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Measurements of soldier head capsules of cuticular hydrocarbon phenotypes of *Reticulitermes* (Blattodea: Heterotermitidae) from Northern California identify morphologically cryptic and sibling species

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Four undescribed species or subspecies of *Reticulitermes* Holmgren 1913 in northern California have been identified by chemotaxonomic and phylogenetic analyses of voucher specimens used for chemotaxonomy. To date, useful morphological keys do not exist. We present eight direct and 22 derived measures and indices of soldier head capsules of multiple colonies of each of five cuticular hydrocarbon (CHC) phenotypes. Our objective was to identify one or more morphological measurements of soldier head capsules that would unequivocally separate taxa previously identified by CHCs and phylogenetic analyses. Phenotypes CA-A, CA-A', and CA-D are found in the coastal areas, whereas phenotypes CA-A, CA-B, and CA-C are found in the foothills of the Sierra Nevada. For all direct measurements, as well as the derived measurements and indices, we discovered extensive variation within all five phenotypes and overlap in values for nearly all variables. The discriminant analysis did not provide unequivocal separation of phenotypes CA-A and CA-D. The discriminant analysis provided unequivocal separation of phenotypes CA-A from both phenotypes CA-B and CA-C. From these results, we consider phenotypes CA-A and CA-D to be cryptic, but not sibling, species because they are very difficult to separate on the basis of soldier head capsule measurements and they are not closely related by phylogenetic analyses. Phenotypes CA-B and CA-C should be considered both cryptic and sibling species because they are impossible to separate on the basis of soldier head capsule measurements and they are separate, but closely related, species on the basis of phylogenetic analyses.

Keywords: discriminant analysis, integrative taxonomic approach (ITA), species identification, subterranean termites, taxonomy

Introduction

Our knowledge of the biology and identification of termite species in the United States was first presented by Banks and Snyder (1920) and greatly expanded by Kofoid et al. (1934). Early descriptions of species of *Reticulitermes* Holmgren 1913 were published in Banks and Snyder (1920). The resulting keys to the species in Banks and Snyder (1920) are, by today's standards, not very useful. For example, one couplet asks whether the gula is twice as large in the front as in the middle without saying exactly where the front of the gula is on the ventral surface of the head capsule. Another couplet asks whether the head is fully twice as long as broad without mentioning whether the mandibles should be included in the measurement (Banks and Snyder 1920, Snyder 1954).

Identification of western species of *Reticulitermes* continues to be hampered by a paucity of reliable morphological keys to species. Nutting (1990) published a key to soldiers of the termites of North America, but it was largely based on keys published earlier and is, therefore, also not very useful. Only a few keys have been published that identify specimens to species based on soldier morphology

(Weesner 1965, Scheffrahn and Su 1994, Lim and Forschler 2012), but these keys are regional in nature. Weesner (1965) did not present a key to soldiers of western *Reticulitermes* in her handbook of the termites of the United States. Furthermore, recent discovery and documentation of additional undescribed species of *Reticulitermes* in California and other southwestern states (Haverty and Nelson 1997, 2007, Copren et al. 2005, Nelson et al. 2008, Tseng et al. 2023) will require additional morphological analyses to make morphological keys useful.

Termites are important ecosystem engineers and only about 4% of the nearly 3,000 living species are considered serious destructive pests (Krishna et al. 2013). Inadequate morphological keys lead to incorrect species identification (Korb et al. 2019). To properly study their behavior, ecology, economic impact, and successful management with new technologies, accurate identification of species is primary (Collins 1988). A thorough and comprehensive revision of the genus *Reticulitermes* has long been recognized as necessary (Weesner 1970, Nelson et al. 2008, 2023, Lim and Forschler 2012). In reality, a vigorous revision of *Reticulitermes* is a daunting task and a more achievable scenario is likely the description of a single (or multiple) new species outside of this context, which would be justifiable in light of the economic and ecological significance of this genus (Lim and Forschler 2012). Johnson and Forschler (2022) emphasized the need for an integrated taxonomic approach (ITA), incorporating multiple character sets to create accurate and well-supported species descriptions.

Renewed interest in the diversity of *Reticulitermes* in the western United States was initiated in the early 1990s with preliminary investigations of the cuticular hydrocarbons (CHCs) of *Reticulitermes* and *Coptotermes* (Haverty et al. 1991). Because of the lack of useful keys to species, ecological and control studies of *Reticulitermes* in northern California necessitated the development of techniques for determining taxa and distinguishing different colonies within species (Haverty and Nelson 1997). Separation of taxa based on CHC profiles of *Reticulitermes* allowed the Haverty Laboratory to conduct field studies on the dispersion of sympatric taxa in wildland and residential situations in northern California (Haverty et al. 2000) and to quantify seasonal foraging and feeding behavior (Haverty et al. 1999b). Furthermore, this new knowledge greatly enhanced our ability to conduct colony elimination studies using baits from different manufacturers (Getty et al. 2000b, 2007, Haverty et al. 2010).

These taxonomic groupings or phenotypes were further validated with studies of geographic distributions in northern and southern California (Nelson et al. 2001, 2008, Copren et al. 2005, Copren 2007), agonistic behavior (Haverty et al. 1999a, Getty et al. 2000a, Delphia et al. 2003), alate flight phenology (Haverty et al. 2003), and genetic correlation with CHC phenotypes (Jenkins et al. 2000, Copren et al. 2005, Tseng et al. 2023). Additional researchers also began investigating the taxonomy of *Reticulitermes* in California using mtDNA and nuclear DNA technology (Austin et al. 2002, 2005, 2008, Su et al. 2006, Szalanski et al. 2006, Tripodi et al. 2006, Dedeine et al. 2016, Tseng et al. 2023). What has been missing are well-defined, reliable morphological characters that correlate with taxonomic determinations, whether they are made with cuticular hydrocarbon or phylogenetic techniques.

Most keys to species of termites are divided into two sections: a key to the alates (imagoes) and a key to the soldiers. If one were to collect alates and needed to identify them to species, then of course, a key to the alates is the answer. Ideally, collections should include both alates and soldiers, however, the seasonal occurrence of alates often makes the concurrent collection of both castes problematic (Chouvenc et al. 2016). However, most collections in wildland situations or in monitoring/bait stations will likely only consist of workers, nymphs, and a small number of soldiers. Keys to workers or nymphs of *Reticulitermes* from western North America are nonexistent. Weesner (1965) only presented a key to species of the alates of *Reticulitermes* of the Northwest and Pacific Coastal Area of the United States. Because field collections seldom include alates, currently one would either need to have CHCs characterized or have a phylogenetic analysis done to distinguish taxa. Because morphology is generally required for field-based identification, a more generally accessible method would be to use one or more measurements or characteristics of the soldier(s) in the sample to identify the species in the collection (Korb et al. 2019).

We need to be careful in the pursuit of morphological keys to the soldiers of *Reticulitermes*. The potential for finding morphologically cryptic species is always a risk. The concept of cryptic species is not without controversy (Zuniga-Reinoso and Benitez 2015, Korshunova et al. 2019). We suggest that for the purposes in this paper, we consider cryptic species to be two or more distinct, biological species incorrectly classified under a single species name because they are impossible or nearly impossible to distinguish by morphology and are not necessarily closely related (Freeman and Herron 2001, Bickford et al. 2006). We consider sibling species to be (i) biological species in that they are reproductively isolated, (ii) they are closely related or sister taxa, and are (iii) difficult (or impossible) to distinguish by morphological characters (also cryptic species). (Mayr 1969, 1982, Futuyma 1998, Freeman and Herron 2001, and Bickford et al. 2006).

Here, we report the morphological examination of soldier samples taken in conjunction with studies of the cuticular hydrocarbon chemistry, ecology, behavior, and control of *Reticulitermes* at two sites in northern California. The study reported here focuses on measurements of the head capsules of soldiers. Our objective was to identify one or more morphological measurements of soldier head capsules that would unequivocally separate taxa previously identified by CHC phenotypes and phylogenetic analyses.

Materials and Methods

Collection of Termites

Samples of *Reticulitermes* were collected from termite monitoring stations (Lewis et al. 1998) at field sites established in California at the Institute of Forest Genetics (IFG), Pacific Southwest Research Station, Forest Service, U.S. Department of Agriculture, near Placerville in El Dorado County, and at two residential sites in Marin County, St. Francis of Assisi Church in Novato and a private residence in Novato. CHC phenotypes were determined for termites collected from each active monitoring station over a 3-yr period. When present in sufficient numbers, samples of 100 to 200 workers and one or more soldiers from every monitoring station were returned to the laboratory. Workers were kept frozen at −15 °C until the CHC mixtures were determined.

The methods of extraction and characterization of CHCs were previously reported (Haverty and Nelson 1997, Nelson et al. 2001, 2008). From these studies, we consistently identified five CHC phenotypes of *Reticulitermes* in northern California: CA-A, CA-A', CA-B, CA-C, and CA-D. Phenotypes CA-A, CA-A', and CA-D were found in the coastal area; phenotypes CA-A, CA-B, and CA-C were found in Placerville in the foothills of the Sierra Nevada (Haverty et al. 1999b, 2000). Voucher samples with soldiers of each of the five CHC phenotypes were used for the study reported here.

To evaluate variation in morphological measurements of soldiers within and among each of five phenotypes, we chose monitoring stations with a minimum of five soldiers in voucher samples from earlier studies (Haverty et al. 1999b, 2000, Getty et al. 2000b, Nelson et al. 2001, 2008). At the Novato sites, we used collections from monitoring stations L4, St18, St57, and St71 for phenotype CA-A', and monitoring stations L6, St253, St314, and St333 for phenotype CA-D. At the Placerville site we used collections from monitoring stations Wc41, Wg7, Xt10, Yh30, YI22, YI28, Yr19, and Ze4 for phenotype CA-A; from monitoring stations Wb36, Wc10, Yq23, Yv34, and Zv31 for phenotype CA-B, and monitoring stations Wi74, Yk27, Yt2, and Zn11 for phenotype CA-C.

For our study of the various head capsule measurements, we used voucher specimens that were kept completely intact, i.e., there were no dissections of the specimens measured. We used two collections each with five soldiers (10 total) for each monitoring station, except St314 and St333; for these two monitoring stations, we only used one collection of five soldiers. In total, we examined 240 specimens and made a total of 1,920 measurements. For one variable, gular width in phenotype CA-B, there was one missing observation because the transcribed measurement was unreadable. Unfortunately, we did not keep individual specimens used in the study in separate vessels; we returned them to the pooled voucher specimens. So, we were unable to remeasure this specimen to obtain the missing value. Therefore, while there were 1920 measurements, we could only use 1919 of those. Voucher specimens (usually soldiers and workers) for the collection dates for each of the colonies in this study were placed in 70% ethanol and are retained by the authors (MIH & LJN).

Soldier Head Capsule Measurements

Direct Measurements of the Head Capsule

Eight measurements were taken from each of the head capsules examined. These measurements were inspired by the work of S.F. Light (1927) in his study of the pan-tropical termite genus, *Coptotermes* Wasmann. Measurements were taken with an Olympus Stereo Microscope (Model SZH10) equipped with an ocular micrometer. The ocular micrometer was calibrated using an Olympus 0.01-mm stage micrometer. Specimens were placed in a 5-cm glass Petri dish filled with fine sand saturated with 70% ethanol. The specimens were positioned such that the plane being measured was parallel to the plane of the microscope stage.

The location of the eight measurements on the head capsules of the specimens can be seen from the ventral aspect (View V), dorsal aspect (View D) and in lateral aspect (View L) (Table 1, Fig. 1). The terminologies we use here are adapted from drawings of a generalized insect in Chapman (1998, Fig. 1.3). The measurements are: (v_1) Maximum *Gular Width* (mm) (View V: cd), (v_2) *Minimum Gular Width* (mm) (View V: ef), (v_3) *Head Length* (mm) is the length of the head from posterior margin to the line which joins the anterior limits of the chitinization of the head capsule, the mandibular articulations, not including the mandibles (View D: ab), (v_4) *Maximum Head Width* (mm) is the distance between the outer edges of the genae (View D: cd), (v_5) *Minimum Head Width* (mm) is the distance between the outer edges of the mandibular articulations (View D: ef), (v_6) *Epistomal Sulcus* (mm) is the distance from the posterior margin of the head capsule to the sulcus between the head capsule and the clypeus (View D: bb_1), (v_7) *Length to Hump* (mm) of the frons is the distance from the posterior margin of the head capsule to the beginning of the raised portion of the frons (View D: bb_2), and (v_8) *Height at Hump* (mm) is the distance from the ventral surface of the head capsule to the extreme height of the hump of the frons (View L: hi).

Additional Characters Derived from Head Measurements

The utility of derived characters was investigated using the measurements of the head capsule. We generated 22 additional characters to evaluate their usefulness, in addition to the original 8 (see Table 1), for separating specimens assigned a CHC phenotype. The list of these derived characters and their formulas are found in Table 2.

The derived characters are as follows: (v_9) *Head Circumference* is the circumference (mm) of the cross section of the head at the height of the hump of the frons; (v_{10}) *Head Cross Section Area* is the area (mm^2) of the cross section of the head at the height at hump of the frons; (v_{11}) *Length of Hump* (mm) is the length of the hump of the frons along the axis of the head capsule; (v_{12}) *Area of the Head* (mm^2) from a dorsal perspective is the maximum head width times the epistomal sulcus; (v_{13}) *Narrow Area of the Head* (mm^2) from a dorsal perspective is the minimum head width times the epistomal sulcus; (v_{14}) *Head Index* is the ratio of the epistomal sulcus to the maximum head width; (v_{15}) *Head Contraction* is the ratio of the minimum head width to the maximum head width; (v_{16}) *Mean Gular Width* (mm) is the mean of the maximum and minimum gular widths; (v_{17}) *Gular Index I* is the head length divided by the maximum gular width; (v_{18}) *Gular Index II* is the head length divided by the minimum gular width; (v_{19}) *Epistomal Sulcus/Height at Hump* is the head length to the epistomal sulcus divided by the height at the hump of the frons; (v_{20}) *Head Length/Height at Hump* is the length of the head capsule divided by the height at the hump of the frons; (v_{21}) *Max to Min Gular Width Ratio* is maximum gular width divided by the minimum gular width; (v_{22}) *Head Length/Max Head Width* is head length divided by the maximum head width; (v_{23}) *Head Length/Min Head Width* is head length divided by the minimum head width; (v_{24}) *Epistomal Sulcus/Min Head Width* is the epistomal sulcus divided by minimum head width; (v_{25}) *Max Head*

Table 1. Various measurements of head capsules of soldiers of *Reticulitermes* from northern California.

Variable Number	Variable	View[a]	Code[b]
v_1	Max Gular Width (mm)	V	*cd*
v_2	Min Gular Width (mm)	V	*ef*
v_3	Head Length (mm)	D	*ab*
v_4	Max Head Width (mm)	D	*cd*
v_5	Min Head Width (mm)	D	*ef*
v_6	Epistomal Sulcus (mm)	D	bb_1
v_7	Length to Hump (mm)	D	bb_2
v_8	Height at Hump (mm)	L	*hi*

[a]Views from Figure 1 (variables v_1 - v_8). V = ventral aspect; D = dorsal aspect; L = lateral aspect.
[b]Code represents the letters on three views in Figure 1. The measurement of the variable is the distance between the letters in each of three views in Figure 1.

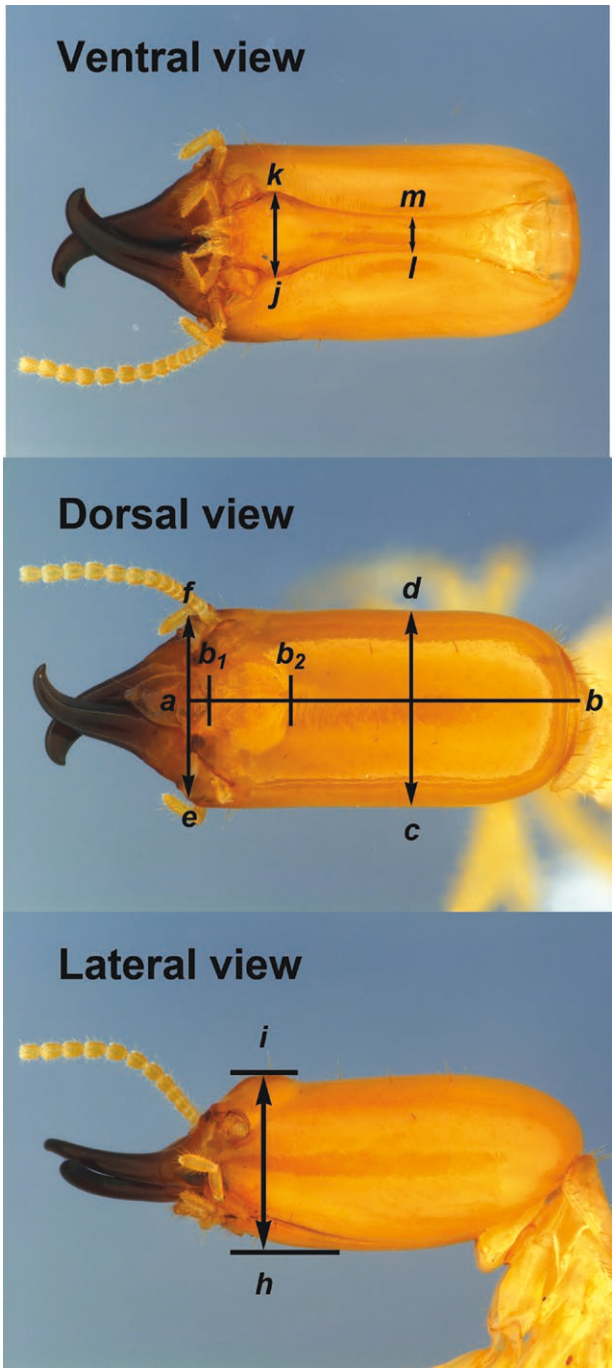

Fig. 1. Head capsule of a *Reticulitermes* soldier from three different aspects: ventral, dorsal and lateral views. Measurements from the ventral view are Maximum Gular Width (*cd*) and Minimum Gular Width (*ef*); from the dorsal view are Head Length (*ab*), Maximum Head Width (*cd*), Minimum Head Width (*ef*), Epistomal Sulcus (bb_1) and Length to Hump (bb_2); and from the lateral view is Height at Hump (*hi*).

Width/Head Length is the maximum head width divided by the head length; (v_{26}) *Max Head Width/Epistomal Sulcus* is the maximum head width divided by the epistomal sulcus; (v_{27}) *Length to Hump/Max Head Width* is the length to the hump of the frons divided by the maximum head width; (v_{28}) *Head Length/Mean Gular Width* is the head length divided by the mean of the maximum and minimum gular widths; (v_{29}) *Length to Hump/Min Head Width* is the length to the hump of the frons divided by the minimum head width; (v_{30}) *Max Head Width/Min Head Width* is the ratio of the maximum and minimum head widths [the reverse of variable number v_{15}].

Table 2. Derived variables using measurements in Table 1.

Variable	Derived Variable	Formula [a]
v_9	Head Circumference (mm)	$\pi * (v_8 + v_4)/2$
v_{10}	Head Cross Section Area (mm^2)	$\pi * ((v_8 + v_4)/4)^2$
v_{11}	Length of Hump (mm)	$v_6 - v_7$
v_{12}	Area of the Head (mm^2)	$v_4 * v_6$
v_{13}	Narrow Area of the Head (mm^2)	$v_5 * v_6$
v_{14}	Head Index	v_6/v_4
v_{15}	Head Contraction	v_5/v_4
v_{16}	Mean Gular Width (mm)	$(v_1 + v_2)/2$
v_{17}	Gular Index I	v_3/v_1
v_{18}	Gular Index II	v_3/v_2
v_{19}	Epistomal Sulcus / Height at Hump	v_6/v_8
v_{20}	Head Length / Height at Hump	v_3/v_8
v_{21}	Max to Min Gular Width Ratio	v_1/v_2
v_{22}	Head Length / Max Head Width	v_3/v_4
v_{23}	Head Length / Min Head Width	v_3/v_5
v_{24}	Epistomal Sulcus / Min Head Width	v_6/v_5
v_{25}	Max Head Width / Head Length	v_4/v_3
v_{26}	Max Head Width / Epistomal Sulcus	v_4/v_6
v_{27}	Length to Hump / Max Head Width	v_7/v_4
v_{28}	Head Length / Mean Gular Width	$v_3/((v_1 + v_2)/2)$
v_{29}	Length to Hump / Min Head Width	v_7/v_5
v_{30}	Max Head Width / Min Head Width	v_4/v_5

[a]Formulas using the 8 variables in Table 1: $v_1, v_2, v_3, v_4, v_5, v_6, v_7, v_8$.

Display of Data and Statistical Analyses

Strip Plots for All Phenotypes for Each Variable.

We created strip plots using R (R Core Team 2023) to display the individual values and the mean for each phenotype-variable combination. The individual data points were jittered vertically and horizontally a small amount to avoid overlap of points with the same value. The value of the mean for each phenotype-variable combination is represented as a larger open circle. These plots provide a visual comparison of the variability within phenotypes and among all phenotypes for each variable. Furthermore, these plots provide a visual of the variation of data points about the mean. From these plots, we also can see the minimum and maximum values and to identify potential outlier data points.

Statistical Analyses

Analysis of Variance

We compared mean values for each variable among all phenotypes using a linear mixed model with repeated observations (generally measurements of five termites) for each collection area and date and analyzed with PROC MIXED (SAS Institute 2023) adjusting for the 6 pairwise comparisons of phenotypes using Tukey's range test at the overall 5% significance level. From these analyses, we determined which variables resulted in statistically significant differences between pairings of phenotypes (CA-A vs. CA-A', CA-A vs. CA-B, CA-A vs. CA-C, CA-A vs. CA-D, CA-A' vs. CA-D, CA-B vs. CA-C).

While we did adjust the overall significance level within an analysis of variance for the pairwise comparisons of phenotypes, we did not adjust the overall significance level among the 30 analyses of variance as commonly done when many multiple comparisons are performed; we were more concerned with not missing potential

Table 3. Summary statistics (sample size, mean, and standard deviation) for 8 measured variables (1-8) and 22 derived variables (9-30) for measurements of soldier head capsules from five cuticular hydrocarbon phenotypes found in northern California: CA-A, CA-A′, CA-B, CA-C and CA-D. Sample sizes for phenotypes CA-A, CA-A′, CA-B, CA-C, and CA-D are 80, 40, 50, 40, and 30, respectively with the exception for Phenotype B where the sample size is 39 for variables v_2, v_{16}, v_{18}, v_{21}, and v_{28}.

		Mean					Standard deviation				
Variable Number	Variable	CA-A	CA-A'	CA-B	CA-C	CA-D	CA-A	CA-A'	CA-B	CA-C	CA-D
v_1	Max Gular Width (mm)	0.48	0.48	0.42	0.42	0.47	0.021	0.021	0.019	0.019	0.014
v_2	Min Gular Width (mm)	0.19	0.20	0.16	0.17	0.19	0.014	0.013	0.011	0.011	0.013
v_3	Head Length (mm)	1.83	1.79	1.60	1.67	1.68	0.121	0.110	0.082	0.112	0.069
v_4	Max Head Width (mm)	1.11	1.10	0.92	0.94	1.07	0.040	0.042	0.038	0.030	0.034
v_5	Min Head Width (mm)	1.02	1.01	0.87	0.89	0.97	0.036	0.039	0.036	0.035	0.026
v_6	Epistomal Sulcus (mm)	1.67	1.64	1.45	1.53	1.50	0.134	0.111	0.079	0.124	0.065
v_7	Length to Hump (mm)	1.13	1.08	1.02	1.10	0.99	0.115	0.089	0.068	0.115	0.056
v_8	Height at Hump (mm)	0.93	0.93	0.80	0.80	0.89	0.031	0.041	0.027	0.017	0.016
v_9	Head Circumference (mm)	3.19	3.19	2.69	2.73	3.08	0.104	0.124	0.097	0.065	0.073
v_{10}	Head Cross Section Area (mm^2)	0.81	0.81	0.58	0.59	0.75	0.053	0.062	0.041	0.028	0.026
v_{11}	Length of Hump (mm)	0.54	0.56	0.43	0.43	0.50	0.033	0.036	0.028	0.030	0.024
v_{12}	Area of the Head (mm^2)	1.86	1.80	1.34	1.44	1.60	0.205	0.180	0.119	0.156	0.106
v_{13}	Narrow Area of the Head (mm^2)	1.72	1.66	1.26	1.37	1.45	0.190	0.166	0.116	0.159	0.084
v_{14}	Head Index	1.51	1.49	1.58	1.63	1.40	0.084	0.065	0.057	0.093	0.052
v_{15}	Head Contraction	0.92	0.92	0.94	0.95	0.90	0.017	0.014	0.019	0.019	0.025
v_{16}	Mean Gular Width (mm)	0.34	0.34	0.29	0.29	0.33	0.013	0.014	0.012	0.011	0.010
v_{17}	Gular Index I	3.78	3.74	3.83	3.94	3.60	0.169	0.148	0.105	0.157	0.156
v_{18}	Gular Index II	9.60	8.93	10.13	10.13	8.71	1.085	0.701	0.783	1.017	0.719
v_{19}	Epistomal Sulcus / Height at Hump	1.81	1.75	1.83	1.92	1.69	0.101	0.078	0.066	0.137	0.071
v_{20}	Head Length / Height at Hump	1.97	1.92	2.01	2.09	1.90	0.086	0.072	0.066	0.119	0.079
v_{21}	Max to Min Gular Width Ratio	2.53	2.38	2.65	2.57	2.42	0.224	0.151	0.188	0.219	0.180
v_{22}	Head Length / Max Head Width	1.65	1.64	1.74	1.78	1.57	0.070	0.061	0.050	0.079	0.059
v_{23}	Head Length / Min Head Width	1.78	1.77	1.85	1.87	1.74	0.076	0.066	0.042	0.070	0.071
v_{24}	Epistomal Sulcus / Min Head Width	1.64	1.62	1.68	1.71	1.55	0.089	0.070	0.050	0.086	0.066
v_{25}	Max Head Width / Head Length	0.61	0.61	0.57	0.56	0.64	0.026	0.023	0.017	0.026	0.024
v_{26}	Max Head Width / Epistomal Sulcus	0.66	0.67	0.63	0.62	0.72	0.037	0.029	0.023	0.036	0.027
v_{27}	Length to Hump / Max Head Width	1.02	0.98	1.11	1.17	0.93	0.079	0.060	0.058	0.094	0.050
v_{28}	Head Length / Mean Gular Width	5.42	5.27	5.55	5.67	5.09	0.317	0.243	0.192	0.286	0.235
v_{29}	Length to Hump / Min Head Width	1.10	1.07	1.18	1.23	1.03	0.085	0.065	0.056	0.089	0.060
v_{30}	Max Head Width / Min Head Width	1.08	1.08	1.06	1.05	1.11	0.019	0.016	0.021	0.021	0.031

differences. We used these differences in our inferences as to whether any of these paired comparisons assist in determining whether the differences are consistent with the conclusions that the CHC phenotypes are separate species. Variables that are significantly different between pairs of phenotypes are potential characters for the morphologicial separation of these phenotypes into different species.

Descriptive Statistics for Each Variable

For each of the 30 variables, we tabulated the number of samples (n) included in the mean and standard deviation using the statistical package R (R Core Team 2023). The purpose of these statistics is to provide quantitative, morphological characters for all phenotypes or phena (Mayr 1969, 1982) to be used in future descriptions of species of these five cuticular hydrocarbon phenotypes of *Reticulitermes* in California.

Discriminant Analysis

In addition, given the 30 variables (based on 1, 2, or 3 measurements), we wanted to determine how well we can predict the correct phenotype based on the eight combinations of a single variable vs. two variables, observations on a single termite vs the mean of five termites from the same location, and whether the samples come from the coast or foothills region.

For each of the eight combinations, we used linear discriminant analysis (Venables and Ripley 2002), to construct a score for each potential phenotype (using SAS PROC DISCRIM; SAS Institute Inc. 2023) based on the variable means and pooled covariance matrix. We assumed that prior to any measurements, the probabilities of correct classification were equal among the phenotypes. A linear score function is created for each phenotype that looks like a linear regression function:

$$Score = c_0 + c_1 x$$

for a single variable value x and

$$Score = c_0 + c_1 x_1 + c_2 x_2$$

for two variables labeled x_1 and x_2. The phenotype with the largest score is declared the predicted phenotype.

To assess the quality of each variable and each pair of variables, we used the jackknife estimate of the correct classification rate in which the scores are constructed leaving out a specific termite sample and that left-out sample is then predicted in an attempt to obtain an unbiased estimate of the probability of the correct classification. (In other words, our model prediction equations did not include the termite sample being predicted.) We then ranked each variable and each pair of variables by the overall correct classification rate. Choice of the 'best' single variable and pair of variables is made by an examination of the correct classification rates for each phenotype and the quality, consistency, and ease of the measurements (in our opinion) of each variable. Because this was considered an exploratory analysis, we did not adjust for multiple comparisons. This allows us to

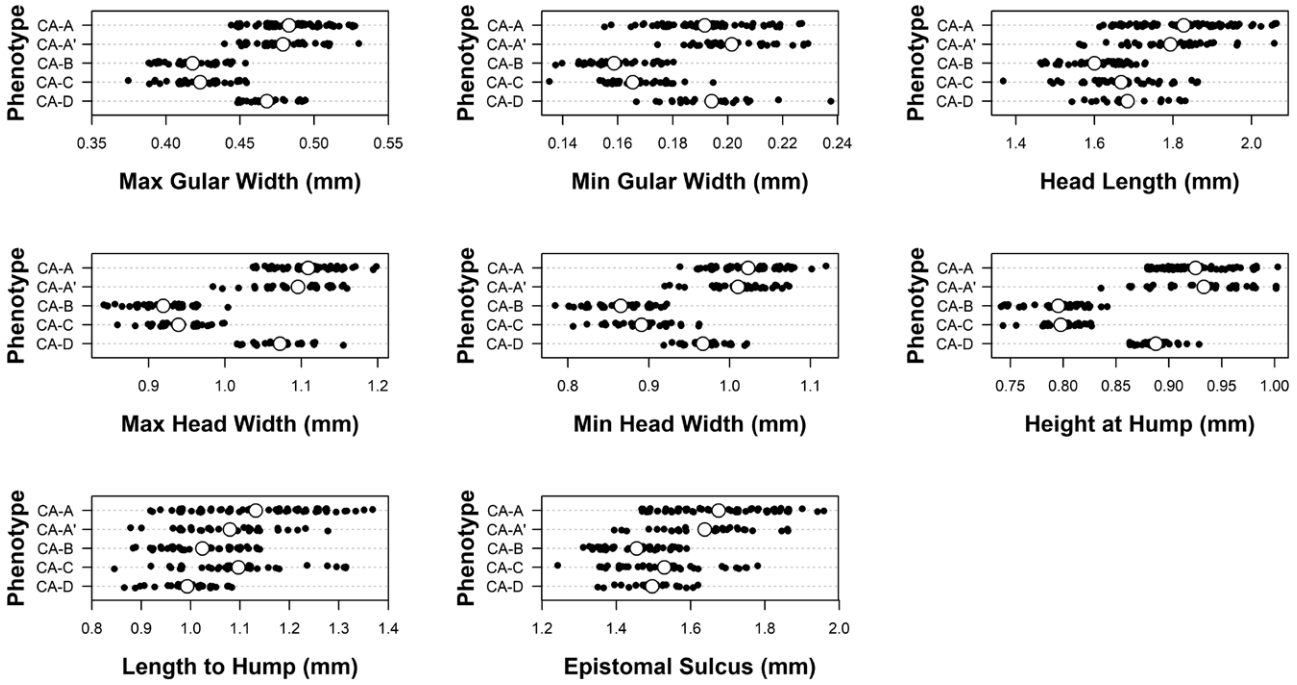

Fig. 2. (a) Strip plots of measurements of soldier heads of five cuticular hydrocarbon phenotypes of *Reticulitermes*, CA-A, CA-A', CA-B, CA-C and CA-D. Measurements include direct measurements identified in Table 1. The plots display the individual values and the mean for each phenotype-variable combination. The individual data points were jittered vertically and horizontally a small amount to avoid overlap of points with the same value. Each black dot represents a measurement on a single termite. The value of the mean for each phenotype-variable combination is represented as an open circle. (b) Strip plots of measurements of soldier heads of five cuticular hydrocarbon phenotypes of *Reticulitermes*, CA-A, CA-A', CA-B, CA-C and CA-D. Measurements include derived measurements identified in Table 2. The plots display the individual values and the mean for each phenotype-variable combination. The individual data points were jittered vertically and horizontally a small amount to avoid overlap of points with the same value. Each black dot represents a measurement on a single termite. The value of the mean for each phenotype-variable combination is represented as an open circle.

determine which variables or combinations of variables have the highest probability of correctly identifying the phenotypes separately for the two sites, coastal and foothill.

Results

Display of Data

In general, for all direct measurements (variables 1 to 8) phenotypes CA-A, CA-A', and CA-D had larger mean values than phenotypes CA-B and CA-C (Table 3, Fig. 2a). The two exceptions were (i) Epistomal Sulcus where the mean value for CA-C exceeded that of phenotype CA-D, but not significantly so, and (ii) Length to Hump where the mean value for phenotype CA-C exceeded that of phenotype CA-D (Table 3). Of the 22 derived variables, 13 had a higher mean value for phenotypes CA-B and CA-C than the other three phenotypes (Table 3, Fig. 2a,b). Two trends in these measurements are quite apparent. First, the measured variables (1 to 8) and derived variables (9 to 30) for phenotypes CA-A and CA-A' are very similar and are not significantly different. Second, all variables for phenotypes CA-B and CA-C are also very similar and none are significantly different (Tables 3 and 4, Fig. 2a,b). Therefore, using the soldier head capsule measurements we present here, we cannot separate phenotypes CA-A from CA-A' nor phenotypes CA-B from CA-C based on these morphological measurements.

The biggest challenge is to identify measurements or derived variables to separate phenotype CA-D from phenotypes CA-A and CA-A'. The most promising direct measures are: Height at Hump, Min Head Width, Head Length, Epistomal Sulcus, and Length to Hump (Tables 3 and 4, Fig. 2a). However, we must point out that for these five direct measurements, there is considerable overlap. Of the derived variables there is an abundance of variables that are significantly different between CA-A vs. CA-D and CA-A' and CA-D, however, there is also considerable overlap in values within these variables (Tables 3 and 4, Fig. 2a and b).

Although CA-B and CA-C cannot be separated from one another, they are unequivocally separable from CA-A using either of two direct measurements (Maximum Head Width and Height at Hump, both of which are larger in CA-A (Table 3 and Fig. 2a). Three derived variables appear to be very useful, with no or little overlap, for separating CA-B and CA-C from phenotype CA-A: Head Circumference, Head Cross Section Area, and Mean Gular Width (Table 3, Fig. 2b).

Statistical Analyses

Analysis of Variance

Of the direct head capsule measurements only Height at Hump resulted in 4 statistically significant differences and six derived variables resulted in 4 statistically significant differences among the six pairs of CHC phenotypes. Minimum Head Width, Head Length, and Epistomal Sulcus were the only direct measurement that resulted in 3 statistically significant differences among pairs of CHC phenotypes: CA-A vs. CA-B, CA-C, and CA-D. Six derived variables had 3 statistically significant differences among the same pairings (Table 4). Overall 22 variables, measured and derived, resulted in statistically significant differences between CHC phenotypes CA-A vs. CA-B and CA-A vs. CA-C; of these the seven direct measurements were Height at Hump, Minimum Head Width, Maximum Gular Width, Maximum Head Width, Minimum Gular Width, Head

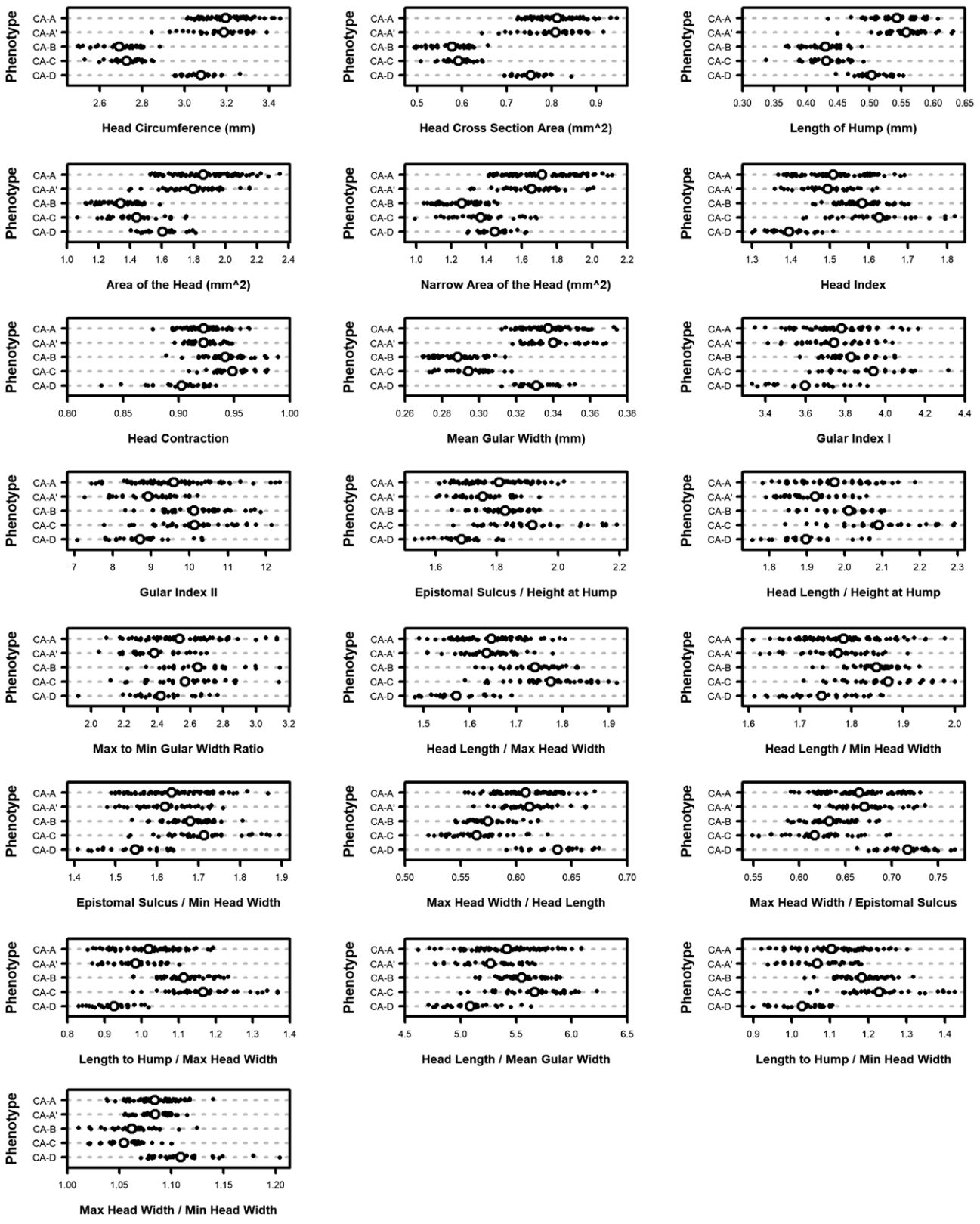

Fig. 2. (b) Continued

Length, and Epistomal Sulcus. Twenty-three variables were statistically significant comparing CHC phenotypes CA-A vs. CA-B, 26 variables were statistically significant comparing CA-A vs. CA-C, and 21 variables were statistically significant comparing CA-A vs. CA-D. Phenotype CA-A' was found to be statistically significant for only 7 variables vs. CA-D (Table 4).

As mentioned in the Methods section to formalize the probability of making correct classifications, we used linear discriminant

Table 4. Analyses of variance of means of five cuticular hydrocarbon phenotypes from northern California resulted in 6 pairwise comparisons. Differences with Tukey adjusted *P*-values < 0.05 are indicated with an * and P-values ≥ 0.05 are indicated with a ·.

	Comparison of Phenotype Pairs						
Variable	CA-A vs CA-A'	CA-A vs CA-B	CA-A vs CA-C	CA-A vs CA-D	CA-A' vs CA-D	CA-B vs CA-C	# of significant differences out of 6
v_8: Height At Hump (mm)	·	*	*	*	*	·	4
v_{11}: Length of Hump (mm)	·	*	*	*	*	·	4
v_{13}: Narrow Area of the Head (mm2)	·	*	*	*	*	·	4
v_{14}: Head Index	·	*	*	*	*	·	4
v_{15}: Head Contraction	·	*	*	*	*	·	4
v_{26}: Max Head Width/Epistomal Sulcus	·	*	*	*	*	·	4
v_{30}: Max Head Width/Min Head Width	·	*	*	*	*	·	4
v_3: Head Length (mm)	·	*	*	*	·	·	3
v_5: Min Head Width (mm)	·	*	*	*	·	·	3
v_6: Epistomal Sulcus (mm)	·	*	*	*	·	·	3
v_9: Head Circumference (mm)	·	*	*	*	·	·	3
v_{10}: Head Cross Section Area (mm2)	·	*	*	*	·	·	3
v_{12}: Area of the Head (mm2)	·	*	*	*	·	·	3
v_{22}: Head Length/Max Head Width	·	*	*	*	·	·	3
v_{25}: Max Head Width/Head Length	·	*	*	*	·	·	3
v_{27}: Length to Hump/Max Head Width	·	*	*	*	·	·	3
v_1: Max Gular Width (mm)	·	*	*	·	·	·	2
v_2: Min Gular Width (mm)	·	*	*	·	·	·	2
v_4: Max Head Width (mm)	·	*	*	·	·	·	2
v_7: Length To Hump (mm)	·	*	·	*	·	·	2
v_{16}: Mean Gular Width (mm)	·	*	*	·	·	·	2
v_{17}: Gular Index I	·	·	*	*	·	·	2
v_{19}: Epistomal Sulcus/Height at Hump	·	·	*	*	·	·	2
v_{23}: Head Length/Min Head Width	·	*	*	·	·	·	2
v_{24}: Epistomal Sulcus/Min Head Width	·	·	*	*	·	·	2
v_{29}: Length to Hump/Min Head Width	·	*	*	·	·	·	2
v_{20}: Head Length/Height at Hump	·	·	*	·	·	·	1
v_{28}: Head Length/Mean Gular Width	·	·	·	*	·	·	1
v_{18}: Gular Index II	·	·	·	·	·	·	0
v_{21}: Max to Min Gular Width Ratio	·	·	·	·	·	·	0

analysis (LDA). We also used separate LDA's for the coastal region phenotypes CA-A, CA-A', and CA-D and the foothills region phenotypes CA-A, CA-B, and CA-C because previous studies (Haverty and Nelson 1997, Copren et al. 2005, Nelson et al. 2008, Tseng et al. 2023) determined that phenotypes CA-A' and CA-D are not found in the foothills region and phenotypes CA-B and CA-C are not found in the coastal region.

Discriminant Analysis

When using a single variable, Height at Hump gives the best overall correct classification rates for the coastal phenotypes (CA-A, CA-A', and CA-D) when measuring a single termite: 60.7% correct classification rate (Table 5). When using the mean of five termites Length of Hump was best with a 68.8% overall correct classification rate (Table 5). Furthermore, CA-D is generally distinguished from phenotypes CA-A and CA-A' in that the key variable mean measurements are smaller except for Max Head Width/Epistomal Sulcus (Figs. 2a, 2b, 3 and 4). For the foothills phenotypes (CA-A, CA-B, and CA-C) Max Head Width provides the best overall correct classification rate at 73.2% for a single termite and Max Head Width provides the best overall correct classification rate at 78.3% when using the mean of five termites (Table 5). Also, for the foothills region phenotype CA-A is easily distinguished from phenotypes CA-B and CA-C because it is much larger for most variables (Figs. 2a, 2b, 5, and 6).

We can see the separation and where the predicted values occur with the variables with the top four correct classification rates in Figs. 3 through 6. The linear discriminant coefficients for those top four models are found in Supplementary Table 1.

When using two variables the overall correct classification rates are much higher than using a single variable (as expected). The correct overall classification rates for the top four pairs of variables are in Table 6. The top overall correct classification rates for the coastal single termite, coastal mean of five termites, foothills single termite, and foothills mean of five termites analyses are 67.8%, 79.2%, 80.3%, and 89.2%, respectively. The associated linear discriminant coefficients for the associated top four pairs of variables for coastal and foothill regions single termite and the mean of five termite combinations are in Supplementary Table 2.

Figures 7 through 10 show the respective separations and predicted phenotype areas. Even with high correct classification rates for CA-A, CA-A', and CA-D, these phenotypes are not unequivocally separated. There is considerable overlap for a single termite/variable and for the mean of five termites/variable (Figs. 7 and 8). However, the separation of CA-A vs. CA-B and CA-C is dramatic for single termite/variable and the mean of five termites/variable (Figs. 9 and 10). Phenotype CA-A is unequivocally separated from CA-B and CA-C for all of the top four variables and pairs of variables; the overlap of CA-B and CA-C is consistent.

Table 5. Percent correct phenotype classification (using a jackknife estimate) with a single variable for the top four variables for each of the 4 combinations of Coastal vs. Foothills and single termites vs. the mean of five termites.

Variable	Name	Percent correct classification			
Coastal (single termite)		CA-A	CA-A'	CA-D	Overall
v_8	Height at Hump (mm)	26.3	62.5	93.3	60.7
v_{13}	Narrow Area of the Head (mm2)	51.3	27.5	90.0	56.3
v_{14}	Head Index	50.0	35.0	83.3	56.1
v_{26}	Max Head Width / Epistomal Sulcus	52.5	35.0	80.0	55.8
Coastal (mean of five termites)		CA-A	CA-A'	CA-D	Overall
v_{11}	Length of Hump (mm)	43.8	62.5	100.0	68.8
v_{14}	Head Index	56.3	62.5	83.3	67.4
v_{26}	Max Head Width / Epistomal Sulcus	56.3	62.5	83.3	67.4
v_{27}	Length to Hump / Max Head Width	62.5	50.0	83.3	65.3
Foothills (single termite)		CA-A	CA-B	CA-C	Overall
v_4	Max Head Width (mm)	100.0	52.0	67.5	73.2
v_5	Min Head Width (mm)	98.8	50.0	67.5	72.1
v_{16}	Mean Gular Width (mm)	97.5	53.1	62.5	71.0
v_{10}	Head Cross Section Area (mm^2)	100.0	50.0	60.0	70.0
Foothills (mean of five termites)		CA-A	CA-B	CA-C	Overall
v_4	Max Head Width (mm)	100.0	60.0	75.0	78.3
v_2	Min Gular Width (mm)	87.5	80.0	62.5	76.7
v_{16}	Mean Gular Width (mm)	100.0	50.0	75.0	75.0
v_{22}	Head Length / Max Head Width	81.3	60.0	75.0	72.1

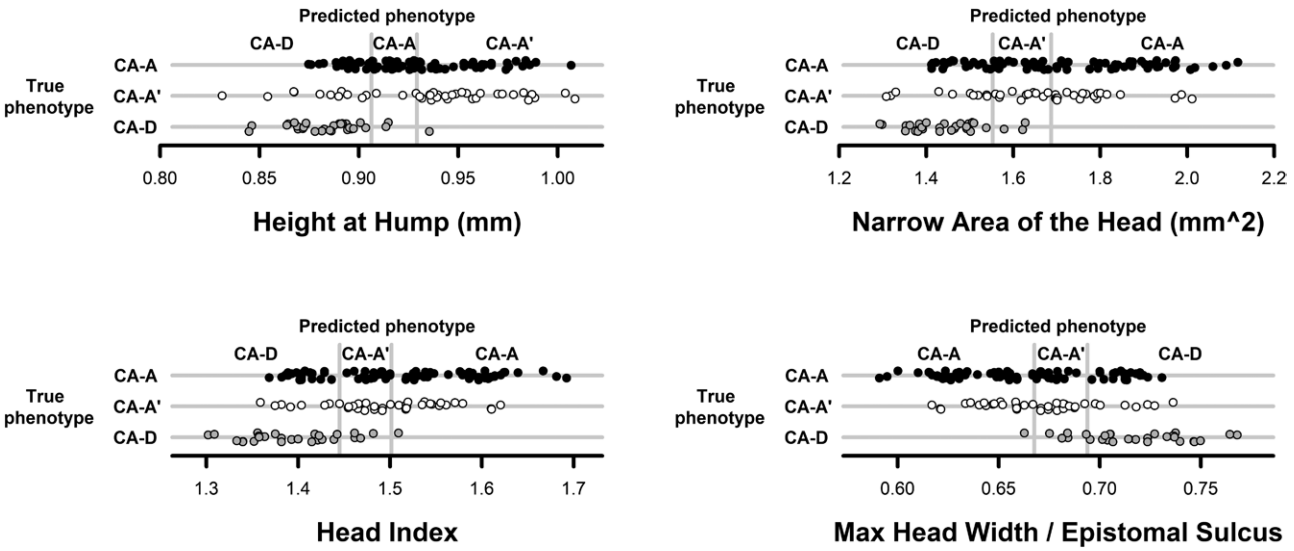

Fig. 3. Top four variables predicting phenotype for the coastal area using a single termite. Each circle represents a measurement on a single termite with black circles representing phenotype CA-A, open circles representing phenotype CA-A', and gray circles representing phenotype CA-D. All variables had an overall jackknifed correct classification of at least 55.8%. The vertical lines divide the variable values into the areas of predicted phenotypes.

Discussion

Accurate identification of species is primary to the successful execution of studies of the ecology and behavior of termites. Morphology is often used for field-based identification to species (Collins 1988, Korb et al. 2019). Our goal in this study was to find easily measured characters that could be used by nearly anyone, students, researchers, and pest management professionals, to identify the species of *Reticulitermes* in northern California.

How are species discrimination characters determined? Do we start with a great number of measurements or observations of a large, geographically extensive sample of specimens or do we examine specimens of 'known' taxa, group them, and take measurements or make observations to find characteristics that consistently separate them? Over the last 100 + years, scholars of termite biology have used both approaches.

Consider the early revision of the Nearctic termites by Banks and Snyder (1920). They started by examining a group of undiagnosed and undescribed specimens from all over North America. They grouped their samples into families, then genera, and finally into species. They developed keys to species for both the alates (imago) and soldier castes and assigned species names. Most of their species designations for *Reticulitermes* are still used today (*R. flavipes*

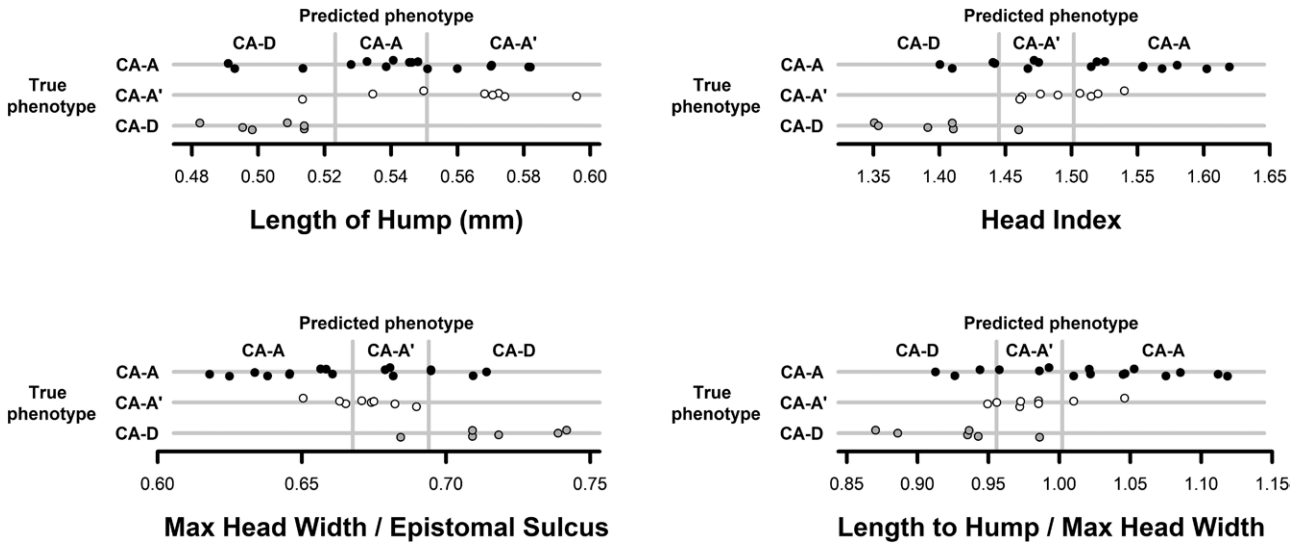

Fig. 4. Top four variables predicting phenotype for the coastal area using the means of 5 termites collected at a single survey. Each circle represents a 5-termite mean with black circles representing phenotype CA-A, open circles representing phenotype CA-A', and gray circles representing phenotype CA-D. All variables had an overall jackknifed correct classification of at least 65.3%. The vertical lines divide the variable values into the areas of predicted phenotypes.

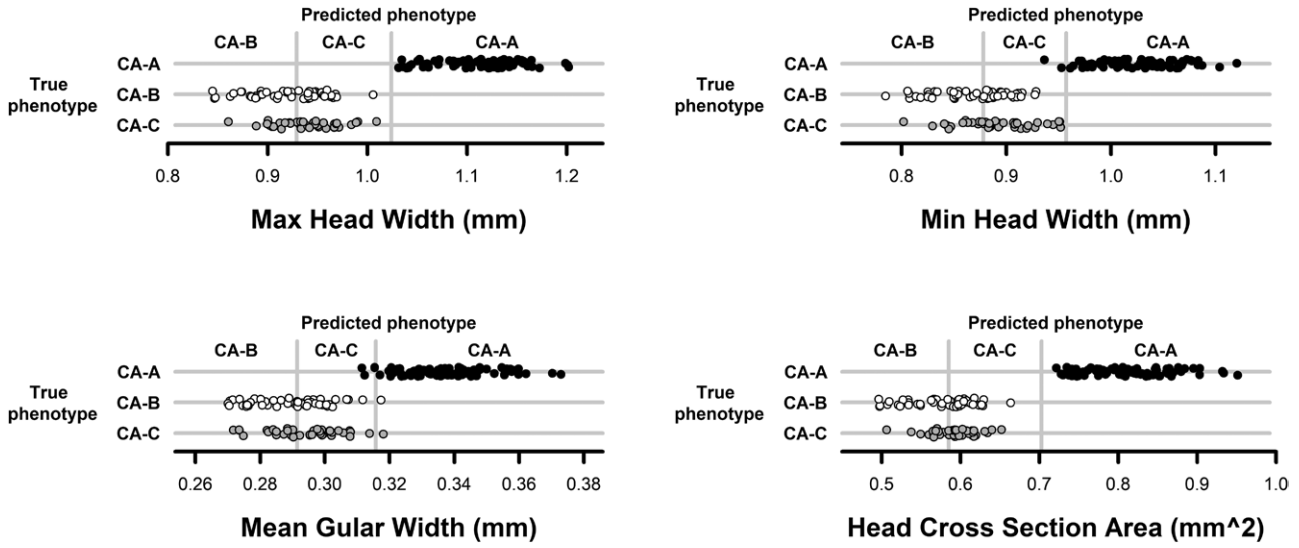

Fig. 5. Top four variables predicting phenotype for the foothills area using a single termite. Each circle represents a measurement on a single termite with black circles representing phenotype CA-A, open circles representing phenotype CA-B, and gray circles representing phenotype CA-C. All variables had an overall jackknifed correct classification of at least 70.0%. The vertical lines divide the variable values into the areas of predicted phenotypes.

(Kollar), *R. hageni* Banks, *R. hesperus* Banks, *R. tibialis* Banks, and *R. virginicus* (Banks). *R. flavipes* and *R. virginicus* were originally assigned to the genus *Termes*, then reassigned to *Reticulitermes* by Banks and Snyder (1920). Other species originally named by Banks and Snyder (1920) have been synonymized with other species (*R. tumiceps* Banks and *R. humilis* Banks with *R. tibialis* Banks, as well as *R. aureus* Banks and *R. humilis* Banks var. *hoferi* with *Heterotermes aureus* (Snyder)). Their work was extensive, and considering the number of unknown species (other than *Reticulitermes*) they were examining and the lack of the modern technologies we have today, they did an exemplary job of dividing this large group of termites into species groups.

Since the monograph by Banks and Snyder (1920) was published, some students of termite identification have used the approach of sorting specimens of soldiers that were identified to species by association with alate identifications then searching for characters or measurements that can be used to identify soldiers to species (Hostettler et al. 1995 , Brown et al. 2005). Lim and Forschler (2012) used evidence from multiple techniques, such as genetics, chemistry, and behavior for the description of *R. nelsonae* Lim and Forschler. These three studies (Hostettler et al. 1995, Brown et al. 2005, Lim and Forschler 2012) have a common feature that we encountered in our study reported here: substantial variability in measurements among individuals from the same colony and among members of the same species or CHC phenotype.

Why would there be such a large amount of variability in the size of soldiers of *Reticulitermes*? Thomas E. Snyder mentioned this phenomenon in *R. hesperus* (Banks and Snyder 1920): 'The long-headed

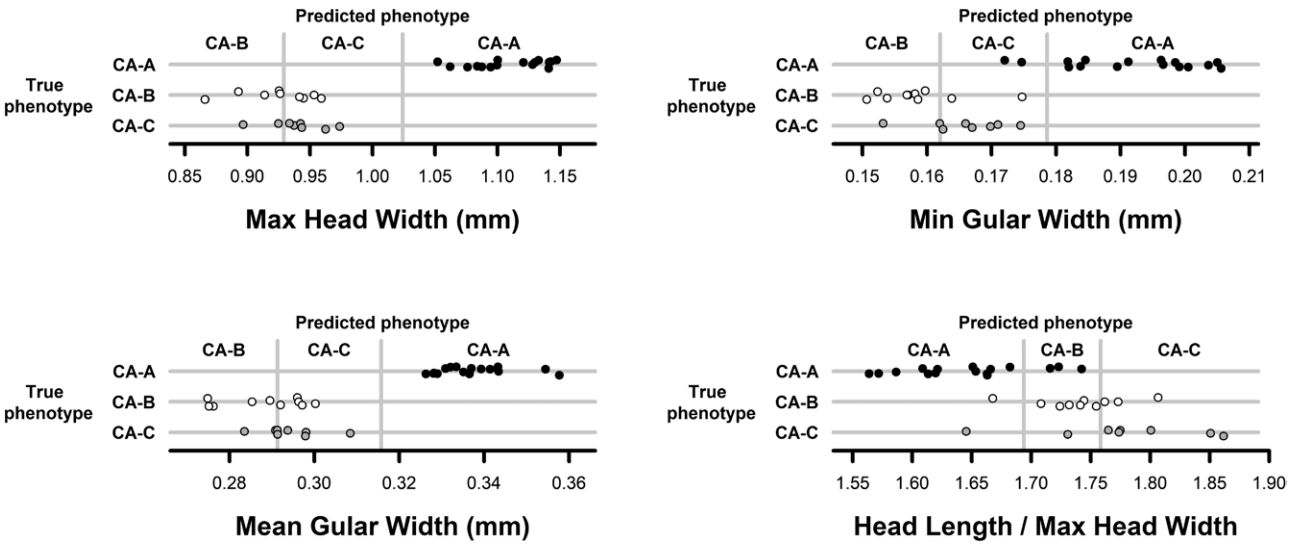

Fig. 6. Top four variables predicting phenotype for the foothills area using the means of 5 termites collected at a single survey. Each circle represents a 5-termite mean with black circles representing phenotype CA-A, open circles representing phenotype CA-B, and gray circles representing phenotype CA-C. All variables had an overall jackknifed correct classification of at least 72.1%. The vertical lines divide the variable values into the areas of predicted phenotypes.

Table 6. Percent correct phenotype classification (using a jackknife estimate) with two variables for the top four variables for the four combinations of Coastal vs. Foothills and single termite vs. the mean of five termites.

Variable 1		Variable 2		Phenotype				
Coastal region: measurements on a single termite				CA-A	CA-A'	CA-D	Overall	# of Measurements
v_{26}	Max Head Width / Epistomal Sulcus	v_{27}	Length to Hump / Max Head Width	55.0	65.0	83.3	67.8	3
v_7	Length to Hump (mm)	v_8	Height at Hump (mm)	56.3	57.5	86.7	66.8	2
v_{11}	Length of Hump (mm)	v_{27}	Length to Hump / Max Head Width	56.3	52.5	90.0	66.3	3
v_{14}	Head Index	v_{27}	Length to Hump / Max Head Width	51.3	60.0	86.7	66.0	3
Coastal region: using mean of measurements on five termites from the same collection				CA-A	CA-A'	CA-D	Overall	# of Measurements
v_{11}	Length of Hump (mm)	v_{19}	Epistomal Sulcus / Height at Hump	62.5	75.0	100.0	79.2	3
v_4	Max Head Width (mm)	v_{11}	Length of Hump (mm)	56.3	75.0	100.0	77.1	3
v_6	Epistomal Sulcus (mm)	v_7	Length to Hump (mm)	56.3	75.0	100.0	77.1	2
v_6	Epistomal Sulcus (mm)	v_{11}	Length of Hump (mm)	56.3	75.0	100.0	77.1	2
Foothills region: measurements on a single termite				CA-A	CA-B	CA-C	Overall	# of Measurements
v_3	Head Length (mm)	v_8	Height at Hump (mm)	100.0	76.0	65.0	80.3	2
v_3	Head Length (mm)	v_{20}	Head Length / Height at Hump	100.0	76.0	65.0	80.3	2
v_8	Height at Hump (mm)	v_{20}	Head Length / Height at Hump	100.0	76.0	65.0	80.3	2
v_{16}	Mean Gular Width (mm)	v_{20}	Head Length / Height at Hump	100.0	71.4	67.5	79.6	4
Foothills region: using mean of measurements on five termites from the same collection				CA-A	CA-B	CA-C	Overall	# of Measurements
v_4	Max Head Width (mm)	v_{21}	Max to Min Gular Width Ratio	100.0	80.0	87.5	89.2	3
v_1	Max Gular Width (mm)	v_3	Head Length (mm)	100.0	90.0	75.0	88.3	2
v_1	Max Gular Width (mm)	v_{17}	Gular Index I	100.0	90.0	75.0	88.3	2
v_3	Head Length (mm)	v_{17}	Gular Index I	100.0	90.0	75.0	88.3	2

soldier of this termite is distinctive. However, there is a difference in the length of the heads of soldiers of *R. hesperus* from different colonies, some being long-headed and others short-headed'. We are not sure whether he was actually looking at different colonies of *R. hesperus* or different taxa like CHC phenotypes of *Reticulitermes* such as CA-B and CA-C. Collins (1988) identified the variability of soldier sizes and their measurements as a problem for taxonomy of termites. She said that 'the variability in soldier size resulted from the flexibility inherent in caste determination in termite colonies. Immature individuals of several instars may enter the pathway of soldier development'.

Miller (1969) described the possible differentiation pathways for species of *Reticulitermes* from egg to the three possible terminal castes: alate (or imago or perfect adult), replacement reproductive, or mature soldier. After hatching from the egg, the first instars increase in size, and are known as larvae (apterous nymphs). They are immature individuals without external evidence of wing buds or soldier morphology. Subsequent molts can result in a morphological pathway to the nymphal stage where individuals increase in size and begin to show external wing buds. Nymphs can continue to molt and increase in size and, after passing through roughly nine instars, will molt into a mature, winged adult. Nymphs can undergo regressive

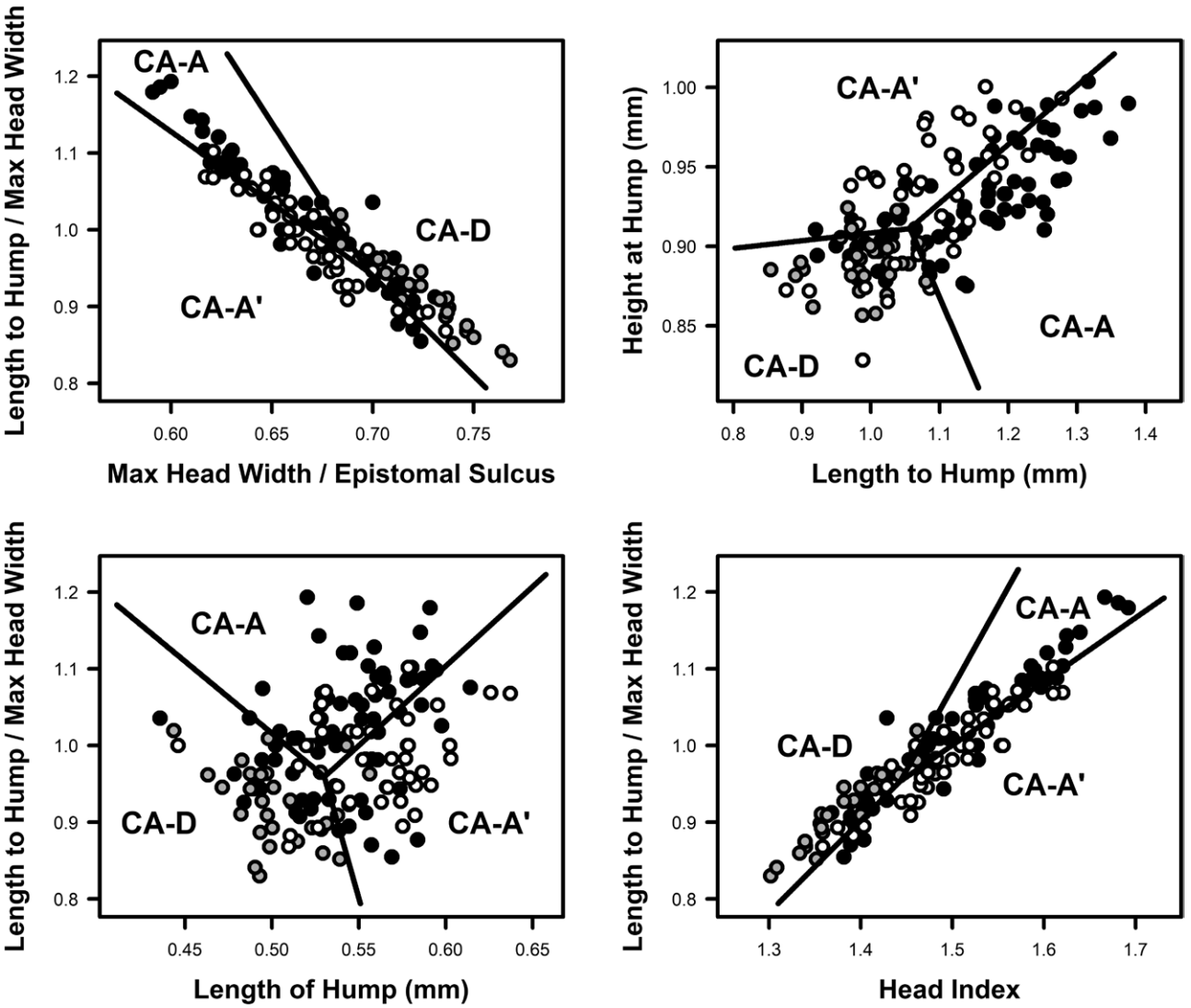

Fig. 7. Fig. 7.Top four pairs of variables predicting phenotype for the coastal area using a single termite. Each circle represents a single termite with black circles representing phenotype CA-A, open circles representing phenotype CA-A', and gray circles representing phenotype CA-D.The areas of the predicted phenotype are shown with the bold phenotype letter. The line segments divide the area into the predicted phenotypes. All variables had an overall jackknifed correct classification of at least 66.0%.

molts that reduce or eliminate their wing buds or develop into a form derived from a larva by undergoing stationary, nondifferentiation molts. This stage is called a pseudergate or literally false worker in *Reticulitermes*.

Soldiers can develop from larvae, nymphs or pseudergates that are in stadia 3 through 9. These individual soldiers will vary in size depending upon the instar or stadium from which they develop. Furthermore, the instar from which they develop or whether they develop from larvae, nymphs or pseudergates depends on the needs of their colony. Production of soldiers in laboratory groups of field-collected pseudergates of *R. flavipes* from Mississippi fluctuated seasonally (Haverty and Howard 1981). For example, after a period of fledging of the alates from the nest, the colony will have a surplus of soldiers. Many of the surplus soldiers will then be eliminated from the colony either by being consumed, slayed, and buried, or simply left outside the nest on the soil surface by their colonymates. It is unknown how the size, age, or origin of the soldiers may affect selection for elimination, however, there is the potential for drastically altering the mix of sizes within each colony. Our findings indicate that averaging measurements from five soldiers per colony would greatly improve classification accuracy compared to using single individuals. Therefore, when feasible, future ecological or management efforts would benefit from incorporating sampling multiple soldiers.

Even with the extensive variation in sizes of soldiers in our samples of multiple colonies of each of five CHC phenotypes, we were able to separate the CHC phenotypes into three groups based on various measurements: (i) CA-A and CA-A'; (ii) CA-D; and (iii) CA-B and CA-C. CHC phenotypes CA-A and CA-A' could not be statistically separated from each other by a difference of means on the basis of any variable. These two phenotypes were extremely similar in soldier head capsule morphology, as well as chemically (Haverty and Nelson 1997, Nelson et al. 2001, 2008, Page et al. 2002, Copren et al. 2005), genetically (Copren et al. 2005, Tseng et al. 2023), and behaviorally (Haverty et al. 2003). Phenotypes CA-A and CA-A' have nearly identical CHC profiles but are distinguishable by the

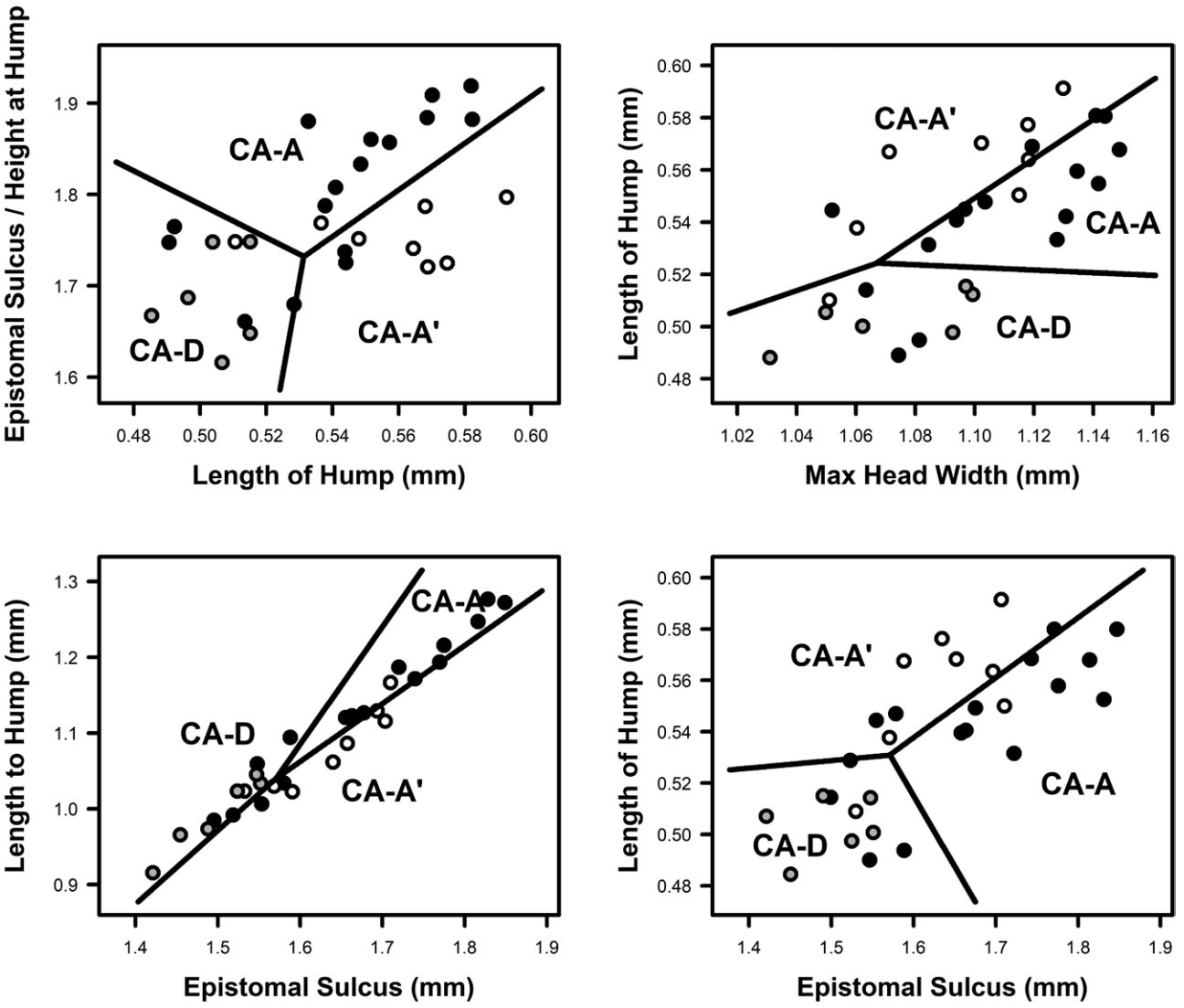

Fig. 8. Top four pairs of variables predicting phenotype for the coastal area using the means of 5 termites collected during a single visit. Each circle represents a 5-termite mean with black circles representing phenotype CA-A, open circles representing phenotype CA-A¢, and gray circles representing phenotype CA-D. The areas of the predicted phenotype are shown with the bold phenotype letter. The line segments divide the areas into the predicted phenotypes. All variables had an overall jackknifed correct classification of at least 77.1%.

presence or absence of several C25 and C27 trienes as indicated by GC-MS spectra (Haverty and Nelson 1997). While they appear to be the same species (*R. hesperus*) phylogenetically (Tseng et al. 2023), there is geographic variation in the quantities of the trienes.

Only samples of CA-A were found at our wildland site in Placerville, none of the collections were determined to be CA-A'. The majority of collections from the residential site in Marin Co. were CA-A' although there were some samples characterized as CA-A. The absence of trienes in the CA-A samples could possibly be an artifact from sample storage, as these compounds are unstable at ambient temperatures (Haverty et al. 1996). Our protocol was to inject samples as soon as possible after extraction and isolation from other cuticular lipid constituents, but on rare occasions, this was not possible, e.g., if equipment issues arose. If the CHC extracts remained at ambient temperatures for an extended period of time, the trienes would be undetectable under our GC-MS conditions. The distinction of these two phenotypes could be confounded by this phenomenon but may be inconsequential given that all other characteristics we have examined indicate they are the same species (Tseng et al. 2023). Samples of *R. hesperus* from southern California were found to vary in the relative proportions of these compounds (Nelson et al. 2008), and were determined to fall in the same clade with CA-A and CA-A' (Tseng et al. 2023). Head capsule measurements of samples from these southern California locations have not been made by us.

Phenotype CA-D soldiers were statistically different from the other coastal phenotypes examined in our study based on numerous morphological measurements (Tables 3 and 4, Figs. 3 and 4). The separation of CA-D from the other coastal phenotypes based on head capsule measurements is consistent with the characterization of CHCs (Haverty and Nelson 1997, Page et al. 2002 , Copren et al. 2005, and Nelson et al. 2008) but is not unequivocal. Genetically, CA-D is separate from the other coastal phenotypes and is much more closely related to CA-B, CA-C, and SC-B than to CA-A/A' (Copren et al. 2005, Tseng et al. 2023). Probably the most convincing evidence that CA-D is a different species from CA-A/A' are the disparate times of the year that these two CHC phenotypes fledge their alates. In the San Francisco Bay Area phenotype CA-A/A' (*R. hesperus*, see Nelson

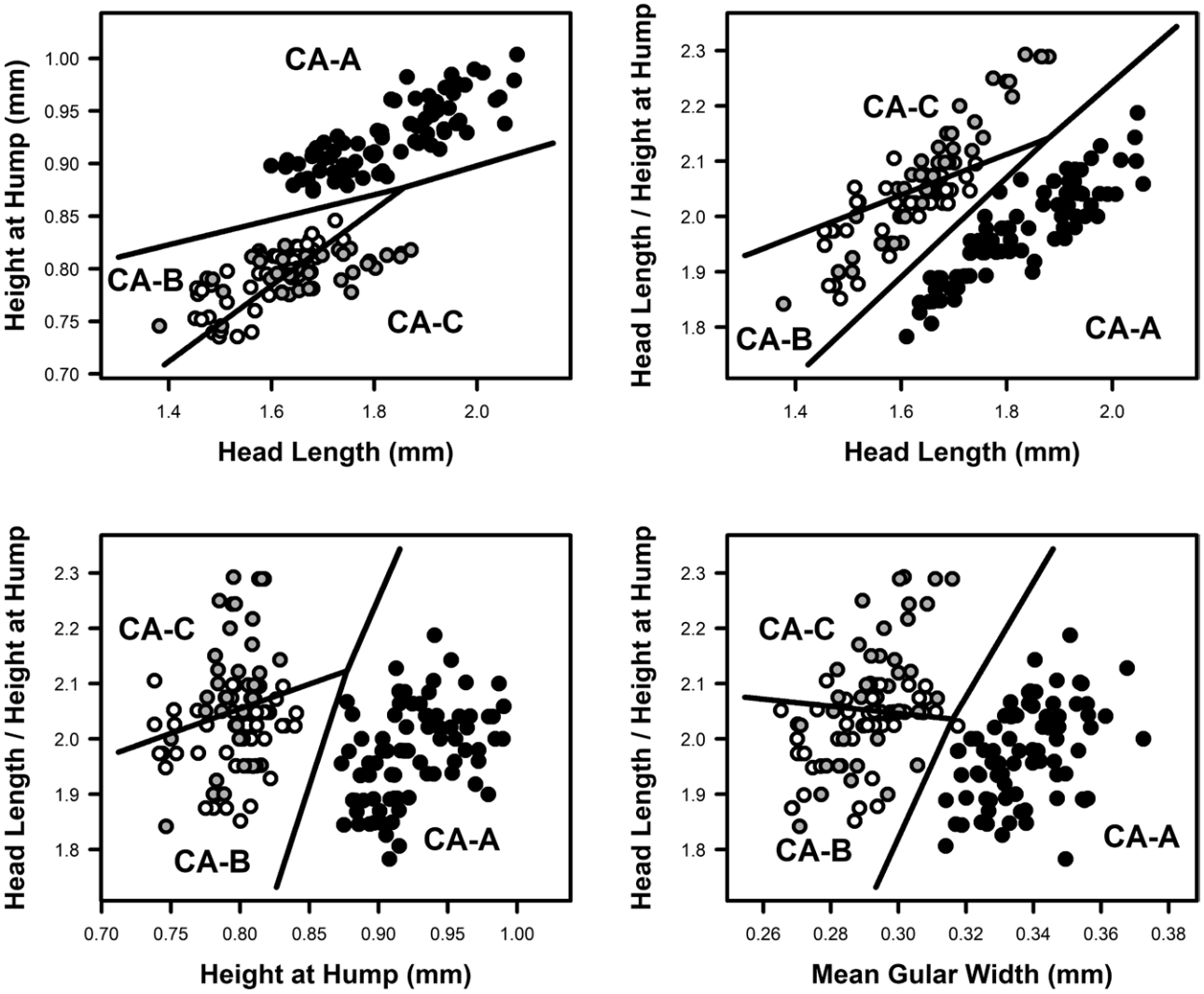

Fig. 9. Top four pairs of variables predicting phenotype for the foothills area using a single termite. Each circle represents a single termite with black circles representing phenotype CA-A, open circles representing phenotype CA-B, and gray circles representing phenotype CA-C. The areas of the predicted phenotype are shown with the bold phenotype letter. The line segments divide the areas into the predicted phenotypes. All variables had an overall jackknifed correct classification of at least 79.6%.

et al. 2008) flights occur in the spring from February to May and CA-D flights occur in the fall/winter from October to January; the flights of both phenotypes are associated with sunny days following rain (Haverty et al. 2003, Tseng et al. 2023). These disparate flight times ensure reproductive isolation of CA-A/A' from CA-D. From all the evidence presented here, we have determined that phenotypes CA-A/A' and CA-D are cryptic species, but because of their genetic differences, we cannot consider them sibling species.

Phenotypes CA-B and CA-C are statistically different from CA-A with respect to many direct and derived measurements (Table 4). The morphological separation of CA-B and CA-C from CA-A is consistent with the characterization of CHCs (Haverty and Nelson 1997, Page et al. 2002, Copren et al. 2005, Nelson et al. 2008), phylogenetic analyses (Copren et al. 2005, Tseng et al. 2023), average weight of workers (Haverty et al. 1999b), and agonistic behavior (Delphia et al. 2003). Alates of phenotypes CA-B and CA-C have never been observed or collected to date, thus there is no information about the timing of reproductive flights for comparison.

Phenotypes CA-B and CA-C are not only similar (almost identical) in soldier head capsule measurements and mean weight of workers (Haverty et al. 1999b), genetically they are closely related, sister taxa (species) and also are more closely related to CA-D than to CA-A (Copren et al. 2005, Tseng et al. 2023). When there is agreement of two or more characters (in this case genetics, CHC mixtures, and agonistic behavior) we can be confident in assigning separate species status to phenotypes CA-B and CA-C. *Reticulitermes* soldiers generally lack ornamentation, and phenotypes CA-B and CA-C are morphologically austere in that they lack features that we can use to effectively distinguish the species. Speciation is not always accompanied by morphological change; cryptic species may result from selection pressures that promote morphological stasis (Mayr 1969, Bickford et al. 2006). We can infer from the phylogenetic analyses of Copren et al. (2005) and Tseng et al. (2023) that CA-B and CA-C are closely related even though their CHC mixtures are quite dissimilar. We are of the opinion that CA-B and CA-C are cryptic species, as well as sibling species, as defined by Bickford et al. (2006).

We (MIH and LJN) have made collections of *Reticulitermes* throughout much of California, as well as Nevada, Utah, Arizona, and New Mexico, and have encountered CA-B and CA-C only in

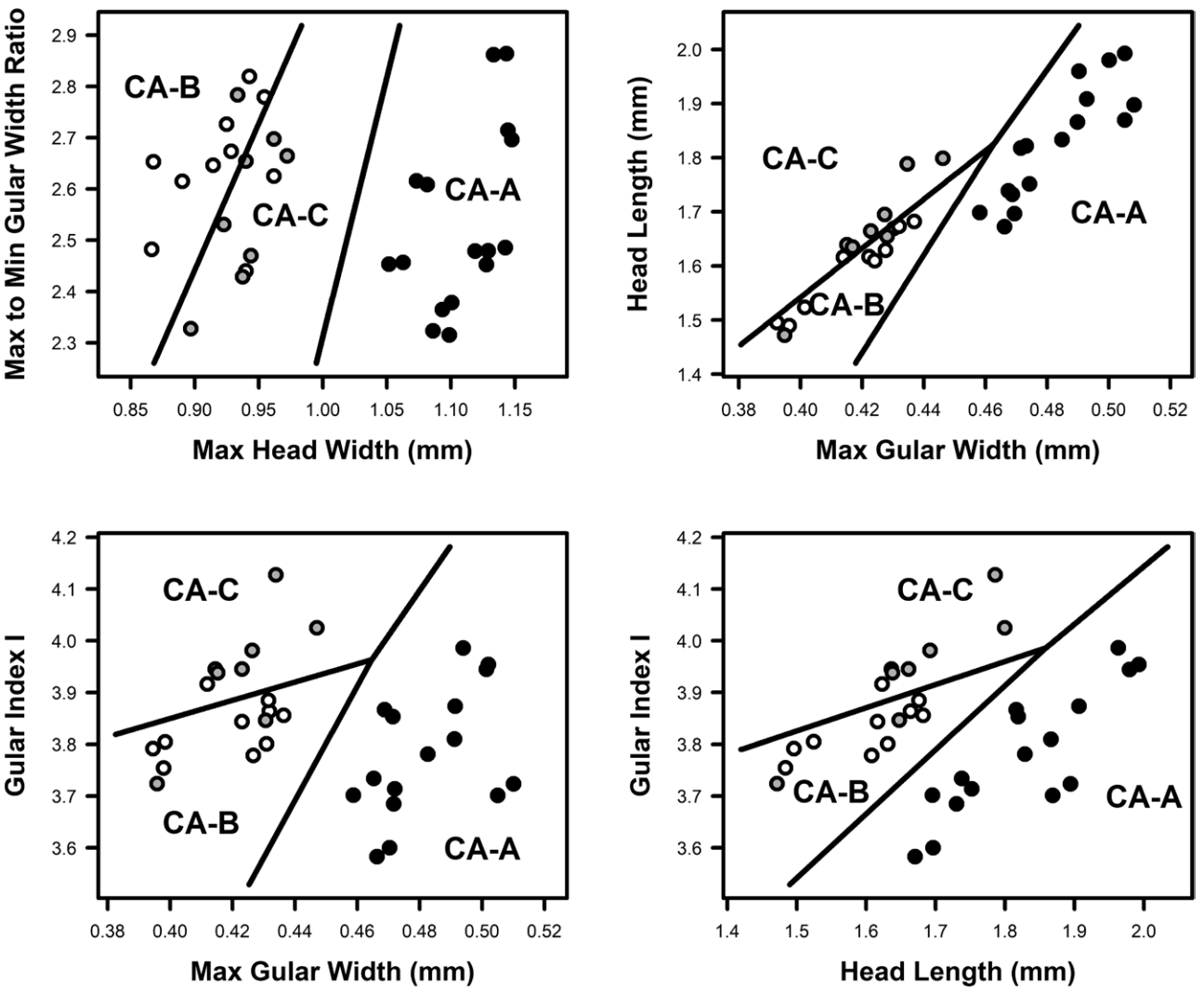

Fig. 10. Top four pairs of variables predicting phenotype for the foothills area using the means of 5 termites collected during a single visit. Each circle represents a 5-termite mean with black circles representing phenotype CA-A, open circles representing phenotype CA-B, and gray circles representing phenotype CA-C. The areas of the predicted phenotype are shown with the phenotype letter. The line segments divide the areas into the predicted phenotypes. All variables had an overall jackknifed correct classification of at least 88.3%.

the vicinity of Placerville. They are sympatric with CA-A at this location. Samples misidentified as *R. hesperus* by Tripodi et al. (2006) grouped with samples of phenotype CA-C in genetic analyses reported by Tseng et al. (2023), expanding the range of this cryptic species to include Sacramento, San Joaquin, and Nevada Counties in California.

The concept of cryptic species is not without controversy (Zuniga-Reinoso and Benitez 2015, Korshunova et al. 2019). We suggest that for the purposes in this paper, we consider cryptic species to be two or more distinct species incorrectly classified under a single species name because they were impossible or nearly impossible to distinguish by morphology, but are not necessarily closely related (Freeman and Herron 2001, Bickford et al. 2006). In our study we consider phenotypes CA-A and CA-A' vs. CA-D to be cryptic species. Phenotypes CA-A and CA-A' are considered chemical variants of *R. hesperus* (Copren et al. 2005, Nelson et al. 2008), and collections of phenotype CA-D soldiers would be misclassified or misidentified as *R. hesperus* due to its sympatric distribution with phenotypes CA-A and CA-A' in northern California (Weesner 1970, Nutting 1990, Haverty and Nelson 1997). However, phenotype CA-D is not closely related genetically to CA-A/A' *(R. hesperus)* (Copren et al. 2005, Tseng et al. 2023). Our study here does not provide unequivocal morphological means for separating the heretofore cryptic species CA-A/A' and CA-D.

For us to develop a definition of sibling species we had to consider definitions from multiple sources (Mayr 1969, 1982, Futuyma 1998, Freeman and Herron 2001, and Bickford et al. 2006). For our purposes we consider sibling species to be (i) biological species in that they are reproductively isolated, (ii) are closely related or sister taxa, and (iii) are difficult (or impossible) to distinguish by morphological characters. In this study, we declare that phenotypes CA-B and CA-C are both cryptic and sibling species.

First of all, CA-B and CA-C should be considered different biological species. We have found no evidence of exchange of genetic material and phylogenetic analyses determined that CA-B and CA-C are monophyletic species, are more closely related to one another than any other *Reticulitermes* species or taxa in California, and are considered sister taxa (Copren et al. 2005, Tseng et al. 2023). Furthermore, in our study here, we can attest that phenotypes CA-B and CA-C are so far impossible to distinguish morphologically. The

concepts of cryptic and sibling species are not unique to termites or insects. Their discovery may be related to differences in the ecology or behavior of multiple taxa. We discovered our cryptic and sibling species by noticing differences in ecology and behavior before we tried to find morphological characters that help future scientists in the field that might not be able to rely on chemistry or genetics that were essential to our studies. It was exciting to find two cryptic species of *Reticulitermes* with different reproductive flight periods. Previously it was thought that *R. hesperus* had a fall/winter and a spring flight (Weesner 1970). We can understand the confusion that was caused by the two species that were not readily separated based on morphology alone.

In conclusion, misidentifying cryptic species could affect pest control efforts. For instance, although phenotype CA-D is morphologically very similar to CA-A, it differs in drastically reproductive flight phenology and potentially in ecological or behavioral traits. Misidentification of these two taxa/species could result in poorly timed baiting strategies or ineffective colony elimination efforts. Unfortunately, our pursuit of measurements of the soldier head capsules of *Reticulitermes* that would provide unequivocal separation of the five CHC phenotypes was not totally successful. We found measurements on the species found in the foothills of the Sierra Nevada that would separate two species from the other sympatric species. Identification of additional morphological features or measurements for separation of all four phenotypes (= species) remains a task for future research and researchers.

Supplementary material

Supplementary material is available at *Journal of Economic Entomology* online.

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Author contributions

Michael Haverty (Conceptualization [Lead], Data curation [Lead], Funding acquisition [Lead], Methodology [Equal], Project administration [Lead], Resources [Lead], Supervision [Lead], Validation [Equal], Writing - original draft [Lead], Writing - review & editing [Equal]), Lori Nelson (Data curation [Supporting], Validation [Supporting], Writing - original draft [Supporting], Writing - review & editing [Equal]), and James Baldwin (Data curation [Supporting], Formal analysis [Lead], Methodology [Equal], Validation [Equal], Visualization [Lead], Writing - original draft [Supporting], Writing - review & editing [Equal])

Conflicts of interest. None declared.

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