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An assessment of function, intraspecific variation, and taxonomic reliability of eremobatid ctenidia (Arachnida: Solifugae)

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ABSTRACT

Camel spiders, members of the order Solifugae, are a diverse but poorly understood order of arachnids. The conserved morphology within many groups in the order makes species delimitation and identification challenging. In the North American family Eremobatidae, swollen setae called ctenidia are a common character employed in species delimitation, diagnoses, and identification. Unlike many other arachnid systems, the traditional use of ctenidia characters in eremobatid taxonomy and species identification does not allow for intraspecific variation, despite variation being common in this character system. To access and document the extent of intraspecific variation in this system, a combination of light microscopy and scanning electron micrographs (SEM) were used to survey and document the inter- and intraspecific variation of ctenidia shaft number, shape, and relative length. Additionally, these characters were also evaluated for species-group level and/or genus level taxonomic utility by evaluating the phylogenetic signal for each using a previously published molecular phylogeny as a context. Lastly, as ctenidia have no known biological function, we also assessed ctenidia shaft morphology for evidence of mechano- and/or chemoreceptive function. Observations from nearly 800 museum specimens indicate that ctenidia characteristics are generally far more variable within individual species than previous taxonomic literature indicates, necessitating increased caution for utilization in species diagnoses, delimitation and identifications. Phylogenetic signal was detected for shaft number and shape, but shaft length was not constrained by phylogenetic proximity. Scanning electron micrographs did not reveal morphology consistent with arachnid mechano- or chemoreception, as evidenced by a lack of pores or shaft mobility. Although the biological function of ctenidia remains elusive, the presence of phylogenetic signal and shifts in shaft number and shape may indicate some functional significance yet undiscovered.

1. Introduction

1.1. Ctenidia as taxonomic characters

Solifuges, colloquially known as camel spiders, comprise the sixth largest order of Arachnida with approximately 1100 described species (Harvey 2003). Due to the notorious difficulty of capturing and maintaining solifuges in captivity, few researchers are focused on this group, leaving many aspects of their biology elusive (Punzo 1998, 2012). Solifuge taxonomy is a particular challenge due to their conserved morphology, and is reliant on the dentition and setation of the chelicerae (see Bird et al. 2015). Outside of the chelicerae, one of the most common taxonomic characters used for species delimitation and identification is ctenidia, modified setae on various opisthosomal sternites of families Ammotrechidae, Daesiidae, Eremobatidae, Galeodidae, Karschiidae, Melanoblossidae, and Mumuciidae (Roewer 1933; Wharton 1981; Maury 1984). In the North American family Eremobatidae, ctenidia characteristics are frequently used for species delimitation, diagnoses and commonly employed in dichotomous keys.

Ctenidia were first described by Kraepelin (1901) as “tubular hairs and tubular bristles. Peculiar, soft, often almost fleshy hair-like structures of lancet, spatula or sickle shape. Often tapering to basis and bulbous in the middle, as they are frequently present on particular segments of the ventral side of the abdomen or on the ventral side of the tarsus of the 4th leg of the male”. The hairs present on the abdominal sternites were then distinguished as

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"ctenidien," by Roewer (1932, 1933, 1934, 1941, 1942) in laying the foundation for both New World and Old World solifuge systematics and continue to be broadly used. Botero-Trujillo (2016) is the only contemporary author to modify this definition, describing ctenidia as flexible, long, non-bifid setae-like structures; though this definition may only apply to the South American family Mummuciidae. Maury (1984) recognized that the term "ctenidia" as currently used is reflective only of its taxonomic utility and not of any hypotheses of homology or shared function among the solifuge families. Ctenidia are generally sexually dimorphic, being present in males and either absent or highly reduced in females (Maury 1984; Muma 1951; Wharton 1981), and may vary in number between developmental stages (Wharton 1981). In Eremobatidae, ctenidia (when present) are found on the posterior margin of the fifth abdominal sternite proximate to an unpaired spiracle, but any functional association with respiration is untested.

1.2. Taxonomic use of ctenidia in Eremobatidae

Eremobatid species generally exhibit a high degree of interspecific variation in ctenidia number and morphology, allowing a certain degree of utility in dichotomous keys and diagnoses (Muma 1951; 1962; 1963; 1970; 1987; 1989; Rowland 1974; Brookhart & Muma 1981; Muma & Brookhart 1988; Brookhart & Cushing 2002; 2004; 2005; 2008; Cushing & Brookhart 2016). As a likely consequence of limited material availability, the majority of eremobatid taxonomic publications rarely indicate the presence or extent of intraspecific variation in ctenidia morphology and number, and typically describe each species as having a set number of ctenidial shafts, relative shaft length, and shaft shape or size. Some dichotomous keys, such as those developed by Brookhart & Muma (1981), Brookhart & Cushing (2004) and Cushing & Brookhart (2016) for the *Eremobates pallipes* and *scaber* species groups rely heavily on ctenidia shaft length, shape or size and number for species identification. Members of the *Eremobates palpisetulosus* species group are the notable exceptions, in which most species are described as having varying numbers of ctenidia despite ctenidia number being used to delimit, diagnose, and identify individual species (Muma 1951; Muma & Brookhart 1988).

Further, descriptions of ctenidia shape are commonly described in undefined subjective, ambiguous, or redundant terms. From their original depiction in Kraepelin (1901), through most works by Muma, Brookhart, and Cushing, nearly twenty different descriptors have been used for eremobatid ctenidia shaft shape. Based on taxonomic hand-drawings, "stiletto-shaped," "hair-like," "needle-like," "peg-like," "tubular," or "trace" all seemingly describe an unmodified cylindrical shaft that gradually tapers in diameter from the base to the tip, but the subtle distinction between them, other than slight differences in shaft thickness or length, are unclear (Roewer 1932; 1934; Muma 1951; 1962; 1970; Brookhart & Muma 1981; Brookhart & Cushing 2004; Cushing & Brookhart 2016). Consequently, these descriptors may only represent subtle gradients in shaft thickness and/or length rather than distinct shapes. Observation of previously undocumented intraspecific variation in ctenidia characteristics during ongoing taxonomic work within the *E. pallipes* and *scaber* species groups, particularly in regards to the number of ctenidia shafts and the stability of shape descriptions, prompted a family-wide survey of ctenidia characters.

Thus far, Eremobatidae is the only solifuge family for which a family-level molecular phylogenetic hypothesis has been published (Cushing et al. 2015). Both molecular hypotheses presented in Cushing et al. (2015) indicate a need for new taxonomic revisions of polyphyletic genera *Eremochelis* Roewer, 1934 *Hemerotrecha* Banks, 1903, and *Eremobates* Banks, 1900 as well as several of their constituent species groups (Cushing et al. 2015). The availability of a molecular hypothesis provides an opportunity to search for morphological patterns and/or synapomorphies among monophyletic clades rendered in the Cushing et al. (2015) analysis.

The objectives of the present study are threefold: a) assess the extent of intraspecific variation in ctenidia number and morphology (specifically shaft shape and relative length) to validate their stability for use in species delimitation and diagnoses; b) determine if ctenidia modal number, shaft shape, and relative length exhibit phylogenetic signal as an indicator of taxonomic utility at a species-group or genus level and/or biological importance; and c) test sensory functional hypotheses by determining if ctenidia morphology is consistent with known mechano- and/or chemoreceptive setae. Due to the extensive use of ctenidia number and morphology in taxonomy, the presence of extensive intraspecific variation would necessitate the need to adjust their taxonomic use appropriately. If ctenidia characteristics exhibit either phylogenetic signal or evidence of a selective process, the former would have taxonomic implications useful in ongoing revisionary efforts and the latter could be indicative of a consequential biological function or directional selection. The presence of pores and/or a highly mobile shaft inset a wide socket, as detected using a tabletop SEM, would prompt further investigation to test functional hypotheses.

2. Material and methods

2.1. Scanning electron micrograph examinations and assessment of functional significance

Specimens were chosen for SEM in order to represent each focal species with at least one individual, and to capture examples of intraspecific variation (see section 2.2), including specimens that were deemed to exhibit highly "unusual" morphology (e.g., unusual shaft placement, unique morphologies). Prior to examination via SEM, the opisthosoma was separated from the prosoma at the mesopeltidium using micro scissors. Sample opisthosomas were cleaned of particulate debris using a Vigor mini-ultrasonic cleaner for three to five minutes. Micrographs were generated using a Hitachi TM400 Plus Tabletop Microscope.

Micrographs of focal specimens were also used to test for morphology consistent with known arachnid sensory setae structures. For ctenidia to be morphologically consistent with arachnid mechanoreception, they would need to be highly mobile shafts inset a wide cuticular depression that are capable of deflection by air movement, physical contact and/or vibrations in the substrate (Foelix & Chu-Wang 1973; Barth 2004). Morphological consistency with arachnid olfaction or contact chemoreception would require the presence of pores on the tip or along the shaft of the setae, allowing for detection of volatile chemicals present in the environment (Slifer 1970; Foelix & Chu-Wang 1973b; Harris & Mill 1973; Zacharuk 1980; Foelix 1985). At least one specimen for each focal taxon was examined for evidence of either sensory modality.

2.2. Survey of intraspecific variation

Focal specimens included males of all terminal taxa represented in the Cushing et al. (2015) analysis, except for *Hemerotrecha nevadensis* Muma, 1951, *Horribates bantai* Muma, 1989, *Eremobates coahuilanus* Muma, 1986, *Eremobates acuitlapanensis* Vazquez and Gavino-Rojas, 2000, *Eremobates pimanus* Muma & Brookhart, 1988, and an undescribed *E. palpisetulosus* species group taxon due to lack of material. Specimens were sampled from the arachnology collection of the Denver Museum of Nature & Science (DMNS) and loan material from Universidad Nacional Autonoma de

Mexico (UNAM), and the College of Idaho (CIDA). A total of 798 individual males were surveyed via light microscopy, representing 75 out of 81 terminal taxa in the Cushing et al. (2015) analysis. Species identifications were verified by RRJ and/or PEC in addition to DMNS research assistant Jack Brookhart. Between one and 20 male records were examined per focal species where material allowed. Data was not recorded when poor specimen condition prohibited confident characterizations of ctenidia number or morphological data. Because the *Chanbria* Muma, 1951, *Eremocosta* Roewer, 1934, and *Eremorhax* Roewer, 1934 genera are defined as lacking ctenidia, a maximum of five specimens per species represented in the Cushing et al. (2015) analysis were examined, and one *Chanbria* was imaged via SEM as a representative for a ctenidia-less taxon.

Recorded observations for each specimen included ctenidia number, shaft length relative to the length of the succeeding sternite, and shaft shape (supplementary material). Because it is not uncommon for shafts to be damaged or otherwise missing, ctenidia number was measured by the presence of the enlarged socket unique to ctenidia (see Results). The length of the ctenidia shaft was determined to be "short" if the shaft was shorter than half the sternite succeeding the ctenidia base, "medium" if they extended beyond half and up to the posterior edge of the succeeding sternite, and "long" if they extended past the length of the succeeding sternite. This coding scheme was implemented in lieu of physical measurements of shaft length in considerations of both the traditional characterizations used in eremobatid taxonomy and the large total sample size, and that variable body size between species would confound comparisons of absolute measurements. The variety of character states observed, the modal character states, and the percent of study specimens exhibiting the modal character state were summarized per species in Microsoft Excel (Table A1). Light microscopic images were also taken of a subsample of focal specimens to demonstrate characterizations of length and shape as they would be typically seen via light microscopy. Compound light microscopic images were captured using a Leica M165C and processed via Leica OEM software.

2.3. Testing for phylogenetic signal & homoplasy, character mapping, and ancestral state reconstruction

Due to the intraspecific variation exhibited in most taxa examined, the modal character state observed for each taxon was used for assessing phylogenetic signal. Of the taxa in which no male records were available for examination, including *E. acuitlapanensis*, *E. coahuilanus*, and *E. pimanus*, the modal character state was assumed to match species descriptions from the taxonomic literature. Three indices of phylogenetic signal were employed within the context of the maximum clade credibility tree resultant from the Bayesian Evolutionary Analysis by Sampling Trees analysis (BEAST; Drummond & Rambaut 2007) from Cushing et al. (2015). Modal ctenidia number counts were evaluated for phylogenetic signal as a continuous character using the Abouheif's C_{mean} test for phylogenetic autocorrelation, performed via the abouheif.moran function from the adephylo package in R (Jombart et al. 2010) with 1000 permutations. Abouheif's C_{mean} is adapted from Moran's I spatial-correlative test and uses the supplied phylogenetic topology to estimate a matrix of relatedness and tests for correlations of character states among related taxa (Abouheif 1999; Jombart et al. 2010). The analysis generates a sampling distribution of C_{mean} parameters by divorcing character states from corresponding terminals, simulating a null hypothesis of no phylogenetic correlation (character states are randomly distributed throughout the phylogenetic tree). The other two indices, Blomberg's K (Blomberg et al. 2003) and Pagel's λ (Pagel 1999) assume a Brownian motion process of trait evolution (Münkemüller et al. 2012), and were employed via the R package phytools with the function phylosig() (Revell 2012). Similar to Abouheif's C_{mean}, null distributions of K and λ values were generated through 1000 permutations of randomly shuffled traits among the tree tips. To evaluate whether the observed K and λ statistics were significantly different from 0 (no phylogenetic signal), a p-value was calculated by dividing the number of permutations where the K and λ were larger than the observed values by 1000. While a p-value of <0.05 indicates the presence of signal, both statistics can indicate weak signal (K or $\lambda < 1$), congruence with a Brownian motion model (K or $\lambda = 1$) or strong signal (K or $\lambda > 1$). Because of the inability for these packages to handle unknown data, species for which no records or taxonomic information was available (*H. nevadensis* Muma 1951, *H. bantai* Muma 1989, and the aforementioned undescribed *E. palpisetulosus* species group taxon) were excluded. These three taxa were coded for unknown character states and included in the analyses of shaft shape and relative length.

While the three indices used here are well-suited for quantitative traits, they are unable to analyze the categorical coding of shaft shape or length. To detect phylogenetic signal in the categorical traits shape and length, the character states per taxa were mapped onto the terminals of the Cushing et al. (2015) BEAST analysis, and then shuffled 1000 times to create a null distribution (simulating random evolution) for each trait. For both ctenidia shaft shape and length, the number of parsimonious steps and Markov k-state 1 (Mk1) parameter model estimates from the observed data were compared to the mean values of the null distributions using Mesquite (Maddison & Maddison 2019). Phylogenetic signal, or non-random character evolution, is indicated when the observed states fall within the lower tail of the null distribution, considering an alpha value of 0.05. To avoid the statistical bias of the absence of ctenidia as a character state, all taxa representing the ctenidia-less genera *Chanbria*, *Eremocosta*, and *Eremorhax* were excluded from these analyses. To provide further context to interpret phylogenetic signal metrics, consistency (CI) (Kluge & Farris 1969) and retention index (RI) (Farris 1989) scores were also calculated for both shape and length as a measure of convergent evolution among the tips of the Cushing et al. (2015) phylogeny. Both indices take a value closer to 0 when the focal character exhibits homoplasy among the tips of phylogenetic tree. A value of 1 would suggest that most branches exhibit an apomorphic character state, or a complete lack of homoplasy.

To visualize the distribution and the evolutionary history of the traits, maximum likelihood ancestral state reconstructions for both shaft shape and length were performed using the rerootingMethod() function from R package phytools (Revell 2012). Maximum likelihood ancestral state reconstruction for the continuous number data was generated using fastAnc(), and plotted via contMap() of phytools, which graphically expresses changes between nodes as gradual shifts in colors, despite number data being scored as integers.

3. Results

3.1. Functional morphology assessment

The ctenidia shaft surface is absent of any processes, depressions, or features and has an otherwise smooth, unremarkable cuticular surface (Figs. 1A and 2). The shaft is joined to the opisthosoma by a raised thick cuticular socket that tightly hugs the base, permitting little to no deflection of the setae (arrows, Fig. 1A). This thickened cuticular socket is a unique morphological feature that allows identification of ctenidia presence even if the shaft has broken off or is otherwise unobservable. There were multiple

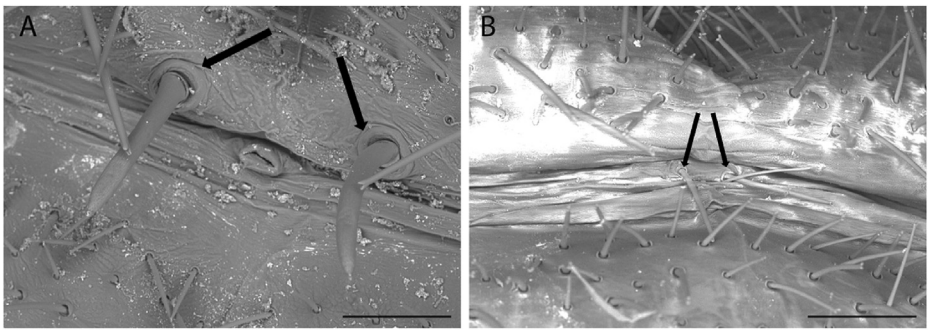

Fig. 1. Ctenidia and serendipitous abdominal setae. Scanning electron micrographs of A) *Eremobates barberi* Muma, 1951 (DMNS ZA.27625), arrows indicating the enlarged cuticular socket used to identify ctenidia; B) *Eremobates pallipes* (Say 1823) (DMNS ZA.37758), arrows indicating "false ctenidia" (abdominal setae in the position where ctenidia are expected). Scale bar is 200 μm.

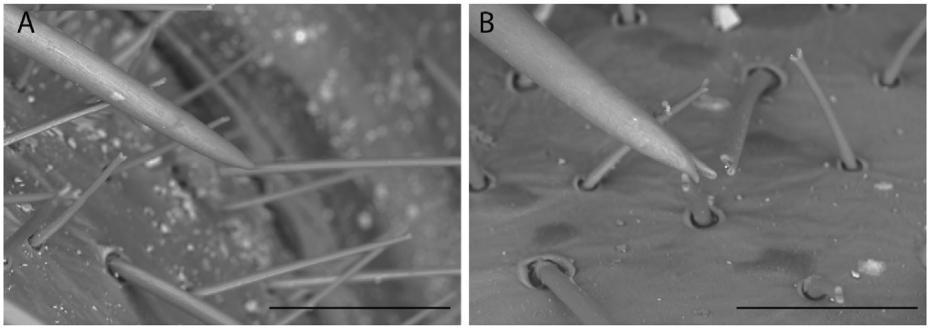

Fig. 2. Ctenidia tip morphology. Scanning electron micrographs of A) *Eremobates kastoni* Muma & Brookhart, 1988, with a simple tip, and B) *Eremobates ctenidiellus* Muma, 1951 with a bifurcated tip. Scale bar is 200 μm.

specimens in which the presence of ctenidia was ambiguous under a light microscope, where it appeared there were thin ctenidia in the correct placement, but these were revealed to be abdominal setae under SEM due to lack of the characteristic ctenidia socket (Fig. 1B). These "false ctenidia" were only found in some specimens of species comprising the *Eremobates scaber* and *E. pallipes* species groups. In some specimens from the *E. palpisetulosus* species group, there were one or multiple ctenidia that appeared to be setae but demonstrated the characteristic enlarged socket (Fig. 3K). The ctenidia shaft terminates in either a simple, blunt tip or a bifurcation like those of the surrounding setae (Fig. 2), but no porous openings or pits in the cuticle were observed (Figs. 1 and 2). Although the shaft shape is variable, this assessment did not identify any observable features that are consistent with either chemoreceptive or olfactory function, nor any features that would necessitate further investigation. However, light microscopy surveys did reveal a distinct white, glandular-like structure of unknown function at the base of both ctenidia in a few specimens (Fig. 3A).

3.2. Intraspecific variation of ctenidia

Of the 75 species examined, 52 (69%) exhibited intraspecific variation in either shaft number, shaft shape, or relative length, or a combination thereof (Table A1). Of the 23 remaining taxa in which ctenidia characteristics were invariable, 20 were invariable due to not having ctenidia at all. Of the 55 species where ctenidia were present, only *Hemerotrecha denticulata* Muma, 1951 (n = 7), *Eremochelis undulus* Muma, 1989 (n = 2), and *Eremochelis imperialis* (Muma 1951) (n = 7) were invariable in ctenidia shaft number, shaft shape, and length. The light microscopy survey, followed by SEM examinations, determined the five shaft shape character states as defined in Table 1 and depicted via light microscopy in Fig. 3 and through SEM in Fig. 4. Most species demonstrated both simple and bifurcated tips; the latter being either prominent and distinct or reduced and barely distinguishable via light microscopy.

No evidence of ctenidia shafts or the characteristic cuticular socket on the opisthosoma was found on any of the species from the *Chanbria*, *Eremocosta*, or *Eremorhax* genera. For *Hemerotrecha* species, variation from the modal state for shape (25% of all *Hemerotrecha* specimens) was not uncommon, but variation was less evident for length (13%) and number (7%). In contrast, variation within *Eremochelis* species was relatively less common between number (8%) and length (12%), with only one *Eremochelis kastoni* Rowland, 1974 specimen deviating from the modal shape. Due to the polyphyletic nature of *Hemerotrecha* and *Eremochelis*, the consistency of ctenidia number, shaft length, and/or shaft shape within strongly supported clades of the Cushing et al. (2015) analysis are summarized in Table 2 and visually identified in Fig. 6.

All members of the monophyletic *Hemerotrecha banksi* species group clade exhibited either stiletto-like or spatulate shaft shapes, with the spatulate shape being far more frequent among all three species and length was highly variable for both *Hemerotrecha hanfordana* Brookhart & Cushing, 2008 (Fig. 4I) and *H. californica* Banks, 1903 (Fig. 4G and H) (Table A1). Although the *H. branchi* species group is not rendered monophyletic by the 2015 analysis (putatively due to *H. milsteadi* and *Hemerotrecha sevilleta* voucher material being misidentified), three constituent species *H. bixleri* Muma, 1989 (Fig. 4A), *H. branchi* Muma, 1951 (Fig. 4C), and *H. xena* Muma, 1951 (Fig. 4B) comprise a monophyletic clade with highly consistent morphology (Fig. 6, clade 1).

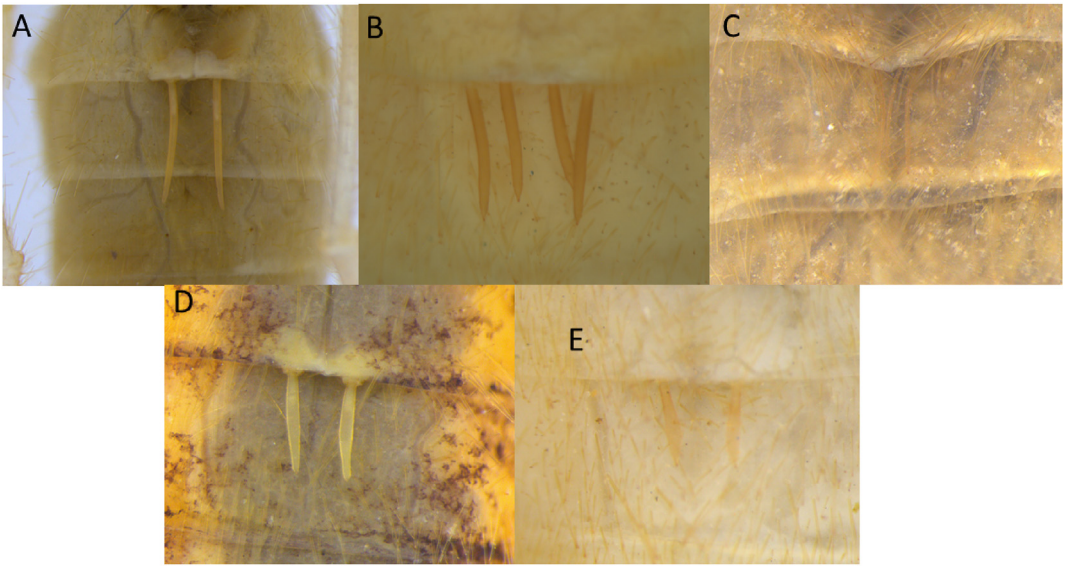

Fig. 3. Light microscopy of ctenidia shaft shape variation, shape morphology as described in Table 1. A) Stiletto-like (*H. branchi* (DMNS. ZA.23784)), B) pin-like (*Eremothera drachmani* (DMNS ZA.37840)); C) Setae-like (*Eremobates marathoni* (DMNS ZA.33676)); D) Flattened (*Eremochelis striodorsalis* (DMNS ZA.22164)); and E) Spatula-like (*Eremobates ascopulatus* (DMNS ZA.33317)).

Table 1
Descriptions of ctenidia shaft shape character descriptions employed in character mapping, ancestral state reconstructions and phylogenetic signal inference.

Stiletto-like	Cylindrical along the entire length of the shaft, with the shaft diameter thickest at the base, gradually tapering towards the distal end and terminating in either a blunt or bifurcated tip. Easily distinguished from neighboring abdominal setae. Stiletto-shaped shafts can vary greatly in length between species as well as conspecifics. Slight differences in thickness give the perception of a "needle-like," "hair-like," or other similar descriptors (see Introduction), but are not considered unique shapes. Originally depicted by Roewer (1932; Abb. 116a). See Figs. 3A; 4A, B, D, E, G, N, and O.
Pin-like	Cylindrical and of uniform diameter extending most of the shaft length, with an abrupt termination in either a simple or bifurcated tip. Similar to stiletto-like, pin-like shafts can vary slightly in thickness. Generally easy to distinguish from abdominal setae, with occasional difficulty due loss of color/opacity from preservation. Originally used by Roewer (1932; Abb. 116d). See Figs. 3B; 4J.
Setae-like	Shaft is very thin, somewhat translucent and can be difficult to distinguish from neighboring abdominal setae using light microscopy, but has the characteristic socket enlarged basal generally visible under LM. See Figs. 3C; 4M and N.
Flattened	Shaft is cylindrical immediately proximate the socket, but flattens dorso-ventrally for the majority of its length and quickly narrows to a simple or bifurcated tip. See Figs. 3D; 4C and F
Spatulate	A short proximate portion of the shaft is cylindrical, while the lateral portion widens laterally and shaft flattens until terminating in a bifurcated tip. See Figure 3E; 4H, I and L.

Four *Eremobates* species groups are represented in the analysis, although only the *E. pallipes* species group is rendered monophyletic. All of the *E. pallipes* species group species that exhibited ctenidia varied in shaft number, but not in shaft shape or length (Table A1; Fig. 6, clade 4). It is worth noting that the majority of *E. pallipes* Say, 1823 and half of the *E. docolora* Brookhart & Muma, 1981 (Fig. 4M) specimens had no ctenidia, but other conspecifics had as many as two. For both species, when ctenidia were present, they exhibited the reduced setae-like shape that is barely distinguishable from neighboring setae. The *E. scaber* species group is rendered paraphyletic by the placement of *E. fisheri* Cushing & Brookhart, 2016, but the clade comprising the remaining *E. scaber* species group taxa are similarly variable in number (25% of all specimens exhibit non-modal character states) and invariable in relative shaft length (excluding *E. fisheri*), but more (15% non-modal) in shaft shape (Table A1; Fig. 6, clade 5). *Eremobates ascopulatus* Muma, 1951 was the most variable in shape, exhibiting both pin-like (Fig. 4J; also has unusual shaft placement) to the most extreme example of the spatulate morphology observed (Fig. 4L) and both morphologies on the same specimen (Fig. 4K). Additional examination of a limited number of *Eremobates ctenidiellus* Muma, 1951, *E. similis* Muma, 1951, and *E. mormonus* (Roewer 1934) (*E. scaber* species group taxa not represented in the 2015 analysis; unpublished data) revealed specimens in which the presence of one or more ctenidia were seemingly ambiguous under light microscopy but were determined to be setae by the lack of the characteristic socket. Considering the entire clade comprising both species groups and *Eremothera* Muma, 1951 species, 19% of specimens deviated from modal character states, while all but one *Eremothera sculpturata* Muma, 1951 specimen deviated from short shafts, and only 5% of all specimens deviated from the pin-like shape per species. Due to putative misidentified specimens used for *H. sevilleta* Brookhart & Cushing, 2002, *H. milsteadi* Muma, 1962, and *H. denticulata* Muma, 1951 in the Cushing et al. (2015) analysis, they are omitted from the summarized results for the clades (Table 2).

The polyphyletic *E. palpisetulosus* species group, as a whole, is far more variable in number (43%) than all other clades or species groups considered. However, one monophyletic clade comprising the *E. palpisetulosus* species group distributed in southern California exceeds the variation in number (62%) for the entire group and in length (24%). All records examined from *E. palpisetulosus* species group taxa exhibited stilleto-like shaped ctenidia, with the exception of *Eremobates marathoni* Muma, 1951, which is the only *palpisetulosus* group species observed to have setae-like ctenidia (Fig. 3C; Table 2; Fig. 6, clade 6). However, several specimens from

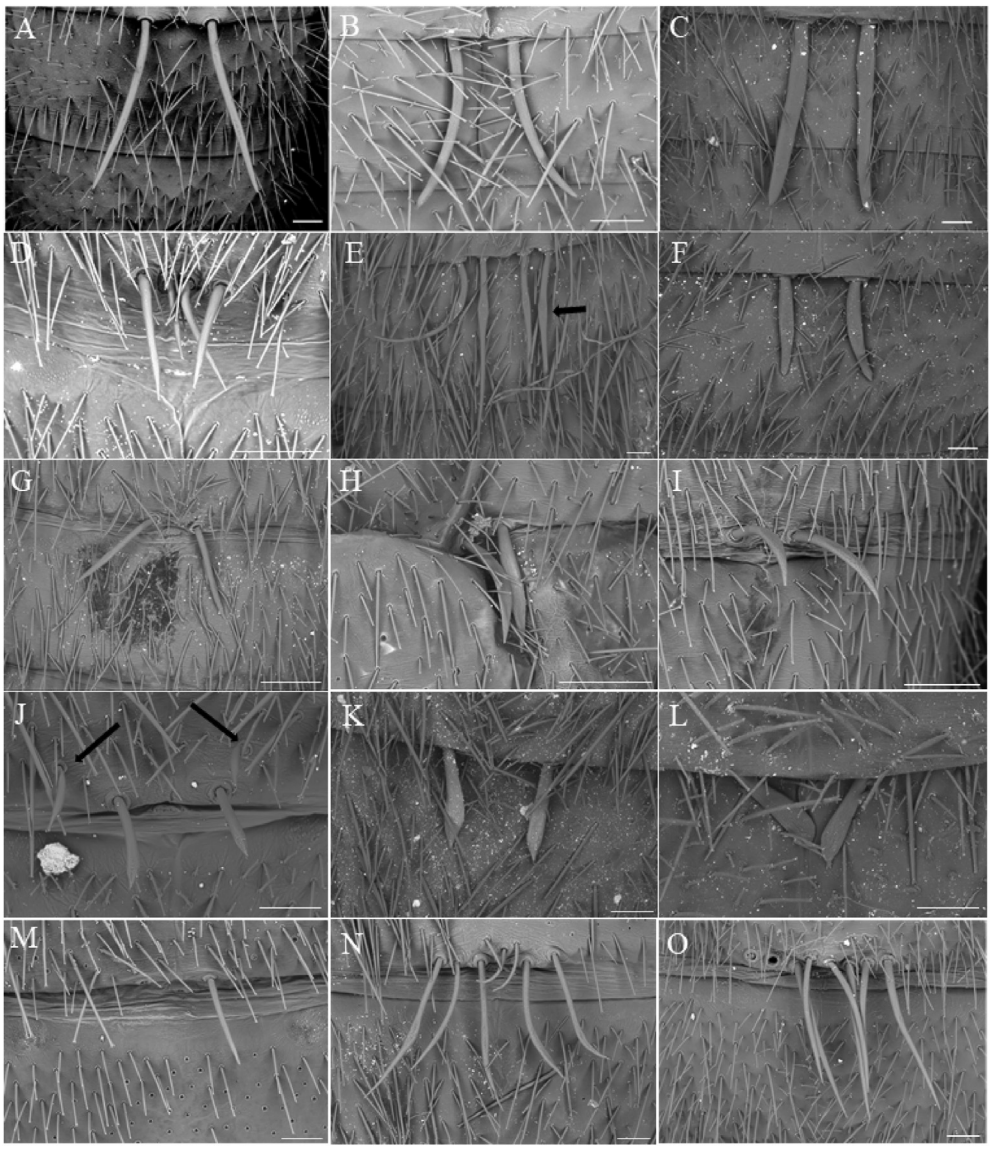

Fig. 4. Scanning electron micrographs of ctenidia shape variation, by descending (most ancient to youngest) node order within the Cushing et al. (2015) analysis. A) *H. bixleri* (DMNS ZA.26445); B) *H. xena* (DMNS ZA.23771); C) *H. branchi* (DMNS ZA.23780); D) *H. elpasoensis* (DMNS ZA.23553); E) *E. larreae* (DMNS ZA.37013 - the compressed portions identified by the arrow are artifacts of sample preparation); F) *E. striodorsalis* (DMNS ZA.22164); G) *H. californica* (DMNS ZA.18288); H) *H. californica* (DMNS ZA.18196); I) *H. hanfordana* (DMNS ZA.17318); representing clade four: J) *E. ascopulatus* (DMNS ZA.35520a), arrows indicating unusual ctenidia placement; K) *E. ascopulatus* (DMNS ZA.38520b); L) *E. ascopulatus* (DMNS ZA.33317); M) *E. docolora* (DMNS ZA.16149); N) *E. gracilidens* (DMNS ZA.16286), arrows indicating additional setae-like ctenidia; and L) *Eremobates leechi* (DMNS ZA.23764). Scale bar is 200 μm.

multiple *E. palpisetulosus* group species, such as *E. palpisetulosus* Fichter, 1941, *E. gracilidens* Muma, 1951 (Fig. 4N), *E. norrisi* Muma & Brookhart, 1988, *E. scopulatus* Muma, 1951, and *Eremobates leechi* Muma & Brookhart, 1988 (Fig. 4O), also appeared to exhibit one or several reduced, setae-like ctenidia in addition to easily distinguishable stilleto-like ctenidia under light microscopy.

3.3. Phylogenetic signal, measures of homoplasy, and ancestral state reconstruction

Abouheif's C_{mean} found that modal ctenidia number is phylogenetically correlated ($C_{mean} = 0.536$, $p = 0.001$). Pagel's λ as well as Blomberg's K similarly detected phylogenetic signal in modal ctenidia number ($\lambda = 0.76$, $p < 0.00001$; $K = 0.12$, $p < 0.001$). Ancestral state reconstructions for modal number, expressed as a gradient along the branch length is in Fig. 5; shape, and length are given in Fig. 6. The observed number of parsimonious steps (22) and Mk1 rate estimate (0.07) for ctenidia shape both fell into the lower tail of the null distribution in Mesquite, indicating that shape is evolving in a non-random fashion. Conversely, length was found to be evolving randomly, with the observed parsimonious steps (33) equaling the average value of the null distribution, and the maximum likelihood rate (317.06) well between the lower tail (0.72) and upper tail (9999.99). Ctenidia shaft number, length, and shape were evaluated to be moderately convergent as measured by the consistency index (CI = 0.23; 0.14; and 0.21; respectively), but contrast to moderately higher RI index values RI = 0.36; 0.52; and 0.44; respectively).

The ancestral state reconstruction estimates that two ctenidia were present in the ancestor to all Eremobatidae taxa. In addition to

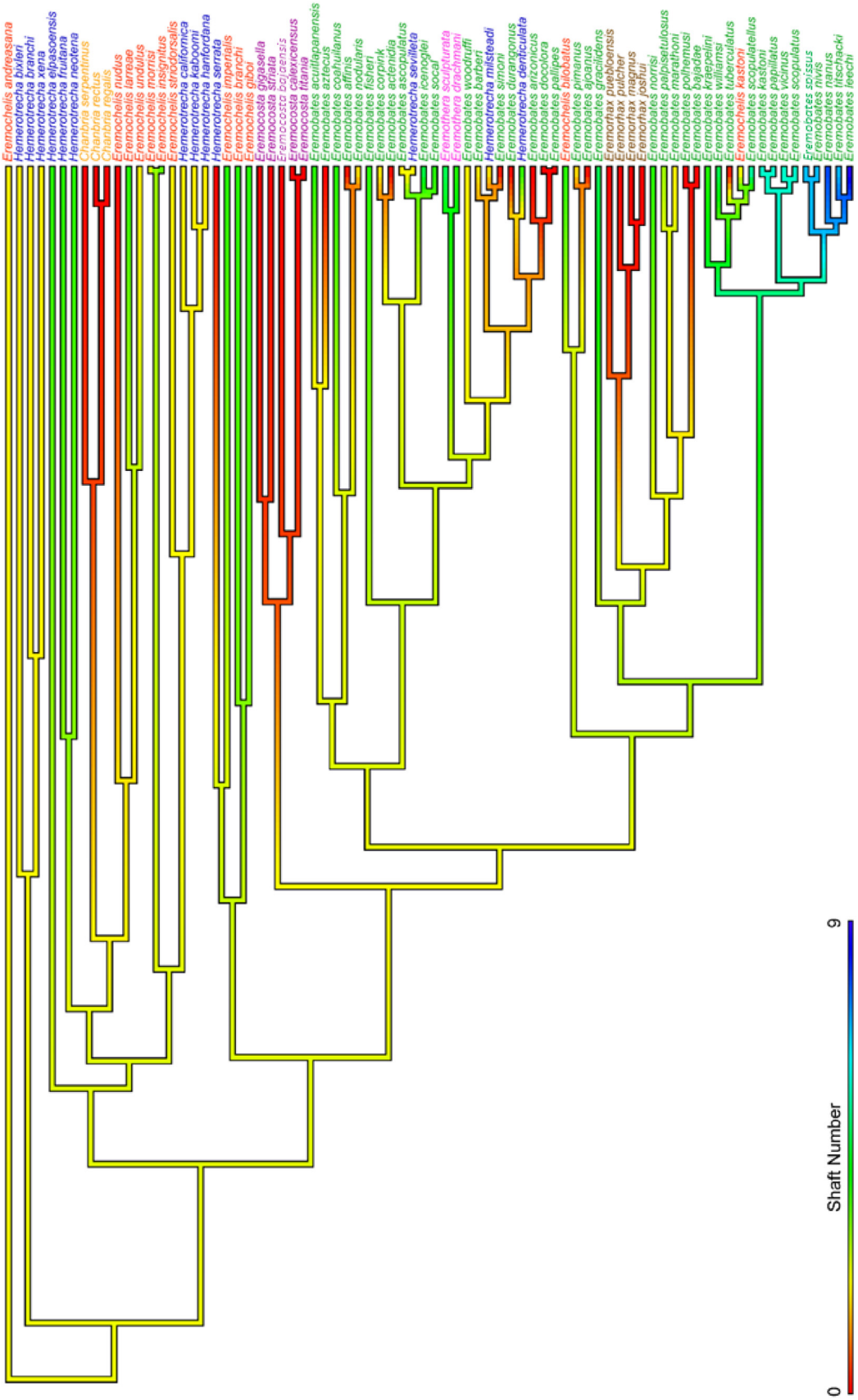

Fig. 5. Ancestral state reconstruction of modal ctenidia number. Terminal tip and node values are integers ranging from zero to ten, with yellow being approximately two, green to four, cyan to six, and light-blue to eight, and dark blue to nine.

Table 2
Patterns of ctenidia number (#), shaft length (L), and shaft shape (S) by clade in the Cushing et al. (2015) Phylogeny. Mode is listed as variable if no modal character state is present. # Range are the minimum and maximum values for shaft number.

Clade	Members	# Range	Mode #	% Mode	Mode L	% Mode	Mode S	% Mode
1	*H. branchi* sp grp	1–2	2	98%	Long	91%	Stiletto-like	82%
2	*E. striodorsalis, H. californica, H. kaboomi, H. hanfordana*	1–2	2	95%	Medium	65%	Spatulate	94%
3	*H. serrata, E. imperialis, E. branchi, E. giboi*	0–5	4	86%	Long	73%	Stiletto-like	100%
4	*E. scaber* sp grp	0–5	N/A	N/A	Short	84%	N/A	N/A
5	*E. pallipes* sp grp	0–3	51%	60%	Short	100%	N/A	N/A
6	CA *E. palpisetulosus* sp grp	0–9	N/A	N/A	Medium	65%	Stiletto-like*	100%

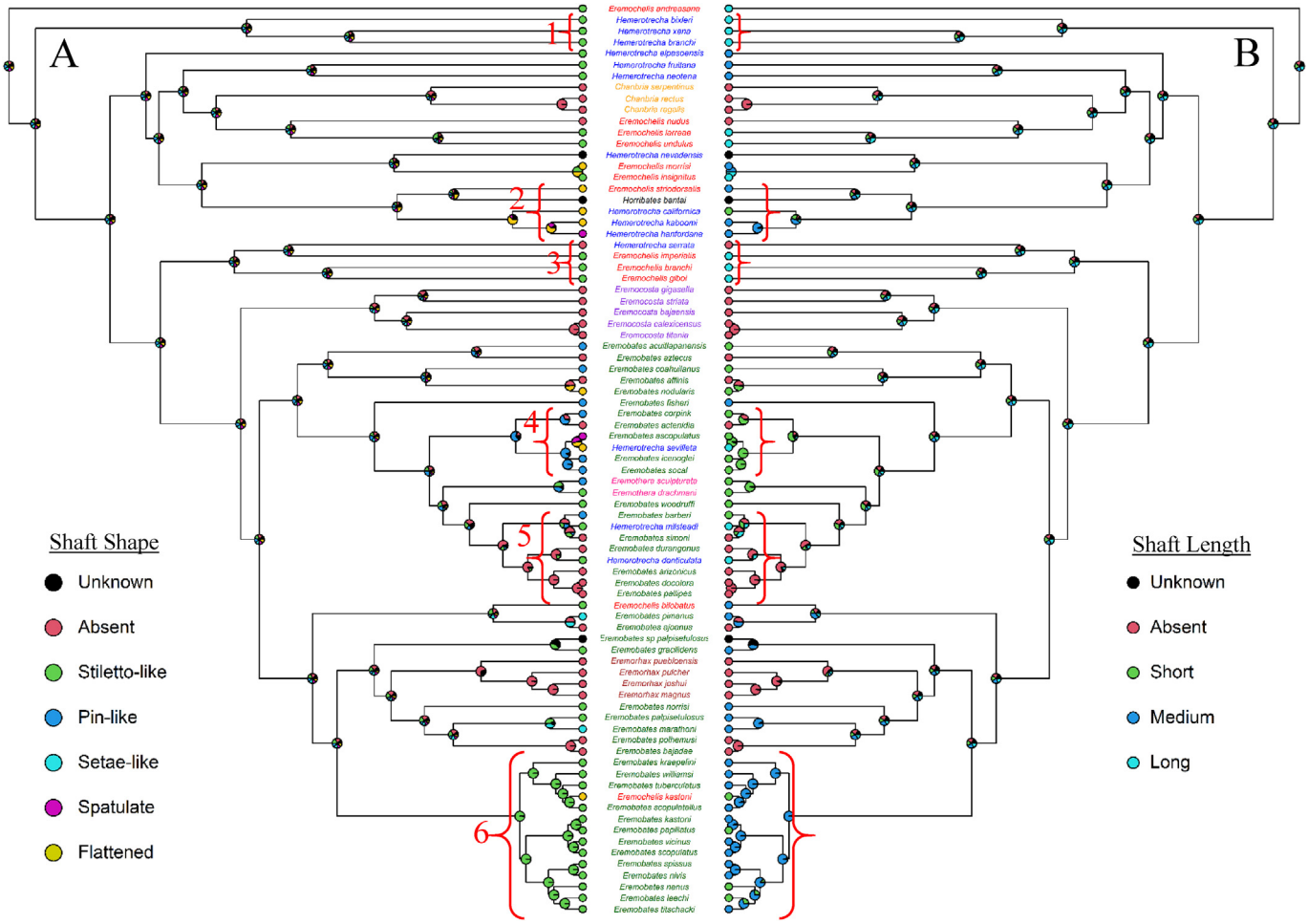

Fig. 6. Ancestral state reconstructions of A) shaft shape, and B) shaft length. Pie charts at internal nodes indicate marginal likelihood estimations of the ancestral state in the common ancestor. Numbered brackets correspond to observations reported in Table 2.

the three genus-wide losses in *Chanbria*, *Eremocosta*, and *Eremorhax*, ctenidia are estimated to be lost eight separate times. The more recently-derived species within the clade comprising the *E. pallipes* species group (clade 5, Fig. 6) appear to have lost ctenidia in a common ancestor if *H. denticulata* is excluded. Conversely, most species of the California clade of the *E. palpisetulosus* species group (clade 6, Fig. 6) exhibit a sharp clade-wide increase in ctenidia number. Ancestral state reconstructions estimate the stilleto-like shaft morphology to be the most likely state for most nodes in which ctenidia are present in descendent species (Fig. 6A). Most ancestral state reconstruction estimates for shaft length were equivocal (Fig. 6B).

4. Discussion

4.1. Ctenidia morphology, intraspecific variation, and implications for taxonomy

Going forward, ctenidia presence in Eremobatidae should continue to be determined through observations of the unique, enlarged socket in the cuticle, rather than the presence of an observable shaft. Currently, there are 11 species within *Eremochelis*, *Hemerotrecha* and *Horribates* described from female types as having “trace” ctenidia that are “barely distinguishable” from other setae present on the sternite, yet no criteria are offered as to how to they are defined as such (Muma 1951, 1962 1963; Muma & Muma 1988).

Similarly, the keys for the *E. scaber* species groups call for distinguishing ctenidia that are "hair-like" (Brookhart & Cushing 2004), "barely discernible from surrounding setae.", or "setal clothing" (Muma 1951). However, since we did not observe setae-like ctenidia in *Eremochelis*, *Hemerotrecha*, *Horribates*, or the *E. scaber* species group, it is likely that the observations in the literature were based on the serendipitous placement of abdominal setae where ctenidia are otherwise expected (e.g., Fig. 1B). In contrast, the presence of one or more reduced, setae-like ctenidia in some *E. pallipes* group taxa and in many *E. palpisetulosus* group taxa indicates that "trace" or "spurious" ctenidia are indeed possible, though the unique socket is still readily observable. The *E. palpisetulosus* group is the only species group that has been previously documented in the literature to exhibit a high degree of intraspecific variation in ctenidia number, and the exclusive presence of these seemingly modified or intermediate forms is curious in light of the clade-wide shift towards an increased number of shafts. This is not a problem unique to eremobatid taxa, as systematic efforts of some groups, such as *Biton* Karsch, 1880 are complicated by the presence of indistinguishable ctenidia (Wharton 1981). Considering this, we offer that ctenidia within Eremobatidae are defined by the characteristic socket, regardless of shaft morphology or placement, and that future taxonomic works should reflect this.

Regarding our designations of shaft shape, we found it challenging to establish stable designations of shape that are not merely functions of shaft thickness or length. As mentioned previously, the variety of terms used to describe ctenidia of arguably similar shape necessitated a means of definitively distinguishing them or uniting them into larger umbrella descriptions. Consequently, we found no reliable way to distinguish between the many descriptors that vary by length and/or diameter of the shaft. Instead, "stiletto-like," "pin-like," and "setae-like" designations all represent slight variations of a cylindrical shape that are reliable enough to be applied to many eremobatid taxa and are not simply gradations of each other. "Stiletto-like" and "pin-like" are borrowed from Roewer (1934) as is "setae-like" from Muma (1951). The observed intraspecific variation does not appear to be driven by population differences; much of the variation exists within the same locality and often within the same collection effort (e.g. multiple conspecifics from one DMNS record) (supplementary material).

Only three of the 55 focal species in which ctenidia were present exhibited invariant

characters, indicating that intraspecific variation in ctenidia characteristics is far more extensive than the literature would indicate. Most intraspecific variation regarding shaft shape is slight and subtle variations in shaft diameter, with most species maintaining modal character states above 70%. Although most focal species did not exhibit extensive intraspecific variation in shaft shape, cross-overs between distinct shapes (e.g. stiletto-like and flattened are both common in *H. xena* and *H. elpasoensis* Muma 1962) are a distinct possibility, seemingly more so within *Hemerotrecha*, and may require more quantitative approaches to improve utility within the genus or its constituent species groups.

The relatively limited intraspecific variation of ctenidia number and length observed within sampled *Hemerotrecha* specimens, and relatively little intraspecific variation overall within *Eremochelis* species may allow for continued but cautious use of all ctenidia characters in species identifications and diagnoses. While ctenidia number appears stable for *Hemerotrecha* and most sampled *Eremochelis* species, we believe that, given the extent of intraspecific variation in number among *Eremobates* species, precludes its continued use in dichotomous keys, in species diagnoses and delimitation between otherwise morphologically similar species. Ctenidia length also seems to vary considerably throughout the family. The use of relative length in this analysis and as a taxonomic character is confounded by variation in sternite lengths among individuals, which can fluctuate based on preservation and distension of the abdomen. Descriptions of new species, and revisions of those previously described, could explore absolute measurements of shaft length, a ratio of the shaft length to total body length, or a ratio to the propeltidium length for utility. Preliminary measurements on several dozen *E. scaber* group specimens have not indicated that the methods suggested above would be elucidating just yet (unpublished data).

Lastly, *E. palpisetulosus* species group taxa exhibited a high degree of intraspecific variation in shaft number, as is expected given the taxonomic literature (Muma & Brookhart 1988). The Cushing et al. (2015) analysis splits the *E. palpisetulosus* species group into multiple clades, with the largest consisting of very closely related species distributed in southern California. Many of these species are sympatrically distributed (as many as five species from one locality) and exhibit very similar cheliceral morphology, which leads us to believe that some of these species may be synonymized in the future. During the course of this study, we redesignated a number of specimens from this clade, principally a redesignation of all DMNS records of *E. villosus* Muma, 1951 to *E. spissus* Muma and Brookhart, 1988, including the voucher the Cushing et al. (2015) analysis. As such, we are less confident in our identifications of species comprising this clade, although we can still confidently conclude that ctenidia number should not be used to delimit or diagnose species in this group as has been done traditionally. The prevalence of additional small, setae-like ctenidia in many sampled *E. palpisetulosus* species will likely continue to confound species identifications and delimitation in this group without further revision given additional morphological characters and/or molecular evidence. Taxonomic revisions for all eremobatid genera (excluding *Eremorhax* and *Eremocosta*) that include UCE phylogenomic and morphological (including total evidence) analyses are ongoing. Increased taxon sampling and the inclusion of more stable morphological character systems in eremobatid systematics are likely to prove fruitful for species delimitation.

4.2. Phylogenetic signal and ancestral state reconstruction

Modal ctenidia number exhibited phylogenetic signal in all three indices used, indicating that closely-related species are more likely to share the same or similar character states. While the p-values of Pagel's λ and Blomberg's K indicate non-randomness in the arrangement of character states on the tips of the phylogeny, their individual statistics provide crucial context for interpretation. Values less than one for both metrics indicate that trait variation is dispersed among the tree tips more than expected under the assumption of Brownian motion evolution, which could be explained byconvergent evolution (Blomberg et al. 2003; Kamilar & Cooper 2013), as is indicated by CI and RI values closer to zero. Following the ancestral state reconstruction, only the California-distributed *E. palpisetulosus* clade appears to strongly diverge from other monophyletic clades in ctenidia number. It is worth noting that, for all three indices, phylogenetic signal detection is

considerably more likely in this analysis given the large number of terminal taxa (Kamilar & Cooper 2013).

The ancestral state reconstruction of modal ctenidia number illustrates that most of the more ancient lineages had two ctenidia, with many well-defined clades experiencing shifts in character states. Although a phylogenetic hypothesis for the entire order is thus far absent, and the sister-family to Eremobatidae is unknown, ctenidia presence may be a plesiomorphic trait due to its presence in the oldest lineages of Eremobatidae and in the majority of solifuge families. If ctenidia were indeed present in the eremobatid common ancestor, then the three genus-wide losses of ctenidia in *Chanbria*, *Eremocosta*, and *Eremorhax* represent three individual evolutionary events. In total, ctenidia appear to be lost independently eight times within the clades comprised of *Eremobates*, *Hemerotrecha*, *Eremochelis* species (Fig. 5). Excluding the placement of *H. denticulata*, all but the oldest *E. pallipes* species group lineages commonly have no ctenidia – although a minority of *E. pallipes* and *E. docolora* specimens still do. Because the absence of ctenidia is not uncommon in eremobatid taxa, a phylogenetic analysis with denser taxon sampling would likely reflect more independent losses. Given the variation in number (or otherwise complete loss) of ctenidia within clades, between closely related species, and within species, it appears as though ctenidia can be gained or lost without much biological consequence.

The estimation of both parsimonious and maximum-likelihood derived values in ctenidia shape as statistically significant was indicative of non-random evolution. However, this could be due to the stilleto-like shape being the prevailing character state within the family. Only one monophyletic clade (Fig. 6, clade 2) experienced a shift in shaft shape. Ctenidia length does not appear to be phylogenetically constrained, as the observed tip data is not meaningfully different from randomly distributed character states. Only two patterns emerge – the maintenance of relatively long shafts among the *H. branchi* species group taxa (clade 1, Fig. 5) and the shift towards short shafts (where ctenidia are present) that coincides with a reduced-shape in the clade comprising the *E. scaber*, *E. pallipes*, and *Eremothera* species.

4.3. Functional morphology

While the distal parts of the shaft appear to be flexible, the enlarged, unique socket at the base of the ctenidia shaft does not appear to allow the deflection consistent with arachnid mechanoreceptive setae (Barth 2004; Foelix 1985; Foelix & Chu-Wang 1973a). Additionally, the lack of pores along the shaft or at the base preclude the ability of volatile airborne or substrate chemicals from permeating the cuticle and being detected. The presence of the bifurcated tips is a character shared with the aptly named "bifurcated seta" (Kraepelin 1901; Cushing & Casto 2012) covering the abdomen, and strengthens the hypothesis that ctenidia are a modified form of these setae. While none of the evidence gathered here conclusively eliminates a modality of sensory function, it does not suggest any such function thus far. Other sensory modalities could include hygro- and thermoreception, but the techniques used in this study are unable to evaluate these possibilities. The white, glandular-like structure seen at the base of the ctenidia in few specimens warrants further investigation/histological examination and/or electrophysiological investigations may be more conclusive in identifying a potential function or lack there-of. Their association with a spiracle could indicate a protective function or perhaps convey flood resistance by enabling the formation of a plastron. However, such a function seems unlikely given that most females do not have ctenidia despite inhabiting the same flood-prone environments as their male counterparts.

5. Conclusion

Generally speaking, ctenidia characters, as they are traditionally used, may not be appropriate for continued use in dichotomous keys, species delimitation and diagnoses. Although we have not exhaustively sampled the eremobatid diversity, we believe the variation documented here is representative of larger trends that warrant further attention and cautious use in taxonomic work. Characteristics of ctenidia are variable enough to, in some taxa such as *Eremobates*, avoid using them for identifications. Future work may do well to attempt morphometric analyses to provide more utility and stability of these characters. Although our analysis indicated that shaft shape and number are somewhat constrained within the phylogeny, we did not observe any exclusive character states among the monophyletic clades for taxonomic use above a species level. Lastly, ctenidia are not morphologically consistent with either arachnid mechano- or chemoreceptive setae, though they cannot be conclusively ruled out. New investigations should establish a functional significance for ctenidia so that these findings can be put into an appropriate biological context.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcz.2021.09.002.

Appendix

Table A1
Observational data for ctenidia characteristics for 75 focal taxa represented in the Cushing et al. (2015) analysis. The genus is abbreviated for all taxa following the first listing for each genus. Shaft shape character states marked with an asterisk indicate the presence of additional reduced, setae-like shafts. N = total number focal specimens; # Range = the minimum and maximum number of ctenidia observed.

Species	Species Group	N	# Range	# Mode	% Modal	Mode Length	% Modal	Mode Shape	% Modal
C. rectus	–	4	0	0	100%	–	–	–	–
C. regalis	-	5	0	0	100%	–	–	–	–
C. serpentinus	-	5	0	0	100%	–	–	–	–
Eremobates aztecus	aztecus	2	0	0	100%	–	–	–	–
E. arizonicus	pallipes	20	0	0	100%	–	–	–	–
E. barberi	pallipes	7	2–3	2	71%	Short	100%	Pin-like	100%
E. docolora	pallipes	4	0–2	0	50%	Short	100%	Setae-like	100%
E. durangonus	pallipes	15	0	0	100%	–	–	–	–
E. pallipes	pallipes	20	0–2	0	70%	Short	100%	Setae-like	100%
E. simoni	pallipes	2	0	0	100%	–	–	–	–
E. woodruffi	pallipes	20	1–3	2	90%	Short	100%	Stiletto-like	100%
E, kraepelini	palpisetulosus	1	6	6	100%	Medium	100%	Stiletto-like	100%
E. affinis	palpisetulosus	2	0	0	100%	–	–	–	–
E. ajoanus	palpisetulosus	20	0	0	100%	–	–	–	–
E. bajadae	palpisetulosus	20	0	0	100%	–	–	–	–
E. gracilidens	palpisetulosus	14	3–8	5	38%	Medium	73%	Stiletto-like*	100%
E. kastoni	palpisetulosus	20	3–9	5	30%	Medium	80%	Stiletto-like*	100%
E. leechi	palpisetulosus	10	7–9	9	50%	Short	60%	Stiletto-like	100%
E. marathoni	palpisetulosus	20	0–3	2	60%	Medium	53%	Setae-like	100%
E. nanus	palpisetulosus	4	6–8	8	50%	Short	100%	Stiletto-like*	100%
E. nivis	palpisetulosus	3	5–8	7	66%	Medium	66%	Stiletto-like*	100%
E. nodularis	palpisetulosus	20	0–3	2	75%	Short	100%	Flattened	75%
E. norrisi	palpisetulosus	18	2–5	2/4	31/31%	Medium	62%	Stiletto-like*	100%
E. palpisetulosus	palpisetulosus	16	2–5	3	38%	Medium	54%	Stiletto-like*	100%
E. papillatus	palpisetulosus	9	4–6	4/5/6	33%	Short	56%	Stiletto-like*	100%
E. polhelmusi	palpisetulosus	8	0	0	100%	–	–	–	–
E. scopulatellus	palpisetulosus	15	4–8	6	45%	Medium	65%	Stiletto-like*	100%
E. scopulatus	palpisetulosus	20	4–7	6	32%	Medium	100%	Stiletto-like*	100%
E. titschacki	palpisetulosus	3	7–10	7	66%	Medium	100%	Stiletto-like	100%
E. tuberculatus	palpisetulosus	2	5–7	5/7	50%	Medium	100%	Stiletto-like*	100%
E. vicinus	palpisetulosus	19	4–9	6	63%	Medium	68%	Stiletto-like*	100%
E. spissus	palpisetulosus	7	5–8	8	42%	Medium	86%	Stiletto-like*	100%
E. williamsi	palpisetulosus	5	5–6	5	80%	Medium	80%	Stiletto-like*	100%
E. actenidia	scaber	20	0	0	–	–	–	–	–
E. ascopulatus	scaber	20	2–4	2	90%	Short	100%	Spatulate	70%
E. corpink	scaber	10	0–2	0/2	40%/40%	Short	100%	Pin-like	75%
E. fisheri	scaber	7	0–4	4	71%	Medium	60%	Pin-like	100%
E. icenoglei	scaber	14	3–5	5	60%	Short	100%	Pin-like	86%
E. socal	scaber	5	4–4	4	100%	Short	100%	Pin-like	100%
Eremochelis bilobatus	bilobatus	20	4–5	4	90%	Medium	90%	Stiletto-like	100%
E. giboi	bilobatus	11	3–5	4	82%	Long	64%	Stiletto-like	100%
E. morrisi	bilobatus	10	2	2	100%	Medium	73%	Flattened	100%
E. nudus	bilobatus	10	0	0	100%	–	–	–	–
E. branchi	branchi	5	4–5	4	80%	Long	60%	Stiletto-like	100%
E. insignitus	branchi	20	2–6	4	75%	Long	95%	Stiletto-like	100%
E. andreasana	imperialis	19	2	2	100%	Long	95%	Stiletto-like	100%
E. imperialis	imperialis	6	4	4	100%	Long	100%	Stiletto-like	100%
E. kastoni	imperialis	19	2	2	100%	Short	100%	Flattened	95%
E. larreae	imperialis	3	3–4	4	67%	Long	100%	Stilleto-like	100%
E. undulus	imperialis	2	2	2	100%	Long	100%	Stilleto-like	100%
E. striodorsalis	striodorsalis	17	2	2	100%	Medium	82%	Flattened	100%
Eremocosta calexicensus	-	5	0	0	100%	–	–	–	–
E. gigasella	-	5	0	0	100%	–	–	–	–
E. bajaensis	-	5	0	0	100%	–	–	–	–
E. striata	-	5	0	0	100%	–	–	–	–
E. titania	-	5	0	0	100%	–	–	–	–
Eremorhax puebloensis	-	5	0	0	100%	–	–	–	–
E. joshui	-	5	0	0	100%	–	–	–	–
E. magnus	-	5	0	0	100%	–	–	–	–
E. pulcher	-	2	0	0	100%	–	–	–	–
Eremothera drachmani	-	3	4–5	4	67%	Short	100%	Pin-like	100%
E. sculpturata	-	10	4–7	5	50%	Short	90%	Pin-like*	100%
Hemerotrecha hanfordana	banksi	20	1–2	2	95%	Medium	55%	Spatulate	90%
H. californica	banski	15	1–2	2	93%	Short	45%	Spatulate	87%
H. kaboomi	banski	20	2	2	100%	Medium	80%	Spatulate	95%
H. bixleri	branchi	15	1–2	2	95%	Long	95%	Stiletto-like	95%
H. branchi	branchi	20	2	2	100%	Long	95%	Stiletto-like	75%
H. milsteadi	branchi	3	2	2	100%	Long	67%	Stiletto-like	100%
H. sevilleta	branchi	9	2	2	100%	Long	78%	Flattened	89%
H. xena	branchi	20	2	2	100%	Long	75%	Stiletto-like	55%
H. denticulata	Denticulata	6	4	4	100%	Long	100%	Stiletto-like	100%

(*continued on next page*)

Table A1 (*continued*)

Species	Species Group	N	# Range	# Mode	% Modal	Mode Length	% Modal	Mode Shape	% Modal
H. neotena	Denticulata	2	4–6	4/6	50%	Medium	100%	Stiletto-like	100%
H. serrata	serrata	8	0	0	100%	–	–	–	–
H. elpasoensis	simplex	8	0–4	4	63%	Medium	63%	Stiletto-like	63%
H. fruitana	simplex	20	0–6	4	86%	Medium	50%	Stiletto-like	86%

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