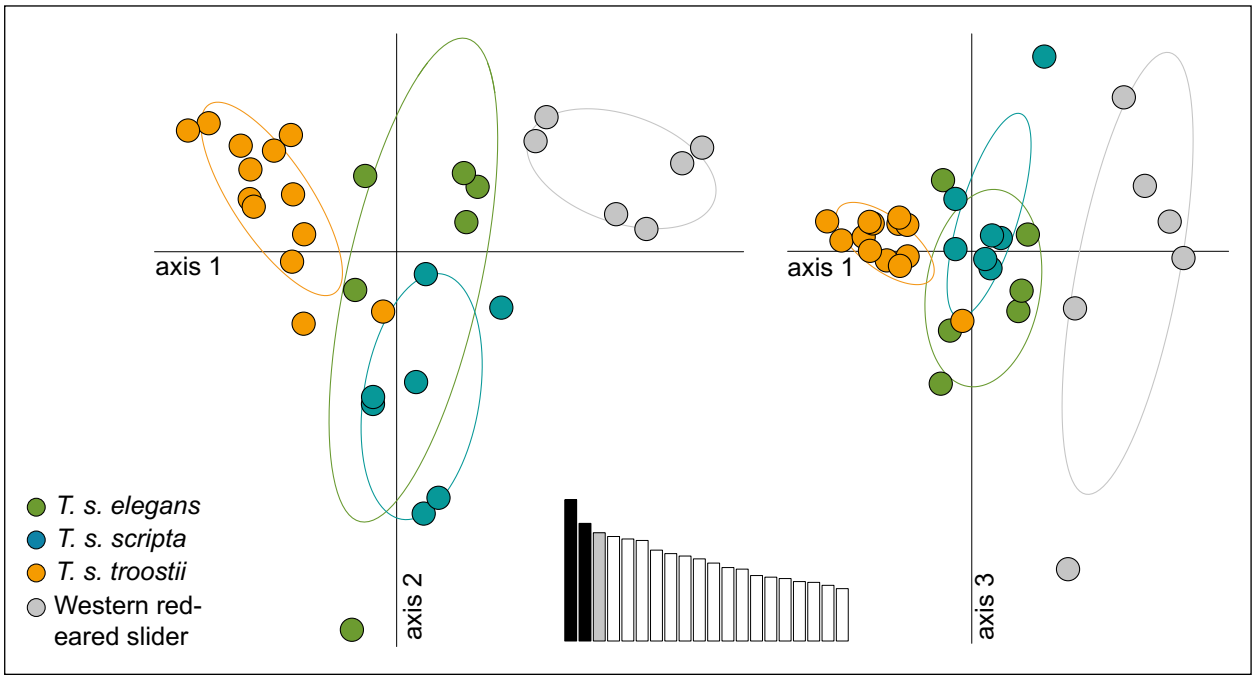

Fig. 5. PCA for microsatellite data of 32 samples of *Trachemys scripta*. The oval outlines correspond to 95% confidence intervals. Axis 1 contributes 12.92%; axis 2, 10.77%; and axis 3, 9.91% of the variance (Eigenvalues: 1–12.92, 2–10.77, 3–9.908, 4–9.562, 5–9.337). Colors correspond to Fig. 2. No microsatellites were available for the *T. s. elegans* × *T. s. scripta* intergrade (9806).

freshwater turtle (*Chrysemys picta*). This species also has morphologically distinct subspecies. The weak genetic divergence in *C. picta* was attributed to a rapid Holocene range expansion, most likely from a single glacial refuge (Reid *et al*., 2019), which led to a continent-wide North American distribution, spanning from the Atlantic to the Pacific, and even crossing the Rocky Mountains. However, as in *T. scripta*, the morphologically distinct subspecies of *C. picta*, with parapatric distributions, suggest instead that several refuges existed. The weak genetic divergences in the two species could imply that the morphological divergence is young and perhaps only of Wisconsin age. Only weak genetic divergence was also discovered in populations of a Western Palearctic snake species (*Natrix natrix*) that were isolated from one another during the last glacial period (Kindler *et al*., 2018), underlining this possibility.

Several species of *Trachemys* have been named from the Pleistocene of North America, including taxa from Florida and Texas (Hay, 1908, 1916). However, subsequent workers have suggested that these all represent the modern species *T. scripta* (Weaver & Robertson, 1967; Jackson, 1988). More recently, an unnamed, potentially durophagous species from the early Pleistocene of Florida (Santa Fe River 1B Locality, Gilchrist County) was referred to cf. *Trachemys* by Parmley *et al.* (2019), based on highly fragmentary material (one lower jaw, two nuchal bones). Furthermore, based on six other nuchal bones, Parmley *et al.* (2019) identified up to two additional species of *Trachemys* at the same fossil site. The possible existence of three syntopic *Trachemys* species is unexpected, particularly given the synonymy of other Pleistocene species of *Trachemys* with *T. scripta* and the extant distribution patterns of *Trachemys* species.

Jasinski (2018) noted the difficulty in differentiating *Graptemys* and *Trachemys* osteologically due to the overlap of some characters previously considered diagnostic for the latter. Some features used by Parmley *et al.* (2019) to assign the lower jaw of the putatively durophagous species to *Trachemys* are also present in *Graptemys*, particularly the fossil species *G. kerneri* from the late Pleistocene of Florida (Ehret & Bourque, 2011). The highly rounded anterior margin of the mandible in dorsal view ('U-shape') would be more common in *Trachemys*, as Parmley *et al.* (2019) mentioned, but has been found in specimens referred to *G. kerneri* as well (Ehret & Bourque, 2011: Fig. 5F). The more pinched anteromedial margin of the triturating surface in the specimen discussed by Parmley *et al.* (2019: Fig. 5A) is also more often present in *Graptemys*, along with the absence of an anterior terminal hook, or the presence of an inconspicuous one. Therefore, without more complete specimens, it seems more likely that the potentially durophagous species of Parmley *et al.* (2019) represents a fossil *Graptemys*.

Pleistocene fossils referred directly to *T. scripta* have been identified from several states in the United States, including Florida, Illinois, Indiana, Kansas, Mississippi, Missouri, Oklahoma, South Carolina, Tennessee, and Texas (Ernst & Lovich, 2009). Although Jasinski (2018) suggested that multiple Pleistocene species may have existed, the morphological variation of living *T. scripta* encompasses that which is seen in Pleistocene *Trachemys* from the United States. This suggests that the individual nuchal elements identified as possibly belonging to multiple species by Parmley *et al.* (2019) probably represent a single early Pleistocene species of *Trachemys*. It seems likely that only one wide-ranging species, *T. scripta*, was

present during the Pleistocene, agreeing with the extant shallow molecular divergence.

Western red-eared sliders are morphologically distinct from eastern *T. s. elegans*, with broken postorbital stripes resembling *T. gaigeae* (CAGLE, 1950; SEIDEL *et al*., 1999; LEGLER & VOGT, 2013; FORSTNER *et al*., 2014). It has been speculated that this similarity is derived from past hybridization of these two parapatric taxa, and perhaps hybridization with *Trachemys venusta cataspila*, another taxon occurring in close proximity (CAGLE, 1950; CARR, 1952; STUART, 1995; LEGLER & VOGT, 2013). A thorough morphological examination of slider turtles from potential contact zones of the distinct taxa revealed that the head and neck patterns varied in all three taxa (SEIDEL *et al*., 1999). Although these authors agreed that western red-eared sliders from the lower Rio Grande and the Pecos River share some similarities with *T. gaigeae*, they concluded that the turtles do not represent hybrids. FORSTNER *et al*. (2014) used mitochondrial DNA and microsatellite loci to assess the genetic impact of native and introduced *T. s. elegans* on *T. gaigeae* in New Mexico and Texas. They revealed limited hybridization of *T. gaigeae* with non-native eastern *T. s. elegans* that had been released in populations of *T. gaigeae* but not with the parapatric native western red-eared sliders.

Our study compared for the first time all three subspecies of *T. scripta* and the western red-eared slider genetically using mitochondrial and nuclear DNA sequences and microsatellite loci. We revealed no genetic differentiation between *T. s. scripta* and *T. s. elegans*, while we found *T. s. troostii* and the western red-eared slider to be divergent using microsatellites. These findings pose new questions and challenges and set the stage for future investigations using denser sampling and preferably genome-wide markers.

Neither the three subspecies nor the western red-eared slider fulfill the recently proposed criteria for the recognition of subspecies (KINDLER & FRITZ, 2018), corresponding to the criteria for Evolutionarily Significant Units as defined by MORITZ (1994). According to these criteria, subspecies should be characterized by distinct mtDNA lineages (except for cases of mitochondrial capture) and distinct nuclear gene pools. Neither the three subspecies nor the western red-eared slider represent a distinct mtDNA lineage, they do not differ in the studied nuclear DNA sequences, and *T. s. scripta* and *T. s. elegans* are not distinct with respect to microsatellite loci. This suggests that the intraspecific taxonomy of *T. scripta* is inflated, and that too many subspecies are recognized based on conspicuous differences in coloration and pattern that reflect population-specific, rather than taxonomic, differentiation. A similar situation is found in the North American map and sawback turtles (*Graptemys* spp.), in which genetic evidence indicates taxonomic inflation (PRASCHAG *et al*., 2017). However, other authors (THOMSON *et al*., 2018) have argued for continuing with the traditional systematics, even though the observed genetic fine-scale differentiation may refer rather to intrapopulational, and not taxonomic, divergence.

For traditionalists and conservationists, abandoning the well-established and morphologically distinct subspecies of *T. scripta* is not desirable. Yet, if subspecies of *T. scripta* continue to be recognized, then the current taxonomy with three subspecies is difficult to justify. Western red-eared sliders are morphologically distinct and differ from *T. s. elegans* and *T. s. scripta*, with respect to microsatellites, as much as *T. s. troostii* does. In view of this morphological and genetic evidence, subspecies status should be considered for western red-eared sliders. None of the available names for North American slider turtles refers to western red-eared sliders (TTWG, 2017), even though an unpublished 19th century manuscript name exists (BOUR, 2017). Thus, if subspecies are recognized within *T. scripta*, western red-eared sliders should be described as a subspecies new to science, with implications for future conservation strategies for slider turtles in the USA and Mexico.

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