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### Research Note

## Host-Induced and Geographical Variation in *Levinseniella cruzi* Travassos, 1920 (Digenea: Microphallidae)

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**ABSTRACT:** Morphological variation in *Levinseniella cruzi* (Digenea: Microphallidae) among the hosts from 3 localities, *Rollandia rolland chilensis* (Podicipedidae), *Himantopus melanurus* (Recurvirostridae), and *Vanellus chilensis lampronotus* (Charadriidae), was analyzed through an ANOVA test and with cluster analysis. A great variation in body shape and size of parasites is noted. Male pocket length and number, sucker diameter, pharynx and genital papillae length, and ratio of suckers appear to be the most constant features and, therefore, valuable for systematic purposes. The morphological variation is discussed in relation to host species and geographical distribution. A new host for *L. cruzi* is reported.

**KEY WORDS:** Digenea, Microphallidae, Aquatic birds, host-induced variations.

*Levinseniella cruzi* was previously reported by Martorelli (1988) from the ceca of 2 birds from Buenos Aires Province: the white tufted grebe, *Rollandia rolland chilensis* Lesson, 1828 (Podicipedidae) and the South American stilt, *Himantopus melanurus* Vieillot, 1817 (Recurvirostridae). We analyzed the morphological variation of *L. cruzi* among avian hosts from various geographic localities.

Definitive hosts were collected from 3 localities related with lentic freshwater environments in Buenos Aires Province (Argentina): Chascomús, a typical "pampa lagoon" which drains in Río Salado system (35°36'S, 58°00'W); Mar Chiquita, a large lagoon by the sea in contact with the Atlantic Ocean (37°46'S, 57°27'W) and Los

Talas, artificial and small lagoons related to the Río de La Plata system (34°52'S, 57°00'W).

Six specimens of each species of bird included in this study were examined: *R. r. chilensis* from Los Talas, *R. r. chilensis* from Chascomús, and *H. melanurus* and *Vanellus chilensis lampronotus* Wagler, 1827 (Charadriidae) from Mar Chiquita.

Voucher specimens of this parasite from different hosts and localities were deposited in the Museo de la Plata, La Plata, Buenos Aires, Argentina, Helminth Coll. no. 3303 a, b; 3304 a, b, c; 3305 a, b, and in USNPC 84905-84908.

All the digeneans measured were recovered alive from the bird's cecum, fixed in Bouin Hollande pressured with a cover glass, stained in Langeron alcoholic carmine, dehydrated in ethanol, cleared in creosote, and mounted in natural Canada balsam. All dimensions were given in millimeters. The morphological variation was studied taking into consideration the measurements shown in Table 1.

One-way analysis of variance (ANOVA) and Tukey's multiple range test were used to appraise differences in these morphological dimensions among 3 groups of specimens: 1) parasites from *R. r. chilensis*, 2) parasites from *H. melanurus*, and 3) parasites from *V. ch. lampronotus* (referred to as groups 1, 2, and 3 hereafter).

Moreover, in order to compare the specimens of *L. cruzi* from different hosts and their localities

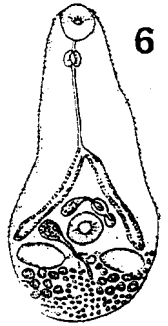
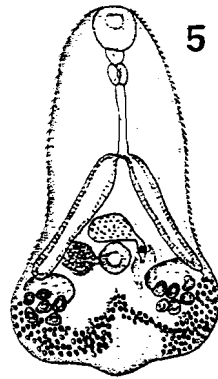
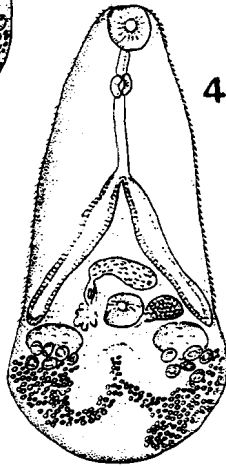
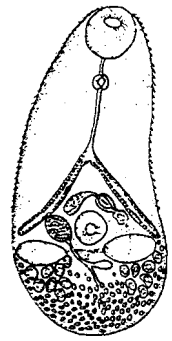
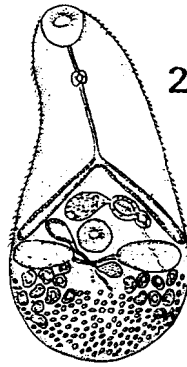
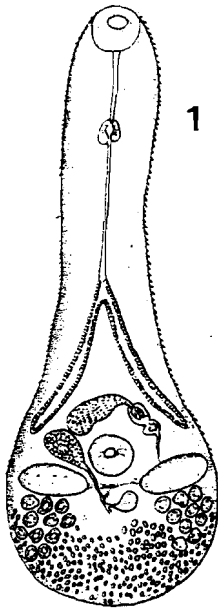
Table 1. Measurements of specimens of *Levinseniella cruzi* from different hosts. All measurements are given in mm.

|                           | Body length        | Body width         | Body length |                           | Oral sucker  | Ventral sucker | Oral sucker    |                        | Pharynx length          | Esophagus length       | Body length |  | Pre-pharynx length | Intestinal ceca length |
|---------------------------|--------------------|--------------------|-------------|---------------------------|--------------|----------------|----------------|------------------------|-------------------------|------------------------|-------------|--|--------------------|------------------------|
|                           |                    |                    | Body width  | Oral sucker               |              |                | Ventral sucker | Forebody               |                         |                        |             |  |                    |                        |
| <i>R. r. chilensis</i>    |                    |                    |             |                           |              |                |                |                        |                         |                        |             |  |                    |                        |
| Mean                      | 0.803              | 0.354              | 2.24        | 0.085                     | 0.066        | 1.292          | 0.041          | 0.094                  | 3.801                   | 0.104                  | 0.241       |  |                    |                        |
| Maximum                   | 1.319              | 0.459              | 2.95        | 0.102                     | 0.079        | 1.593          | 0.048          | 0.199                  | 5.841                   | 0.179                  | 0.329       |  |                    |                        |
| Minimum                   | 0.512              | 0.239              | 1.66        | 0.066                     | 0.059        | 0.881          | 0.031          | 0.028                  | 2.709                   | 0.039                  | 0.168       |  |                    |                        |
| SD                        | 0.245              | 0.069              | 0.36        | 0.011                     | 0.006        | 0.208          | 0.003          | 0.044                  | 1.017                   | 0.034                  | 0.053       |  |                    |                        |
| N                         | 20                 | 20                 | 20          | 19                        | 20           | 19             | 19             | 17                     | 17                      | 16                     | 14          |  |                    |                        |
| <i>H. melanurus</i>       |                    |                    |             |                           |              |                |                |                        |                         |                        |             |  |                    |                        |
| Mean                      | 0.702              | 0.285              | 2.52        | 0.078                     | 0.061        | 1.321          | 0.038          | 0.139                  | 2.719                   | 0.041                  | 0.25        |  |                    |                        |
| Maximum                   | 1.014              | 0.468              | 2.94        | 0.108                     | 0.078        | 1.818          | 0.048          | 0.168                  | 3.346                   | 0.072                  | 0.357       |  |                    |                        |
| Minimum                   | 0.592              | 0.208              | 2.05        | 0.059                     | 0.044        | 1.108          | 0.024          | 0.084                  | 2.141                   | 0.028                  | 0.179       |  |                    |                        |
| SD                        | 0.114              | 0.072              | 0.28        | 0.013                     | 0.011        | 0.179          | 0.007          | 0.029                  | 0.407                   | 0.012                  | 0.058       |  |                    |                        |
| N                         | 14                 | 14                 | 14          | 14                        | 13           | 13             | 14             | 11                     | 11                      | 12                     | 10          |  |                    |                        |
| <i>V. ch. lampronotus</i> |                    |                    |             |                           |              |                |                |                        |                         |                        |             |  |                    |                        |
| Mean                      | 0.902              | 0.429              | 2.12        | 0.088                     | 0.069        | 1.271          | 0.043          | 0.159                  | 3.175                   | 0.041                  | 0.317       |  |                    |                        |
| Maximum                   | 1.329              | 0.539              | 2.81        | 0.109                     | 0.088        | 1.492          | 0.052          | 0.286                  | 4.527                   | 0.072                  | 0.403       |  |                    |                        |
| Minimum                   | 0.649              | 0.304              | 1.51        | 0.064                     | 0.057        | 1.016          | 0.032          | 0.084                  | 2.645                   | 0.019                  | 0.209       |  |                    |                        |
| SD                        | 0.159              | 0.063              | 0.32        | 0.012                     | 0.008        | 0.125          | 0.006          | 0.044                  | 0.461                   | 0.013                  | 0.059       |  |                    |                        |
| N                         | 25                 | 25                 | 25          | 24                        | 24           | 24             | 22             | 22                     | 22                      | 20                     | 20          |  |                    |                        |
|                           | Male pocket number | Male pocket length | Egg length  | Body length<br>Egg length | Ovary length | Right testis   | Left testis    | Seminal vesicle length | Genital papillae length | Genital papillae width |             |  |                    |                        |
| <i>R. r. chilensis</i>    |                    |                    |             |                           |              |                |                |                        |                         |                        |             |  |                    |                        |
| Mean                      | 7.2                | 0.016              | 0.019       | 40.71                     | 0.074        | 0.102          | 0.089          | 0.089                  | 0.023                   | 0.018                  |             |  |                    |                        |
| Maximum                   | 10                 | 0.023              | 0.024       | 73.33                     | 0.091        | 0.114          | 0.104          | 0.108                  | 0.024                   | 0.019                  |             |  |                    |                        |
| Minimum                   | 6                  | 0.011              | 0.018       | 23.33                     | 0.048        | 0.081          | 0.082          | 0.066                  | 0.023                   | 0.016                  |             |  |                    |                        |
| SD                        | 1.4                | 0.003              | 0.001       | 13.89                     | 0.013        | 0.011          | 0.008          | 0.012                  | 0.0005                  | 0.002                  |             |  |                    |                        |
| N                         | 12                 | 9                  | 20          | 20                        | 8            | 6              | 4              | 10                     | 2                       | 2                      |             |  |                    |                        |
| <i>H. melanurus</i>       |                    |                    |             |                           |              |                |                |                        |                         |                        |             |  |                    |                        |
| Mean                      | 6.8                | 0.016              | 0.019       | 35.51                     | 0.077        | 0.061          | 0.054          | 0.086                  | 0.023                   | 0.018                  |             |  |                    |                        |
| Maximum                   | 8                  | 0.016              | 0.021       | 49.78                     | 0.108        | 0.069          | 0.054          | 0.088                  | 0.035                   | 0.019                  |             |  |                    |                        |
| Minimum                   | 6                  | 0.016              | 0.018       | 30.39                     | 0.052        | 0.053          | 0.054          | 0.083                  | 0.012                   | 0.016                  |             |  |                    |                        |
| SD                        | 0.8                | 0                  | 0.001       | 6.005                     | 0.017        | 0.008          | 0              | 0.002                  | 0.009                   | 0.002                  |             |  |                    |                        |
| N                         | 8                  | 1                  | 14          | 14                        | 5            | 2              | 1              | 3                      | 2                       | 2                      |             |  |                    |                        |
| <i>V. ch. lampronotus</i> |                    |                    |             |                           |              |                |                |                        |                         |                        |             |  |                    |                        |
| Mean                      | 7.8                | 0.018              | 0.019       | 47.22                     | 0.081        | 0.107          | 0.102          | 0.141                  | 0.031                   | 0.021                  |             |  |                    |                        |
| Maximum                   | 10                 | 0.035              | 0.024       | 78.24                     | 0.093        | 0.121          | 0.109          | 0.152                  | 0.039                   | 0.024                  |             |  |                    |                        |
| Minimum                   | 6                  | 0.01               | 0.017       | 31.57                     | 0.075        | 0.099          | 0.087          | 0.131                  | 0.026                   | 0.018                  |             |  |                    |                        |
| SD                        | 1.3                | 0.007              | 0.002       | 11.22                     | 0.007        | 0.009          | 0.007          | 0.006                  | 0.004                   | 0.002                  |             |  |                    |                        |
| N                         | 17                 | 13                 | 23          | 23                        | 10           | 8              | 8              | 3                      | 5                       | 7                      |             |  |                    |                        |

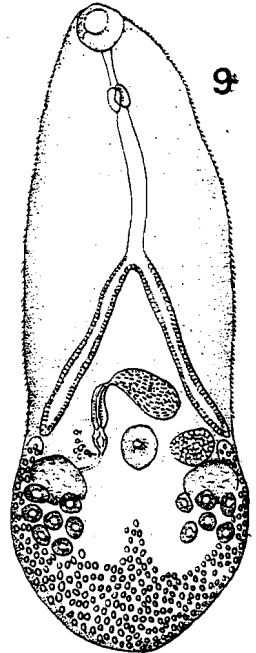
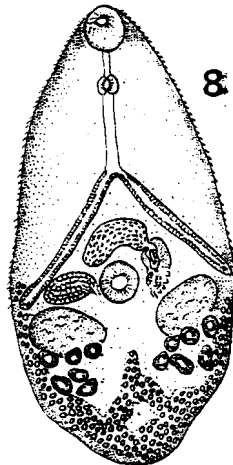
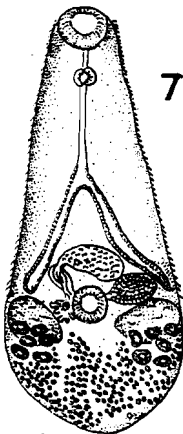
SD = Standard deviation, N = number of parasites.

Figures 1-9. *Levinseniella cruzi*. 1, 2. Specimens of *L. cruzi* from *R. r. chilensis* in Chascomús (ventral view); 3. Specimens of *L. cruzi* from *R. r. chilensis* in Los Talas (ventral view). 4-6. Specimens of *L. cruzi* from *H. melanurus* in Mar Chiquita (Fig. 4: dorsal view; Figs. 5 and 6: ventral view). 7-9: Specimens of *L. cruzi* from *V. ch. lampronotus* in Mar Chiquita (Figs. 7 and 9: dorsal view, Fig. 8: ventral view).

Figure 10. Similarity dendrogram of specimens of *L. cruzi* from different hosts and localities. 1 = parasites from *R. r. chilensis* in Chascomús, 2 = parasites from *R. r. chilensis* in Los Talas, 3 = parasites from *H. melanurus* in Mar Chiquita, 4 = parasites from *V. ch. lampronotus* in Mar Chiquita.



200  $\mu$ m



CCC: 0.86

-0.4      0.0      0.4      0.8      1.2

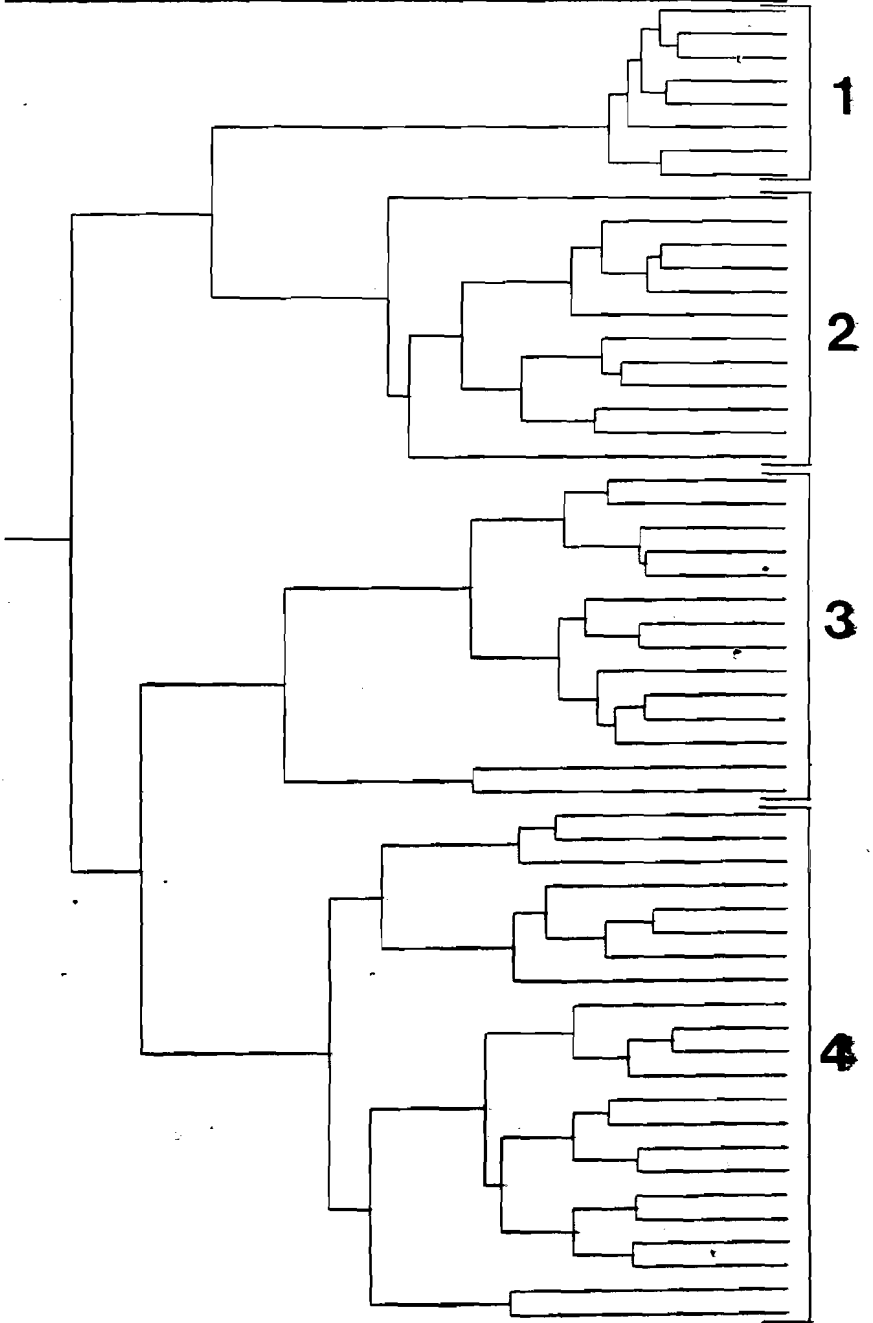


Table 2. Summary of one-way analysis of variance (ANOVA).

| Source of variation     | df | MS                   | F ratio | Significance level |
|-------------------------|----|----------------------|---------|--------------------|
| Body length (Bl)        | 56 | 0.0359               | 5.149   | 0.008              |
| Body width (Bw)         | 56 | 0.0048               | 19.924  | 0.000              |
| Bl/Bw                   | 56 | 0.113                | 6.311   | 0.003              |
| Oral sucker (Os)        | 54 | $1.4 \times 10^{-4}$ | 2.96    | 0.063              |
| Ventral sucker (Vs)     | 54 | $6.8 \times 10^{-3}$ | 4.765   | 0.012              |
| Os/Vs                   | 53 | 0.0305               | 0.350   | 0.706              |
| Prepharynx length       | 45 | 0.0005               | 40.40   | 0.000              |
| Pharynx length          | 52 | $3.7 \times 10^{-3}$ | 2.615   | 0.082              |
| Esophagus length        | 47 | 0.0018               | 11.08   | 0.000              |
| Intestinal caeca length | 41 | 0.0034               | 8.43    | 0.000              |
| Right testis diameter   | 13 | 0.0082               | 3.999   | 0.044              |
| Left testis diameter    | 11 | $6.4 \times 10^{-3}$ | 29.22   | 0.000              |
| Ovary diameter          | 20 | $1.7 \times 10^{-4}$ | 0.487   | 0.621              |
| Seminal vesicle length  | 13 | $1.2 \times 10^{-4}$ | 25.63   | 0.000              |
| Genital papillae length | 6  | $6.2 \times 10^{-3}$ | 1.112   | 0.388              |
| Genital papillae width  | 6  | $5.8 \times 10^{-4}$ | 3.797   | 0.099              |
| Male pockets number     | 34 | 1.6351               | 1.677   | 0.202              |
| Male pockets length     | 20 | $4.1 \times 10^{-3}$ | 0.355   | 0.705              |
| Bl/Forebody length      | 47 | 0.5153               | 8.094   | 0.001              |
| Egg length (El)         | 54 | $2.1 \times 10^{-4}$ | 1.120   | 0.333              |
| Bl/El                   | 54 | 134.39               | 4.654   | 0.013              |

df = degrees of freedom, MS = mean square.

taking into consideration all the measurements at the same time (Table 1), a cluster analysis was applied. In order to obtain a matrix with a low number of missing data, measurements such as testes and ovary diameter, male pockets, genital papillae, and seminal vesicle length were excluded from the analysis because such characters could not be measured in specimens where the eggs overlapped them. The product-moment corre-

lation coefficient ( $r$ ) was applied and the resulting dendrogram was constructed using the UPGMA method. The distortion caused by this method was measured by calculating the cophenetic correlation coefficient (CCC) (Rohlf, 1970).

We found *L. cruzi* in the cecum of the southern lapwing *V. ch. lampronotus* from Mar Chiquita as well as in *R. r. chilensis* and *H. melanurus* as previously reported by Martorelli (1988). In Figures 1-9, specimens of *L. cruzi* in different hosts are shown at the same magnification.

A summary of the ANOVA for the 3 groups is presented in Table 2. In Table 3, the contrasts among the groups of parasites for mean measurements are shown.

As noted in Figure 10, the results of the cluster analysis of *L. cruzi* specimens show the 2 largest clusters separated at a low value of correlation ( $r = -0.27$ ). One of them contains specimens from *H. melanurus* and *V. ch. lampronotus* from Mar Chiquita, and the other specimens from *R. r. chilensis* from Chacomús and Los Talas. This result might suggest the presence of geographical variation, as Kennedy (1980a) found for *Hematoloechus* sp.

The former cluster is divided into 2 groups ( $r = -0.12$ ): 1 contains specimens from *H. melanurus* and the other those from *V. ch. lampron-*

Table 3. Significant differences between groups (Tukey's multiple range test).

| Source of variation     | <i>R. r. chilensis</i> -<br><i>H. mel-anurus</i> | <i>R. r. chilensis</i> -<br><i>V. ch. lampronotus</i> | <i>H. melanurus</i> -<br><i>V. ch. lampronotus</i> |
|-------------------------|--|---|--|
| Body length (Bl)        |  |   | *  |
| Body width (Bw)         | *  | *   | *  |
| Bl/Bw                   |  |   | *  |
| Ventral sucker          |  |   | *  |
| Prepharynx length       | *  | *   | *  |
| Esophagus length        | *  | *   | *  |
| Intestinal caeca length |  | *   | *  |
| Right testis diameter   | *  | *   | *  |
| Left testis diameter    | *  | *   | *  |
| Seminal vesicle length  |  | *   | *  |
| Bl/Forebody length      | *  | *   | *  |
| Bl/Egg length           |  |   | *  |

otus, which indicates host-induced variation (Blankespoor, 1974; Kennedy, 1980b).

The latter cluster is formed on one hand by specimens from *R. r. chilensis* in Chascomús and on the other by specimens from this host in Los Talas ( $r = 0.02$ ).

The great variation in body size and shape of specimens in *L. cruzi* was one of the most notable features. Parasites from *R. r. chilensis* are usually pear-shaped with the forebody clearly prolonged in some specimens. Those from *H. melanurus* have a pear-shaped body but are smaller in size. Pear-shaped, oval, and tongue-shaped specimens could be seen parasitizing *V. ch. lampronotus* (Figs. 1-9).

The most important taxonomic features of *L. cruzi* include terminal genitalia and the position of vitelline glands (Deblock, 1971). We suggest that male pocket length and number, oral sucker and ovary diameter, pharynx and genital papillae length, and the oral sucker/ventral sucker ratio are less subject to variation and, therefore, valuable for systematic purposes.

Finally, the report of a new definitive host for *L. cruzi* confirms the low specificity for this group of parasites. As far as we know, *L. cruzi* has been reported from a mammalian host, *Scapteromys aquaticus* (Cricetidae), by Sutton and Lunaschi (1994), and avian hosts of 4 different families: *Annas bahamensis* (Anatidae) by Travassos (1920), *R. r. chilensis* (Podicipedidae) and *H. melanurus* (Recurvirostridae) by Martorelli (1988),

and *V. ch. lampronotus* (Charadriidae) in this study.

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