

INTRACOLONY MORPHOMETRIC VARIATION AND LABRAL
SHAPE IN FLORIDA *RETICULITERMES* (ISOPTERA:
RHINOTERMITIDAE) SOLDIERS: SIGNIFICANCE FOR
IDENTIFICATION

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ABSTRACT

Measurements of pronotal width, broadest gular width, and labral length of *Reticulitermes* soldiers collected in San Felasco State Preserve, Florida, showed considerable intracolony variation and interspecific overlap. The termites collected did not differ in size from termites collected state wide. The reported 0.81 mm pronotal width threshold failed to separate soldiers of *R. flavipes* from *R. virginicus*. An average pronotal width measurement ($n < 5$) ≥ 0.90 mm denotes *R. flavipes*, ≤ 0.70 mm *R. hageni*, and 0.71 - 0.80 mm *R. virginicus*. A threshold at 0.85 mm ($n > 5$) separates *R. flavipes* from *R. virginicus*. A new character, labral shape, was found to be very reliable for separating the three species.

Key Words: Pronotal width, measurements, gula, subterranean termites,

RESUMEN

Las medidas del ancho pronotal, máximo ancho gular y longitud del labrum de soldados de *Reticulitermes*, colectados en la Reserva Estatal de San Felasco en Florida, mostraron considerable variación y sobreposición interspecífica. Las termitas colectadas no presentaron diferencia en tamaño de aquellas colectadas en el resto del estado. La medida del ancho pronotal registrado de 0.81 mm como límite para separar los soldados de *R. flavipes* de *R. virginicus*, no fue de utilidad. El promedio de las medidas del ancho pronotal ($n > 5$) ≥ 0.9 mm hace referencia a *R. flavipes*, ≤ 0.7 mm a *R. hageni*, 0.71 - 0.80 mm a *R. virginicus*, y un límite de 0.85 mm ($n > 5$) separa *R. flavipes* de *R. virginicus*. Un nuevo carácter encontrado, la forma del labrum, fué muy confiable para separar las 3 especies.

Three species of subterranean termites in the genus *Reticulitermes*, *R. flavipes* (Kollar), *R. virginicus* (Banks) and *R. hageni* Banks, occur in Florida (Miller 1949, Scheffrahn et al. 1988). These species are prominent biotic elements in the confluence of wood and soil in Florida and the southeastern United States, and are of considerable economic importance (Scheffrahn et al. 1988). Confirmatory species identification has been based on characters of the winged imago (alate) which, unfortunately, is a seasonal caste and usually not collected simultaneously with soldiers and workers. The soldiers, present in the colony throughout the year, possess more subtle and, in some cases, rather variable characters making their correct identification more difficult than alates.

In their key to *Reticulitermes* soldiers, Banks & Snyder (1920) differentiated the three species occurring in Florida by size: the larger being *R. flavipes*, the smaller either *R. virginicus* or *R. hageni*. Banks (1946), Emerson & Miller (1943), and Miller (1949) used soldier pronotal width to separate *R. flavipes* (0.81-1.10 mm) from *R. virginicus* and *R. hageni* (0.67-0.81 mm). Soldiers of the latter two species were separated by these authors using the shape of the gula (postmentum) and mandibles. They reported that the gula of *R. virginicus* had more abrupt inward curvatures of its lateral margins than those of *R. hageni* and that the degree of curvature of the mandibular points was greater in *R. virginicus* than in *R. hageni*. In his key, Snyder (1954) used the total length of soldiers to separate *R. flavipes* (6.7 mm) from *R. virginicus* (4.5 - 5.0 mm) and he specified the gular characters mentioned above to separate *R. virginicus* from *R. hageni*.

In his attempt to identify diagnostic characters for soldiers of eastern U.S. species of *Reticulitermes*, Banks (1946) compared 13 morphometric measurements taken from three or more soldiers per colony (number of colonies not specified) of each species, and found overlap for all three species in 11 of the measurements. The two remaining measurements, head length with mandibles and posterior head width, yielded overlap among the largest *R. hageni* and the smallest *R. virginicus* and among the largest *R. virginicus* and the smallest *R. flavipes*.

Based on specimens collected throughout Florida, we found that the three species differ in size, with soldiers of *R. flavipes* being the largest and those of *R. hageni* the smallest. In their key to termite soldiers of Florida, Scheffrahn & Su (1994) use *R. flavipes* pronotum width (> 0.90 mm) and head length (≥ 2.8 mm) to separate this species from *R. virginicus* (pronotum width < 0.85 mm and head length ≤ 2.7 mm). Soldiers of *R. hageni* could be separated fairly reliably from those of the other two species by the shape and degree of curvature of the mandibles and its diminutive pronotal width (≤ 0.70 mm).

In previous studies of *Reticulitermes* soldiers, intracolony variation was not explored as an additional source of morphometric variation within a species. Such measurements would be useful in confirming the reliability of morphometric characters. Intracolony measurements ($n=2-3$) taken of slide-mounted pronota, gula, and labra revealed colony specific variation which contributed to character overlap. Interspecific overlap was found in all measurements except for the broadest gular width and labral length. Proportions calculated from our measurements were inconclusive for species separation.

This paper reports on the intracolony variation of the soldiers of the three *Reticulitermes* species recognized in Florida for pronotal width, broadest gular width, and labral length, and assesses the limitations of using pronotal width to separate *R. virginicus* from *R. flavipes*. We also describe the use of labral shape as an additional character for differentiating soldiers of the three *Reticulitermes* species found in Florida.

MATERIALS AND METHODS

A total of 67 *Reticulitermes* colonies (31 *R. flavipes*, 26 *R. virginicus*, 9 *R. hageni* samples, and one unknown) from San Felasco State Preserve, Alachua Co., Florida, were sampled by collecting foraging groups (i.e. soldiers, workers, and, if present, alates) associated with each colony. Alates were used to identify ten *R. flavipes*, four *R. virginicus*, and one *R. hageni* sample. The remainder were grouped by pronotal width and identified by the labral character described herein. Voucher specimens from each colony were preserved by killing in hot water, fixing overnight in 2.5% glutaraldehyde with a trace of Triton X or Watsol (wetting agents) in cacodylate buffer

(0.1M, pH 7.2), rinsing with water, and storing in 75% ethanol with 5% glycerol. The labrum, gula, and pronotum from 2-3 soldiers of each colony were mounted on microscope slides under cover slips in Hoyer's medium (Kranz 1987). Preliminary measurements of pronotal width were taken of preserved soldiers ($n \leq 10$) using a stereomicroscope at 50x equipped with an ocular micrometer. Later measurements also were taken of pronotal width and length, labral width and length, and gular widths (broadest and smallest) and length of the slide mounts using a compound microscope at 100x equipped with an ocular micrometer.

Based on the pronotal measurements above, ten colonies were selected to assess intracolony measurement variation and interspecific measurement overlap for the *Reticulitermes* populations at San Felasco. The *R. hageni* colonies consisted of typically-sized and large-sized soldiers, 89 and 49 were measured respectively; the *R. virginicus* colonies consisted of small-sized and typically-sized soldiers, 88 and 80 were measured respectively; and the *R. flavipes* colonies of small-sized and typically-sized soldier, 87 and 80 were measured respectively. Because no *R. virginicus* colonies consisting of large-sized soldiers with a sufficiently large number of soldiers were available, additional four sample sets of 12-13 soldiers from colonies consisting of large-sized *R. virginicus* soldiers were prepared. A greater representation of large-sized *R. virginicus* soldiers was sought because these were most likely to be confused with *R. flavipes*. The labra, gula, and pronota of these soldiers were dissected and mounted on microscope slides under cover slips in Hoyer's medium. Measurements were made of pronotal width, broadest gular width, and labral length using a compound microscope at 100X and an ocular micrometer.

Soldier pronotal width measurements in the five colonies having ≥ 80 soldiers were used to estimate a minimum sample size to calculate a reliable, species-specific measurement. Assuming a random sample of the sequentially obtained measurements, a running mean and the deviation of this mean from the overall mean was calculated.

To compare the 67 San Felasco samples with those of *Reticulitermes* state-wide, the pronotal width of soldiers from 29 colony samples (13 *R. flavipes*, 13 *R. virginicus*, and 3 *R. hageni*) from the Florida Counties of Hillsborough, Broward, Washington, Pinellas, Brevard, Palm Beach, Alachua, Okeechobee, Volusia, Orange, and Dade were measured. Alates were used for primary identification and either one or two soldiers from each colony sample was measured. These samples were from the termite collection at the University of Florida, Ft. Lauderdale R.E.C.

Soldiers and workers from alate-identified San Felasco colonies were examined for external morphological differences using a Hitachi scanning electron microscope at 15kV. The specimens were dehydrated through an ethanol series, transferred to hexamethyldisilazane (Nation 1983), mounted on SEM stubs, and sputter-coated with gold.

RESULTS AND DISCUSSION

Measurements (mm) of pronotal width, gular width, and labral length of soldiers from the ten San Felasco *Reticulitermes* colonies are presented in Fig. 1. Because we mounted the pronota on slides which flattened these weakly convex structures, our measurements are slightly larger than those of published keys, but should be more accurate for relative comparisons. The colonies selected consisted of both larger and smaller soldiers within the same species, probably determined by intrinsic genetic factors or by external factors such as nutritional status and colony age. No overlap in pronotal width between *R. hageni* and *R. virginicus* was observed. However, there was significant overlap in broadest gular width, labral length, and pronotal width among

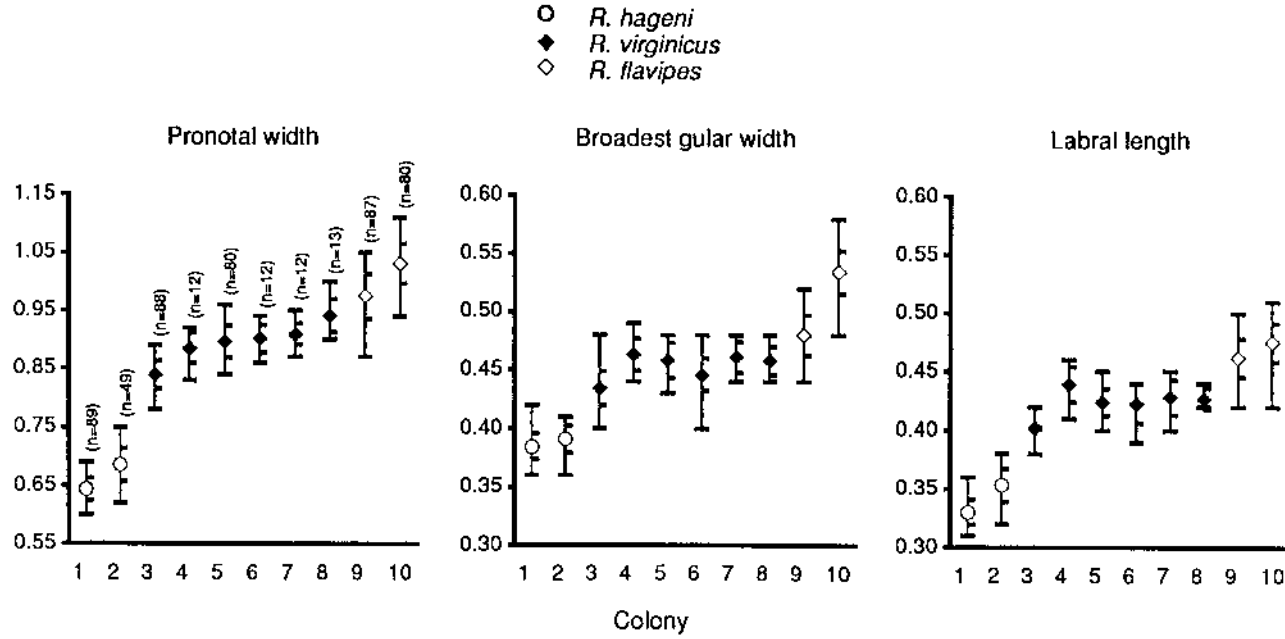


Fig. 1. Average (+SD), minimum, and maximum measurements (mm) of soldiers from 10 *Reticulitermes* spp. colonies from San Felasco State Preserve. Measurements from slide mounted pronota, labra, and gula; sample size given in figure.

R. virginicus and *R. flavipes* soldiers. In fact, the mean pronotal width of one of the *R. virginicus* colonies ($n = 13$) falls within one standard deviation of the mean for one of the colonies ($n = 87$) of *R. flavipes*. Furthermore, the ranges of all six colonies of *R. virginicus* overlap the range of one ($n = 87$) of the *R. flavipes* colonies for all three measurements.

The labral length and broadest gular width were selected because no intracolony specific overlaps were previously encountered. However, the data in Fig. 1 show that these measurements are of little value for separating the *R. virginicus* and *R. flavipes* colonies and demonstrate the inherent danger of making inferences about size using measurements obtained from only a few individuals.

Although Fig. 1 shows pronotal width overlap between *R. virginicus* and *R. flavipes*, the means ($n = 10$) taken of whole soldiers showed three distinct groups each corresponding to a species (Table 1). While these ranges may not be representative of all populations in Florida, the San Felasco measurements do not differ from those obtained from termites collected throughout Florida (Table 2). Even though some pronotal width measurements do overlap, it does show that with some limitation, this measurement is useful for species separation.

In order to use the pronotal width mean value with some degree of confidence, a certain minimum colony sample size is needed. The frequency distributions of the data used for Fig. 1 indicated that the measurements were normally distributed. As the sample size increases, an extreme value has less effect on the mean. Fig. 2 shows the deviation of a running mean calculated from the overall mean, calculated in exact order as the measurements were obtained to assure a random sampling. With a sample size of $n=10$, the deviation is within 0.020 mm, 0.014 mm for *R. hageni*, 0.012 mm for *R. virginicus* and 0.018 mm for *R. flavipes*. This result, and the standard deviations in Fig. 1, indicate that a mean obtained from less than 5 specimens should be used with caution. Measuring more than ten specimens probably will not yield a much more accurate mean.

A fairly reliable characteristic that we found to be useful in separating these species is the shape of the labrum viewed on slide mounts (Fig. 3). The labrum of *R. hageni* is elongated with the ratio of length beyond the anteclypeus (i.e. sclerotized area) to greatest width > 1.2 . The lateral margins of the distal 3/4 of the labrum are straight or slightly concave and converge at a uniformly rounded tip (Figs. 3a and 4a). The labrum of *R. virginicus* is diamond-shaped with angular lateral margins converging into an obtuse point. The ratio of length:width is < 1.2 . If the two lines forming the anterior margin were extended they would intersect beyond the tip (Figs. 3b and 4b).

TABLE 1. RANGE OF COLONY PRONOTAL WIDTH MEANS (MM) OF *RETICULITERMES* SOLDIERS^a.

Species	Smallest	Largest
<i>R. flavipes</i>	0.88	1.03
<i>R. virginicus</i>	0.73	0.83
<i>R. hageni</i>	0.58	0.67
Unknown	0.80	

^aWhole soldiers measured.

TABLE 2. PRONOTAL WIDTH MEASUREMENTS^a (MM) OF INDIVIDUAL *RETICULITERMES* SOLDIERS.

Species	San Felasco State Preserve				Florida			
	N ^b	Colonies	Range	Average±SD	N ^c	Colonies	Range	Average±SD
<i>R. flavipes</i>	298	31	0.81 - 1.11	0.95 ± 0.051	23	13	0.86 - 1.13	0.95 ± 0.16
<i>R. virginicus</i>	250	26	0.71 - 0.87	0.79 ± 0.030	24	13	0.70 - 0.84	0.78 ± 0.10
<i>R. hageni</i>	79	9	0.55 - 0.71	0.62 ± 0.036	5	3	0.65 - 0.71	0.68 ± 0.08
Unknown	9	1	0.80 - 0.81	0.80 ± 0.010				
Total	636	67			52	29		

^aWhole soldiers measured.
^bMaximum 10 soldiers per colony.
^cMaximum 2 soldiers per colony.

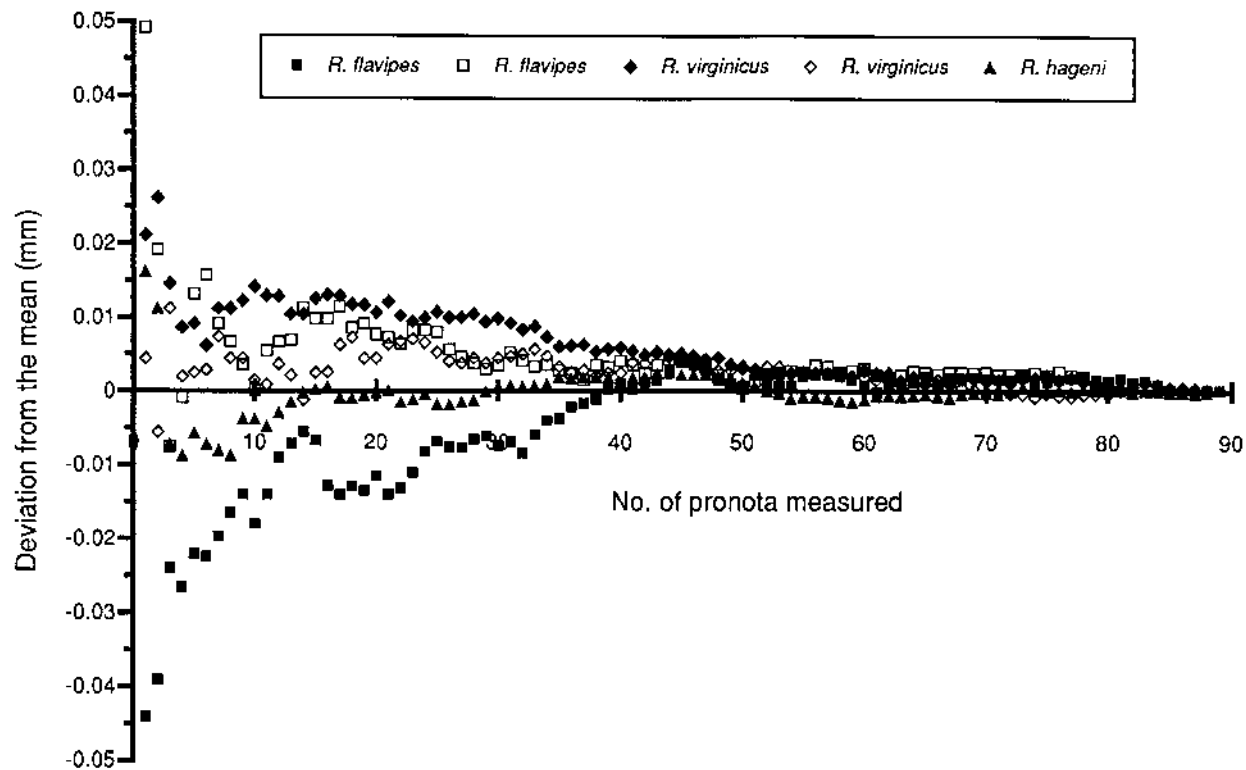


Fig. 2. Deviation of the running pronotal width mean from the overall mean ($n = 80-89$) of soldiers from five *Reticulitermes* spp. colonies.

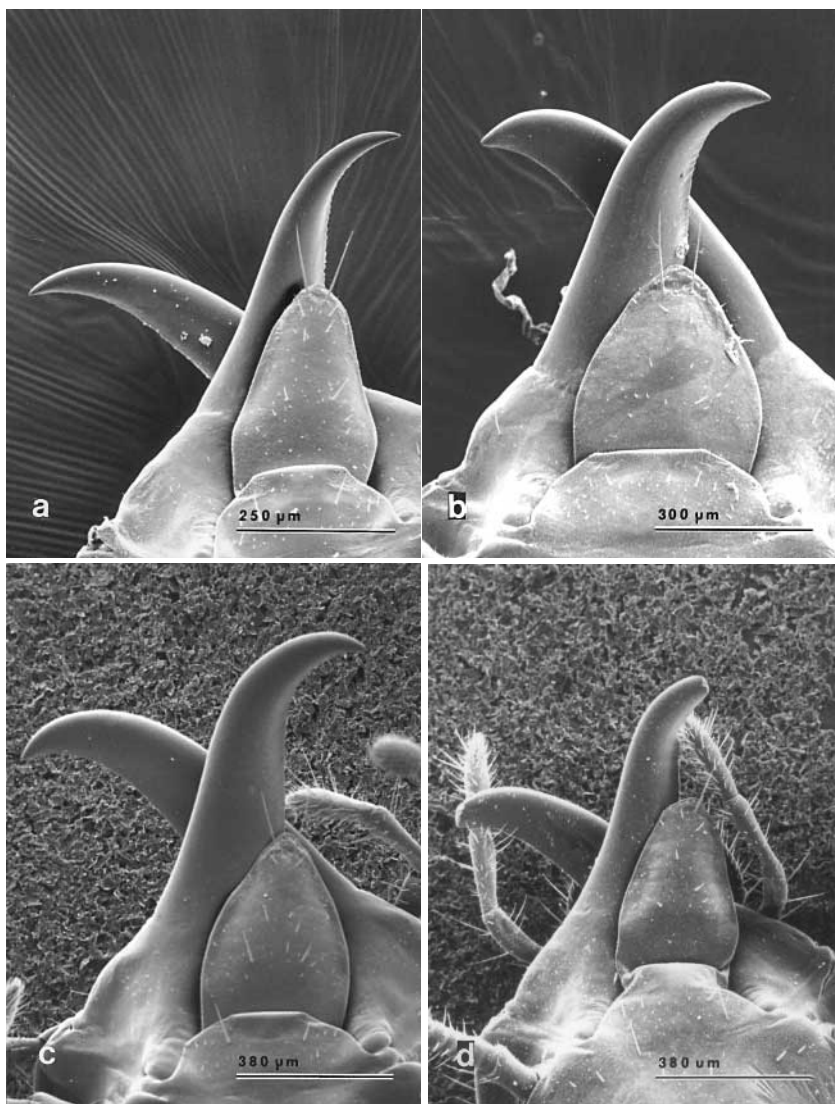


Fig. 3. Electron microphotographs of *Reticulitermes* spp. soldier labra: a) *R. hageni*, b) *R. virginicus*, c) *R. flavipes*, d) *Reticulitermes* sp. unknown.

The labrum of *R. flavipes* forms an outline resembling a flatiron with a frontal projection. The lateral margins are evenly convex from their bases to their distal 2/3-3/4 where a more or less distinct concavity precedes a rounded or an acutely pointed triangular tip. If the two lines forming the anterior lateral margins were extended they would intersect short of the tip (Figs. 3c and 4c).

Labral proportions are relatively constant within a colony for *R. hageni* but variable for *R. virginicus* and *R. flavipes*. The sides of *Reticulitermes* labra are rather

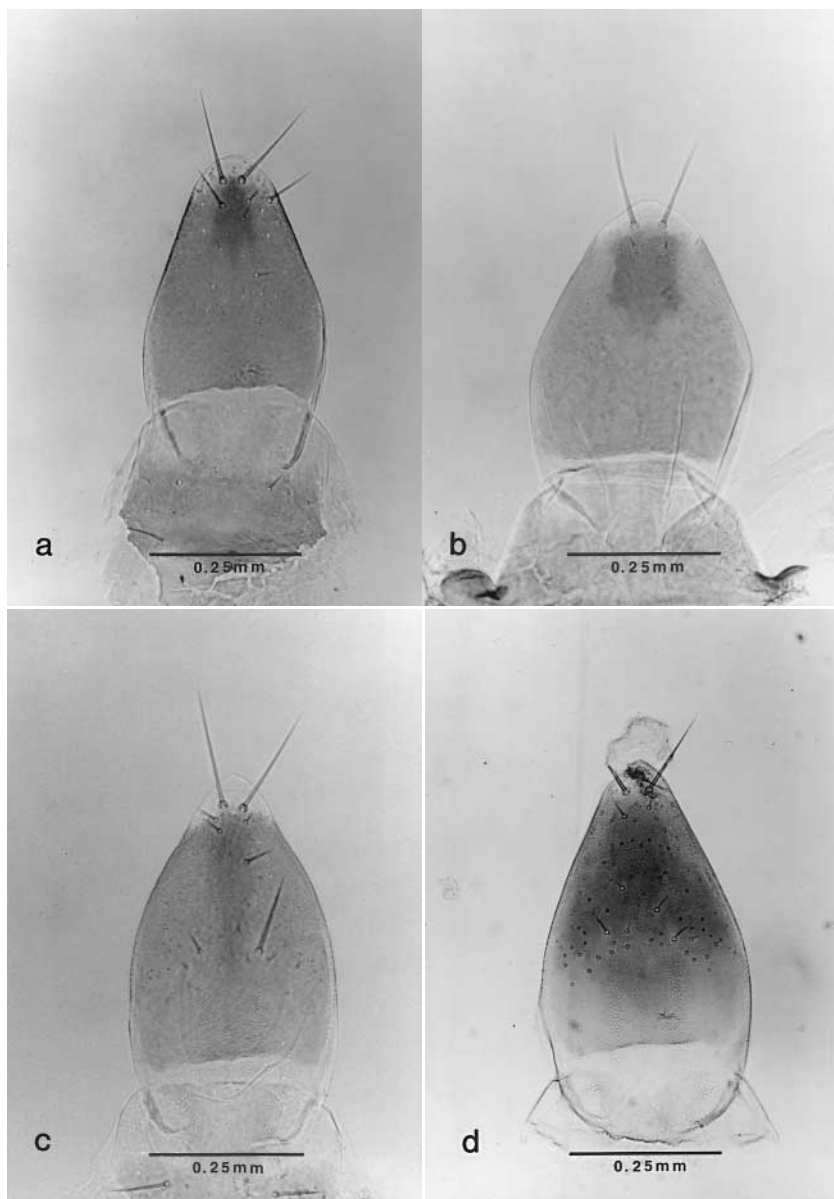


Fig. 4. Light microphotographs of microscope slide-mounted *Reticulitermes* spp. soldier labra: a) *R. hageni*, b) *R. virginicus*, c) *R. flavipes*, d) *Reticulitermes* sp., 100x.

asymmetrical and the characters described above are often more apparent on one side. A mounted labrum usually lies slightly to one side amplifying its apparent asymmetry. The tip of the labrum is subject to wear and tear and therefore sometimes dam-

TABLE 3. NUMBER OF SLIDE MOUNTS NEEDED FOR POSITIVE IDENTIFICATION.

	No. Colonies	No. (cumm.%) of Colonies Identified From:			Tentative ID
		1 Mount	2 Mounts	3 Mounts	
<i>R. flavipes</i>	31	18 (58)	11 (94)	2 (100)	-
<i>R. virginicus</i>	26	20 (76)	5 (96)		1
<i>R. hageni</i>	9	9 (100)	-	-	-
Total	66	47 (71)	16 (95)	2 (98)	1

aged. Aberrant individuals can occur in a colony but most conform to the basic characteristics described.

When examining whole soldiers in alcohol using a stereo microscope, it may be difficult to see the labral characteristics, but these helpful rules generally apply: the posterior sides of the labrum of *R. flavipes* are never angular, and the labral tip of *R. virginicus* is never an acute point; the labral tip is always narrow for *R. hageni* and always broad for *R. virginicus*; and the length:broadest width ratio is > 1.2 for *R. hageni* and < 1.2 for *R. virginicus*. It is imperative to position the whole soldier such that the viewing angle is perpendicular to the labrum. Freely detached labra in alcohol viewed at 50x reveal the shape distinctly enough to make an identification most of the time. The shape is usually more evident if the removed labrum is viewed ventral side up. Table 3 shows the frequency of positive identifications of the slide mounted labra for the San Felasco collection. Correct identification frequencies were found not to differ among other *Reticulitermes* collections examined.

Our standard procedure is to make a microscope slide mount of the labrum and the pronotum from two soldiers, and from a third soldier if no confident identification was possible with two (see Table 3). The pronotal width is an easily obtained, very useful reference that has been widely used. Pronotal width reflects the size of soldiers in a colony, and therefore is a strong suggestion of species (Tables 1 and 2). Based on our Florida *Reticulitermes* collections, it is always possible to find an unusually small individual in a colony, but an upper size limit for the species appears to be the norm. Allowing for a slight margin of error, a pronotal width measurement (whole soldier, $n < 5$) larger than 0.90 mm denotes *R. flavipes*, less than 0.70 mm *R. hageni*, and the range of 0.71 - 0.80 mm *R. virginicus*. A threshold at 0.85 mm ($n > 5$) separates *R. flavipes* from *R. virginicus*.

During the study, a single colony with intermediate-sized soldiers which could not be identified was collected from a very dry, 4 ft tall standing stem of *Pinus palustris* Mill. The mandibles were identical to those of *R. virginicus* and *R. flavipes*, but the shape of the labrum was similar to that of *R. hageni* (Fig. 3d & 4d). These characters were consistent among all soldiers.

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