

Morphometrics of a neotropical sandfly subspecies, *Lutzomyia carrerai thula*

Jean-Pierre Dujardin^{a*}, François Le Pont^b

^a UMR Orstom-CNRS 9926, Montpellier, 911, avenue Agropolis, 34000 Montpellier, France

^b Orstom La Paz, CP 9214, La Paz, Bolivia

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Abstract – In spite of obvious and frequently observed morphological variation in geographic populations of sand flies, taxonomists are reluctant to create subspecies. In doing so they avoid confusion caused by the accumulation of new taxa, but they also obscure natural processes, some of them related to probable species emergence. We investigated here how far the partitioning of size, shape and form could be a relevant technique for exploring early evolutionary processes. We used a typical case of species, subspecies and cryptic species among three related taxa: *Lutzomyia carrerai*, its subspecies *L. carrerai thula* and *L. yucumensis*, a cryptic species of *L. carrerai*. Univariate and multivariate metric analyses showed global size differences, even between sympatric taxa (*L. carrerai* and *L. yucumensis*). When allometric effects were discounted, the subspecies *L. c. thula* still disclosed important differences from its putative type species, more pronounced than those existing between the two cryptic species, *L. yucumensis* and *L. carrerai*. A similar result was obtained after removing isometric size. These analyses question the validity of *L. carrerai thula* as a subspecies, and show that morphometrics may be a useful tool for separating simple geographic variation from evolutionary divergence. © 2000 Académie des sciences/Éditions scientifiques et médicales Elsevier SAS

Lutzomyia / *L. carrerai thula* / *L. carrerai* / *L. yucumensis* / Diptera / Psychodidae / size / form / shape / subspecies / cryptic species / Bolivia / Ecuador

Résumé – Morphométrie d'une sous-espèce de phlébotome du Nouveau-Monde, *L. carrerai thula*. Malgré l'existence de variations intraspécifiques de la morphologie des phlébotomes, les systématiciens sont réticents à créer des sous-espèces. Ils évitent avec raison l'accumulation de nouveaux taxons, mais peuvent ipso facto occulter des processus naturels dont certains par exemple liés à la formation d'espèces. Nous voulons montrer ici l'intérêt de la morphométrie traditionnelle pour mettre en évidence de tels processus. Nous présentons un cas typique d'espèces affines appartenant au sous-genre *Psychodopygus* et considérées comme sous-espèce (*L. carrerai thula*), espèce princeps (*L. carrerai*) et espèce cryptique (*L. yucumensis*). Ces taxons, dont deux sympatriques (*L. carrerai* et *L. yucumensis*), présentent entre eux des différences globales de taille, mais ces dernières n'expliquent pas leurs différences de forme. Chez les femelles, l'analyse révèle que la sous-espèce *L. c. thula* n'est une extension allométrique ni de l'espèce princeps (*L. carrerai*) ni de *L. yucumensis*. Chez les mâles, où les données ne se prêtent pas à une analyse semblable, on montre que les différences de conformation sont plus accentuées que celles qui apparaissent entre les deux espèces jumelles (*L. yucumensis* et *L. carrerai*). Cette analyse remet donc en question le statut de

* Correspondence and reprints: dujardin@mail.megalink.com

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L. c. thula. Elle confirme l'intérêt de la morphométrie pour distinguer dans la variation géographique les changements morphologiques attribuables aux variations de taille [environnement], de ceux qui pourraient être associés à l'émergence d'espèces. © 2000 Académie des sciences/Éditions scientifiques et médicales Elsevier SAS

Lutzomyia / *L. carrerai thula* / *L. carrerai* / *L. yucumensis* / Diptera / Psychodidae / taille / forme / conformation / sous-espèce / espèce jumelle

Version abrégée

1. Introduction

Lutzomyia carrerai thula est une sous-espèce de *L. carrerai*, désignée ainsi parce que seules des différences de taille étaient apparentes entre deux populations allopatriques. Si tel était effectivement le cas, les différences de conformation qu'on pourrait éventuellement constater seraient entièrement attribuables à des phénomènes d'allométrie. Par ailleurs, *L. carrerai* et *L. yucumensis* sont deux espèces cryptiques vivant en sympatrie, comme l'ont démontré des analyses génétiques antérieures. Pour deux espèces différentes, même si elles sont de taille similaire, on s'attend à rencontrer des variations morphologiques indépendantes de l'allométrie. En réunissant ces trois taxons dans une même analyse, nous avons tenté ici de vérifier sur des bases morphométriques la validité de la sous-espèce *L. c. thula*, l'une des cinq sous-espèces décrites sur les 450 taxons de Phlebotominae du Nouveau Monde.

2. Matériels et méthodes

Trente femelles de chaque taxon, vingt-trois mâles de *L. carrerai*, vingt-trois mâles de *L. c. thula* et dix-neuf mâles de *L. yucumensis* (soit un total de 155 insectes adultes) ont fait l'objet d'une analyse morphométrique traditionnelle portant sur six caractères : cinq de la tête et un caractère de l'aile. L'exploitation statistique de ces données a fait appel aux concepts de « conformation » et de « forme ». Les différences de conformation se réfèrent aux variations métriques qui demeurent après l'élimination des différences de taille isométrique. La conformation peut résulter en tout ou partie d'effets allométriques: une vérification a posteriori est réalisée par des analyses de régression des variables de conformation sur la variable de taille. Les différences de forme résultent à la fois de différences de taille et de conformation: c'est « ce qui reste » quand on a éliminé les allométries.

2.1. Forme

Pour détecter des différences métriques non attribuables à l'allométrie, nous avons appliqué la méthode des

composantes principales communes dérivée de celle de Burnaby (1966). Nous avons d'abord recherché l'existence d'un axe allométrique commun. Seules les données femelles ont pu se prêter à ce modèle. La projection orthogonale des variables sur cet axe commun a permis de générer ensuite des variables indépendantes des changements allométriques, c'est-à-dire des variables de forme pure. Ces dernières ont alors été utilisées dans une analyse discriminante classique.

2.2. Conformation

Chez les mâles, pour les données utilisées, les axes d'allométries des différents groupes n'ont pas montré de parallélisme ($p = 0.01$), de sorte que le modèle d'un axe allométrique commun n'a pas été applicable. Par ailleurs, l'inégalité des matrices de variance-covariance nous a empêché d'utiliser la matrice consensus. Nous avons donc choisi une approche indirecte. La taille a été éliminée par le biais de ratios logarithmiques de conformation (*log-shape ratios*). Les variables de conformation ainsi obtenues ne sont pas nécessairement dépourvues d'influences allométriques. Elles ont été utilisées dans une analyse discriminante et, dans une dernière étape, on a vérifié a posteriori à quel point la discrimination était encore influencée par les différences de taille.

3. Résultats

L'élimination des différences allométriques chez les femelles n'a pas annulé les différences métriques existant entre les trois taxons. En particulier la distinction entre *L. c. thula* et les deux autres espèces reste très nette, plus accentuée que celle observée entre les deux espèces cryptiques (*L. carrerai* et *L. yucumensis*). Les différences de conformation séparent également les mâles des trois taxons, à nouveau de façon plus nette pour *L. c. thula*. Toutefois, ces différences ne sont pas totalement indépendantes de l'allométrie, qui contribue pour 47 % aux variations de la première fonction discriminante, et pour 13 % à celles de la seconde.

4. Discussions et conclusions

Malgré la sympatrie et la variation des tailles, les deux espèces cryptiques *L. carrerai* et *L. yucumensis*

présentent des différences non attribuables à la simple allométrie. Cela est complètement vérifié pour les femelles, partiellement pour les mâles, et correspond à ce que l'on attend des différences métriques séparant des espèces différentes. En revanche, les résultats concernant *L. c. thula* ne sont pas compatibles avec l'idée d'une population géographique de *L. carrerai*-

qui serait simplement plus grande. Des différences de forme pure existent, qui sont importantes, vérifiées chez les femelles, et partiellement chez les mâles. Sur la base d'une étude morphométrique qui prend en compte le phénomène d'allométrie, il apparaît donc que le statut de sous-espèce attribué à *L. c. thula* ne se justifie pas.

1. Introduction

Out of the 450 taxa of the New World sand flies, five are subspecies, three of them described in one subgenus only (*Psychodopygus*), out of the 26 known subgenera and species groups [1]. The subgenus *Psychodopygus* is composed of 28 species and three subspecies, including *Lutzomyia* (*Psychodopygus*) *carrerai thula* (Young, 1979). This latter has never been compared by genetic techniques with *L. carrerai* (Barretto, 1946), but fits well with the hypothesis of a subspecies: it is allopatric, having a *trans*-Andean distribution (west of the Andes from Ecuador to Central America) versus a *cis*-Andean distribution (Amazon Basin and southwards) for *L. carrerai*. Male specimens are difficult to separate, but female *L. c. thula* are readily recognized from female *L. carrerai* by minor but consistent univariate metric differences [2].

In Bolivia we described a sister species, *L. yucumensis* Le Pont et al., 1986 on the basis of infuscated colour of the mesonotum, and of an isoenzyme analysis detecting diagnostic alleles in close sympatry [3, 4]. These two species presented fewer differences in their general dimensions [5] than those showed by *L. carrerai* and its subspecies, *L. carrerai thula*.

Comparing metric differences excluding allometric trends, we tried to verify whether multivariate morphometrics could accord with such classification.

2. Materials and methods

2.1. Insects

L. carrerai and *L. yucumensis* were collected by human bait catches in primary forest near Yucumo (350 m a.s.l., Beni, Bolivia) in August 1984. *L. c. thula* was collected using a CDC light trap in primary forest near La Tablada (150 m a.s.l., Esmeraldas, Ecuador) in September 1991. We studied a total of 155 specimens: 30 females of each taxon, 19 males of *L. yucumensis*, 23 males of *L. carrerai* and 23 of *L. carrerai thula*. All the insects were mounted on slides in Euparal according to the technique of Abonnenc [6].

2.2. Data collection

Using a camera lucida, we measured the following six, non-redundant features of the wing (WL, wing length) and

the head (AIII, third antennae segment; EP, labrum-epipharynx; ID, inter-ocular distance; P3, third palpomere; HH, head height). Thus, the same set of characters was used in both sexes.

2.3. Univariate analyses

Univariate analyses used analysis of variance and non-parametric Kruskal-Wallis comparisons. Among-group differences were visualized by the 'profile' graphs [7, 8] reporting for each character and group the difference between the within-group and the total mean, divided by the common standard deviation (figure 1).

2.4. Multivariate analyses

In the hypothesis of one group (*L. c. thula*) being a subspecies of another one (*L. carrerai* or *L. yucumensis*) it was expected that allometric changes alone were responsible for possible differences in shape. In each sex separately, we thus tested the data for their compatibility with a common allometric axis, that is with the model of a common principal component [9, 10]. Female samples seemed to fit with such a model ($P = 0.65$). The influence of within-group allometries was then removed by performing an orthogonal projection of the data onto the plane orthogonal to the first common principal component [11]. The resulting allometry-free variables were submitted to a canonical variate analysis (CAV).

In males, for the same set of characters (WL, A3, EP, P3, ID, HH), there was no parallelism of within-group allometric axes so that a common model was rejected ($P = 0.01$). Dispersion matrices were significantly different ($P = 0.000$), so that the pooled within-group principal component analysis could not be used. As an alternative for removing size effects on differences, the isometric size was discounted from the data [12]. This was achieved by subtracting the mean of each row (one row contains the values of six characters for one individual) from the log-data. The resulting 'log-shape ratios' (isometry-free) were submitted to a principal component analysis, and the principal components – except the last one – were used as input to a CAV. In order to estimate the amount of allometric trends explaining shape discrimination, the first and second canonical factors were regressed on the isometric size (i.e. the mean of each row), and the coefficient of determination was computed [13].

We thus performed two different approaches: one, in females, excluding within-group allometric influences,

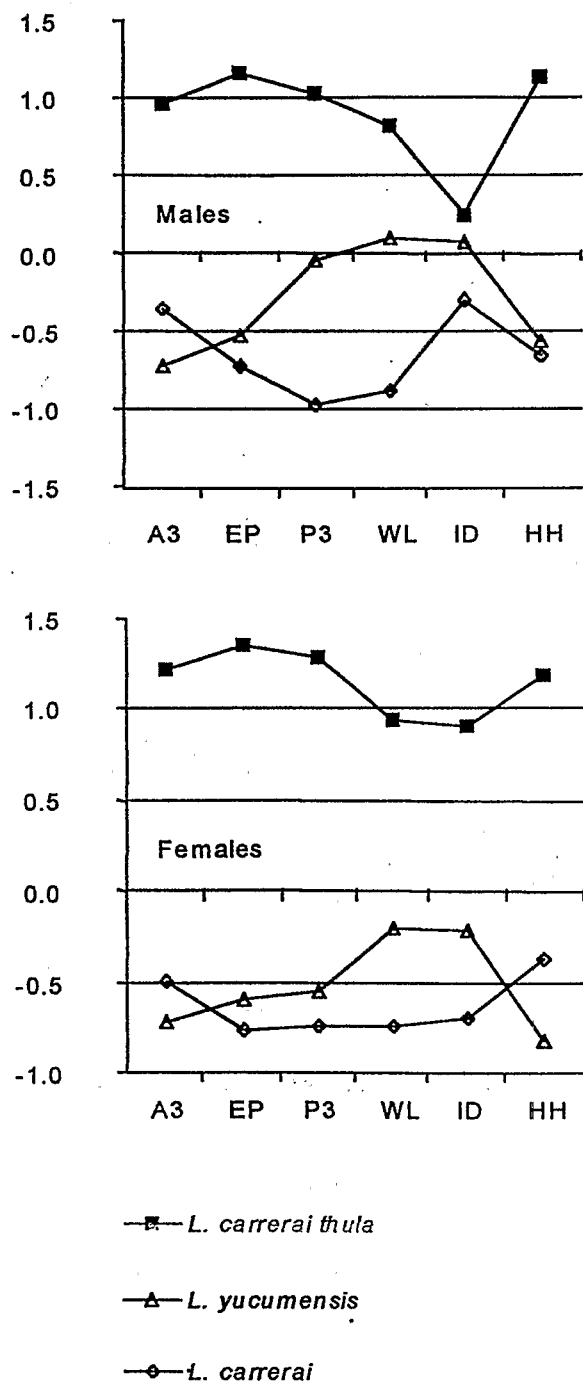


Figure 1. Profiles sensu Guillaumin 1972 [7]. For each variable, the point (black square for *L. c. thula*, white diamond for *L. carrerai* and white triangle for *L. yucumensis*) represents the difference between the within-group and the among-groups means, divided by the common standard deviation. Lines are traced between these points to demonstrate possible trends in relative variation of characters. Unless sampling biases are too important, conspecific populations are expected to present more or less parallel variations, near to the zero level.

and the second one, in males, removing isometric size differences. Results were illustrated by plotting the indi-

viduals on the two canonical variables (CV1 and CV2, figure 2) with polygons enclosing each group ('convex hulls'), as well as by the corresponding discriminant classifications. Statistical significance of multivariate analyses was estimated by the Wilks statistics [14].

For univariate and common principal component analyses, data were log-transformed prior to analyses. Calculations used the NTSYS [15], STATA [16] and JMP [17] packages.

3. Results

3.1. Univariate analyses

In females, and in males except for ID, each variable showed significant differences between *L. carrerai* and its subspecies (*L. carrerai thula*), as well as between this latter and *L. yucumensis*. The sympatric, cryptic species (*L. carrerai* and *L. yucumensis*) showed less frequently significant differences: at WL, ID and HH in females, at P3 and WL in males (table I). The 'profiles' sensu Guillaumin (1972) clearly showed an increased general size for *L. c. thula* (less so for ID in males) and, while not of the same magnitude, size differences between *L. carrerai* and *L. yucumensis*. The pattern of these differences, as designed by the 'profiles', showed opposite trends: some variables showed a relative increase in one group while they decreased in another one (see HH for instance, figure 1).

3.2. Multivariate analyses

Dispersion matrices were significantly different among groups in both sexes ($P = 0.000$ for males and $P = 0.039$ for females, detailed results not shown). The common principal component model, however, does not require homoscedasticity: it was acceptable in females ($P = 0.65$) while not in males ($P = 0.01$), probably as a consequence of ID variation (figure 1).

In both sexes, canonical variate analyses were highly significant and produced an almost perfect reclassification of individuals (table II).

In females, differences were significant after the removal of within-group allometric influences. In males, significant differences were found after removing isometric size. Forty-seven per cent of the variability of the first canonical factor, and 13 % of the second one, were explained by size variation.

Thus, *L. c. thula* showed size, shape and form differences with both its mother species and *L. yucumensis*. These differences were more pronounced than those existing between the sympatric biological species *L. yucumensis* and *L. carrerai*.

4. Discussion and conclusions

The size differences between the sympatric species *L. carrerai* and *L. yucumensis* (except at A3 in males, A3

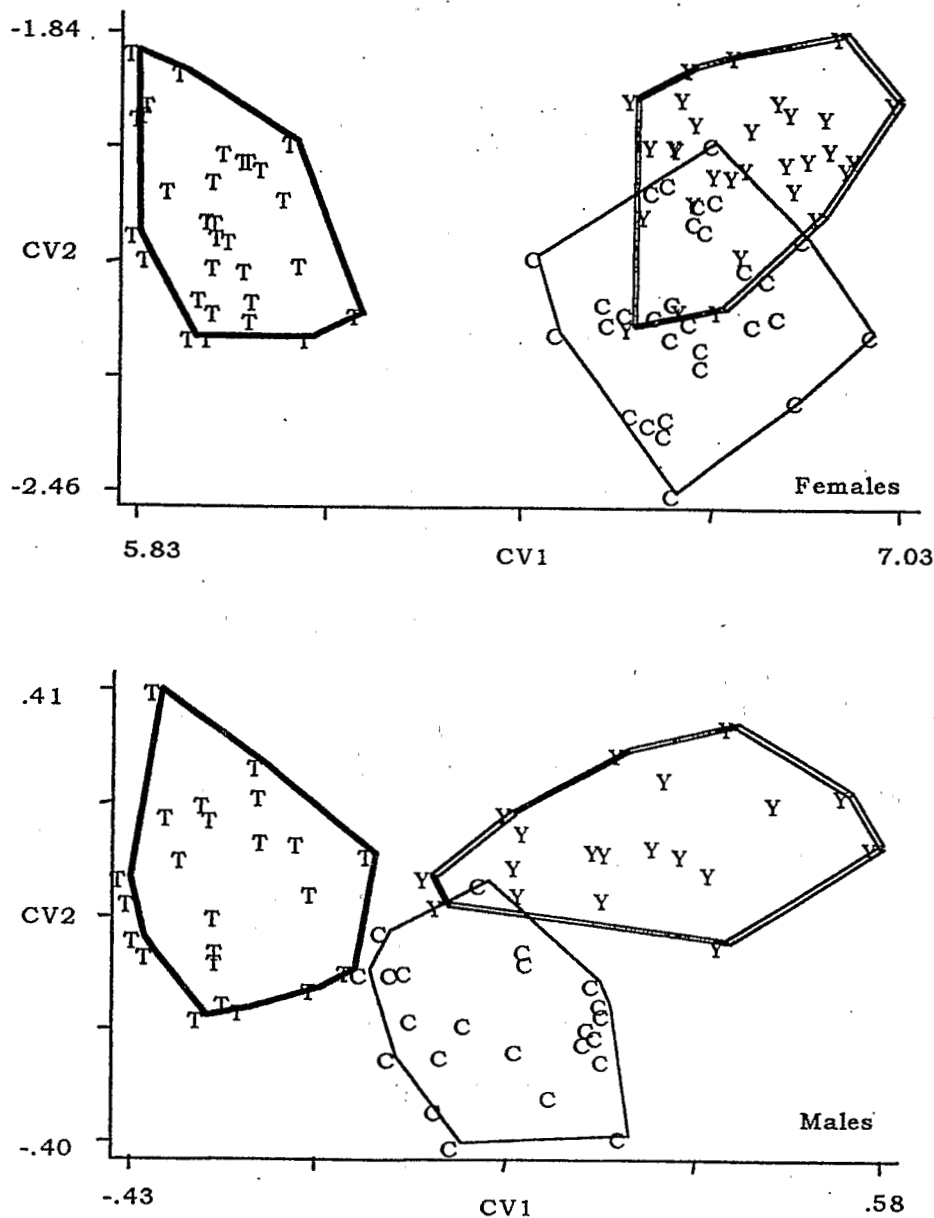


Figure 2. Canonical variate analyses of *L. carrerai*, *L. c. thula* and *L. yucumensis*.

Top: discrimination on allometry-free (form) variable in females. Bottom: log-shape discrimination in male specimens. Polygons enclose each taxon. CV1 and CV2, first and second canonical factors (100 % of contribution to the total heterogeneity).

and HH in females, figure 1) may have taxonomic significance because these taxa are living in close sympatry. Even limited to one character, such circumstance may indicate speciation [18]. Additional evidence here was the significant form (females) and shape (males) differences (figure 2). For these two taxa, species status had been confirmed by genetic studies [3, 4].

The present study focused on the taxonomic status of a subspecies, *L. c. thula*. This population had been considered as a subspecies because it was thought that only size differences existed with the mother species (*L. carrerai*). Such differences between allopatric populations may result

indeed from environmental variation, especially when altitudes – actually, temperatures [19] – are different, as stated by the Bergmann's rule [20]. However, *L. carrerai* and its subspecies live at the same altitudes in similar subtropical climates, so that their size differences have no obvious environmental explanations and could thus question their common taxonomic status [21].

Removal of size as a differentiation factor was intended to reveal patterns of variation that would be less influenced by environmental factors [22], hence more related to evolutionary differences [23]. In females, the existence of significant differences after discounting the within-group

Table I. Univariate comparisons*.

	<i>L. carrerai</i>	<i>L. carrerai</i>	<i>L. yucumensis</i>	Bartlett
	<i>L. c. thula</i>	<i>L. yucumensis</i>	<i>L. c. thula</i>	
AIII	0.000/0.000	0.216/0.252	0.000/0.000	0.344/0.000
EP	0.000/0.000	0.053/0.489	0.000/0.000	0.526/0.008
P3	0.000/0.000	0.189/0.000	0.000/0.000	0.444/0.278
WL	0.000/0.000	0.018/0.000	0.000/0.006	0.366/0.002
ID	0.000/0.173	0.049/0.478	0.000/0.842	0.717/0.756
HH	0.000/0.000	0.005/0.860	0.000/0.000	0.324/0.113

* Scheffe multiple-comparison tests after ANOVA. For each variable and comparison, *P* values are reported for females and males, in this order. Bartlett's test for equal variances are reported in last column. In the cases where variances were not equal (AIII, EP and WL in males), a Kruskal-Wallis equality of populations rank test was applied.

Table II. Canonical variate analysis*.

	PW	Observed agreement (%)	Expected agreement (%)	Kappa	Pr
Females	0.644	88	33.33	0.82	0.000 0
Males	0.010	94	33.79	0.91	0.000 0
r ² CV1 and isometric size = 47 %					
r ² CV2 and isometric size = 13 %					

* 'PW' refers to significance of the Wilks statistics for each canonical variate analysis, on isometry-free variables in males and on allometry-free variables in females, of *L. carrerai*, *L. c. thula* and *L. yucumensis*. 'Observed agreement', indicates the proportion of individuals that have been correctly attributed to their respective group by the model; 'expected agreement', indicates the proportion of individuals that could have been correctly classified by chance only. The 'Kappa' statistics measure the agreement between observed and expected classifications. It is scaled to be 0 when the agreement is as expected by chance and 1 when agreement is perfect. For intermediate values, Landis and Koch (1977) suggest the following interpretation: 0.61–0.80, substantial; > 0.80, almost perfect [24]. Pr is the significance of the difference between observed and expected agreement. The coefficient of determination (r²) was computed after linear regression between canonical factors and isometric size. CV1, first canonical factor; CV2, second canonical factor.

allometric trends (figure 2 top) indicated that *L. c. thula* was not simply an allometric extension of *L. carrerai*, as expected for a subspecies, nor was it of *L. yucumensis*. In males, the same statistical procedure was not possible, probably because of the opposite variations at ID (figure 1). The removal of isometric size to obtain shape variables was then intended to tentatively reduce the effects of allometric changes. The canonical factors derived from these shape variables clearly isolated *L. c. thula* (figure 2 bottom). Their regression on size indicated that these differences were partially (47 and 13 %) due to allometry, suggesting between *L. c. thula* and the remaining taxa the existence of size-independent divergence.

Thus, the hypothesis of *L. c. thula* being conspecific with *L. carrerai* was not supported by the study of their

morphometrical variation: important size differences had no obvious environmental cause, and significant multivariate differences could not be explained by size variation. As long as pure shape mutability is expected to have evolutionary significance, we are authorized to think that the morphological differences exhibited by *L. c. thula* fit with a distinct species more than with a simple geographic variation of *L. carrerai*.

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