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Multivariate tools for distinguishing of cryptic species of mites of the genus *Sancassania* (Acari: Acaridae)

Deutonymph (A) and female (B) of *Sancassania salasi* sp. n. associated with beetle *Xylorycles lobicollis* (Scarabaeidae) in Costa Rica. Sibling species, *S. ochoai* sp. n., was collected from *Passalus spiniger* (Passalidae) in Costa Rica

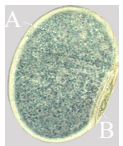
Context: The influence of habitat on morphology is particularly important for mites that are associated with different hosts and feeding substrates. The cosmopolitan genus *Sancassania* is among of the most biologically diverse groups of mites. Its host associations include Coleoptera (mainly Scarabaeoidea), Hymenoptera, Myriapoda, and Crustacea. Species associated with synanthropic habitats are of agricultural, medical and veterinary importance. Despite differences in biology, the genus is morphologically conservative but notorious for its intraspecific variability.

As a result of the lack of quantitative evaluation of characters and small sample sizes, prior *Sancassania* taxonomists have used characters that are likely to be influenced by environmental variation: 75 species have been described but 79% of them are known from the original descriptions only.

To quantify the degree to which morphological characters could discriminate between species in relation to factors that influence their morphology in a laboratory and field, we conducted multivariate analyses of variance of two sibling species, *Sancassania ochoai* sp. n. and *S. salasi* sp. n. Traditional comparison or univariate analyses could not correctly differentiate the two species.

1. CROSSING EXPERIMENTS

Reciprocal crossing experiments demonstrated a **postzygotic reproductive isolation** between the two species. Although mating occurred, the hybrid zygotes died in early developmental stages before birth. At this stage, an egg still contains many yolk spherules (**A**) and blastoderm cells (**B**) on the surface.



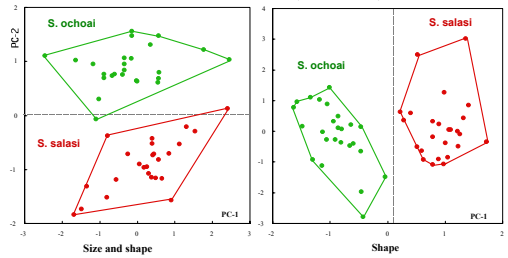
2. DNA SEQUENCE

28S nuclear rDNA gene sequence revealed **1.4% difference** in the genes (793 bp each) of the two species.

3. MULTIVARIATE ANALYSES

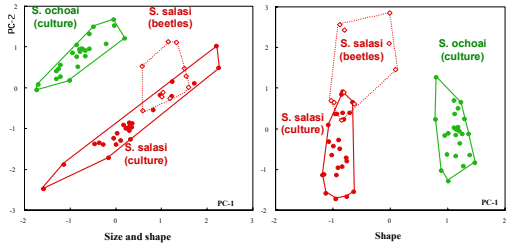
Using principal component analysis, the variance of each character has been partitioned into shape (Darroch & Mosimann, 1985) and size components. The size component should be influenced mostly by environmentally dependent variance. An accounting for the overall size differences (approximately 50%), resulted in the complete separation of the two species:

Females (n=50, 61 variables)



Deutonymphs of *S. salasi* sp. n. reared in culture showed significant shape differences from those taken directly from the host. This indicates the presence of a non-genetic component in the shape variation. In other words, cultured specimens differed in shape from their wild type, causing a **problem with correlation of specimens from culture and nature:**

Deutonymphs (n=60, 101 variables)



Discriminant function analyses were done to identify characters that are influenced the interspecific shape variation. The characters that provided the largest contribution to species discrimination were used in the formal description of the two species.

4. INTRASPECIFIC SHAPE CHANGES

A separate discriminant analyses was done to identify characters that influenced the intraspecific shape variation in deutonymphs of *S. salasi* sp. n. The strongest contributions to the first discriminant function were provided by length of gnathosoma, diameter of posterior conoids (ps_1) of attachment organ, length of *d* IV, and others. Use of these characters in taxonomic distinction is inappropriate, at least for this data set.

Gnathosoma of *S. salasi* from culture (**A-C**) and from beetle host (**D-F**). The variable length of gnathosoma was proven to be the most unreliable character for identification of this species.

Mating *S. ochoai* (left) x *S. salasi* (right)

Results

- **The use of a multivariate morphometric approach based on Darroch & Mosimann (1985) shape variables allowed for the objective quantitative assessment of characters and for development of a clear definition of the boundaries between two sibling species of the genus *Sancassania*.**
- **The species were demonstrated to be reproductively incompatible (postzygotic isolation) and have 1.4% difference in 28S nuclear rDNA.**
- **Deutonymphs of *S. salasi* sp. n. from culture and host show significant shape differences - a potential problem for correlation of specimens of other *Sancassania* species from culture and nature.**

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