Morphology of the shell of *Happiella* cf. *insularis* (Gastropoda: Heterobranchia: Systrophiidae) from three forest areas on Ilha Grande, Southeast Brazil

Amilcar Brum Barbosa[1] & Sonia Barbosa dos Santos[1,2]

[1] *Laboratório de Malacologia Límnica e Terrestre, Departamento de Zoologia, Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro. Rua São Francisco Xavier 524, PHLC sala 525-2, 20550-900 Rio de Janeiro, RJ, Brazil.*

[2] *Corresponding author. E-mail: milkabrum@yahoo.com.br*

ABSTRACT. We conducted a study on shell morphology variation among three populations of *Happiella* cf. *insularis* (Boëttger, 1889) inhabiting different areas (Jararaca, Caxadaço, and Parnaioca trails) at Vila Dois Rios, Ilha Grande, Angra dos Reis, state of Rio de Janeiro, Brazil. Linear and angular measurements, shell indices representing shell shape, and whorl counts were obtained from images drawn using a stereomicroscope coupled with a camera lucida. The statistical analysis based on ANOVA (followed by Bonferroni's test), Pearson's correlation matrix, and discriminant analysis enabled discrimination among the populations studied. The variable that most contributed to discriminate among groups was shell height. Mean shell height was greatest for specimens collected from Jararaca, probably reflecting the better conservation status of that area. Good conservation is associated with enhanced shell growth. Mean measurements were smallest for specimens from Parnaioca, the most disturbed area surveyed. Mean aperture height was smallest for specimens from Parnaioca, which may represent a strategy to prevent excessive water loss. Discriminant analysis revealed that the snails from Jararaca differ the most from snails collected in the two other areas, reflecting the different conservation status of these areas: shells reach larger sizes in the localities where the humidity is higher. The similarities in shell morphology were greater between areas that are more similar environmentally (Caxadaço and Parnaioca), suggesting that conchological differences may correspond to adaptations to the environment.

KEY WORDS. Conchology; discriminant analysis; ecology; morphometry; threatened biome.

Land snails are exceptionally diverse in morphology, for instance they display great polymorphism in shell color and variations in shell dimensions. For this reason, they are a good subject for evolutionary biology studies (CLARKE *et al.* 1978). Differences in size, morphology and growth rates are associated with ecological conditions, natural selection, and phylogenetic history (VERMEIJ 1971, CLARKE *et al.* 1978, EMBERTON 1994, 1995b, COOK 1997, PARMAKELIS *et al.* 2003, TESHIMA *et al.* 2003). According to GOULD (1984), the low mobility of land snails influences character variability. The literature shows that habitat alterations, which result in fragmentation, are an important factor affecting shell morphological differentiation (COOK 1997, GOODFRIEND 1986, EMBERTON 1982, 1994), which can be accelerated in degraded environments (CHIBA 2004, CHIBA & DAVISON 2007).

Ilha Grande, a continental island in the southern portion of the state of Rio de Janeiro, harbors large, continuous and conserved fragments of Atlantic Forest (ROCHA *et al.* 2006), which is among the most threatened biomes in the world (MYERS *et al.* 2000). Over 50% of Ilha Grande is covered by ombrophilous dense forest, now at different levels of regeneration (ALHO *et al.* 2002, OLIVEIRA 2002, ALVES *et al.* 2005, CALLADO *et al.* 2009) from disturbances caused by a range of human activities over the past five decades, being now a natural laboratory to study shell morphological differentiation induced by in environment conditions.

The focus of this study was to investigate variations in the morphology of the shell of *Happiella* cf. *insularis* in three different environments (Table I). This species was described by BOËTTGER (1889) based on a single shell collected from the type locality, Ilha das Flores, São Gonçalo city, Rio de Janeiro, where additional specimens have not been found (SANTOS *et al.* 2010). BOËTTGER's (1889) description, which was not accompanied by illustrations, highlighted the following diagnostic features: maximum diameter with 5.25 mm, shell height 2 mm, large umbilicus, one-fourth the size of the shell base; shell pebble-shaped, thin, white, polished, spire apex slightly prominent, with ½ whorls, slightly convex; borders distinct, mildly striated, last border over the third, approximately as wide as shell, less arched at top than bottom, angled below central region;

suture deeply impressed. Aperture elliptical-lunular, with small slit, aperture height with 2 mm, aperture width with 2.25 mm, simple peristomatic edge, with curved, spherical, sub-angular syphunculus [*sic*] protruding to right side of base.

THIELE (1927), in addition to the type locality of *H.* cf. *insularis*, also listed it in Piracicaba (state of São Paulo), Blumenau (state of Santa Catarina) and Porto Alegre (state of Rio Grande do Sul); MORRETES (1949) also listed it only in Ilha das Flores and SIMONE (2007) to Xanxerê and São Carlos (state of Santa Catarina).

In the present study, we analyzed the shell morphology of three populations of *H.* cf. *insularis* subjected to different environmental conditions, with the goal to assess variability in shell morphology, as detailed morphology and range of variation can prove useful for refining species diagnoses.

MATERIAL AND METHODS

The specimens used in this study were collected from three areas, known as the Jararaca, Caxadaço, and Parnaioca trails, located in Vila Dois Rios, on the ocean side of Ilha Grande, Municipality of Angra dos Reis, southern region of the state of Rio de Janeiro (23°04′25″ to 23°13′10″S, 44°05′35″ to 44°22′50″W). In each collecting site (Fig. 1), a distinct level of forest regeneration (VERA-Y-CONDE & ROCHA 2006) can be found, making them suitable for investigations on the influence of environmental factors on shell morphology.

Table I contains a summary of the environmental parameters measured at the three areas studied.

We selected intact shells from 102 adults, grown to approximately three whorls and proportionally similar to each other. Thirty-three shells from the Jararaca Trail were selected, in addition to 34 and 35 shells from the Caxadaço and Parnaioca trails, respectively.

Material examined. *Happiella* cf. *insularis*. BRAZIL, *Rio de Janeiro*: Angra dos Reis, Ilha Grande, Vila Dois Rios, Trilha da Jararaca, 14.VI.1998, S.B. Santos *leg.* (Col. Mol. UERJ 942-1); 27.IX.1996, S.B. Santos *leg.* (Col. Mol. UERJ 977); 11.I.1996, V.C. Queiroz *leg.* (Col. Mol. UERJ 980-4 and 5); 12.I.1996, S.B. Santos *leg.* (Col. Mol. UERJ 990-1 and 2); 21.III.1997, S.B. Santos *leg.* (Col. Mol. UERJ 1132); 23.III.1997, S.B. Santos *leg.* (Col. Mol. UERJ 1133-1 and 2); 20.IX.1997, S.B. Santos *leg.* (Col. Mol.

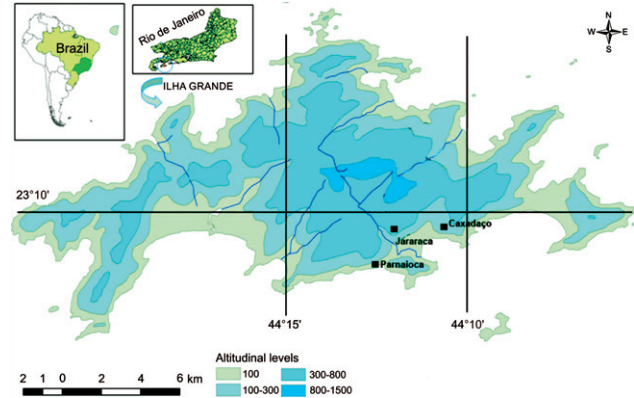
Figure 1. Location of Ilha Grande, in Angra dos Reis Municipality, state of Rio de Janeiro, Brazil, showing the Jararaca, Parnaioca, and Caxadaço trails. Map: Luiz E.M. Lacerda.

UERJ 1155); 30.XI.1997, D.P. Monteiro *leg.* (Col. Mol. UERJ 1168-1 and 2); *ditto*, 26.VI.1999, S.B. Santos *leg.* (Col. Mol. UERJ 1241-1 and 2); 21.III.1997, S.B. Santos *leg.* (Col. Mol. UERJ 1252-2); 14.I.1998, D.P. Monteiro *leg.* (Col. Mol. UERJ 1617-2); 17.II.1998, A.S. Alencar *leg.* (Col. Mol. UERJ 1618-2); 17.II.1998, D.P. Monteiro *leg.* (Col. Mol. UERJ 1646); 15.I.1998, S.B. Santos *leg.* (Col. Mol. UERJ 1647-2); 17.II.1998, M.A. Fernandez *leg.* (Col. Mol. UERJ 1650); 14.I.1998, A.S. Alencar *leg.* (Col. Mol. UERJ 1651); 14.I.1998, D.P. Monteiro *leg.* (Col. Mol. UERJ 1653-2); 17.I.1998, S.B. Santos *leg.* (Col. Mol. UERJ 1656-2 and 3); 17.II.1998, D.P. Monteiro *leg.* (Col. Mol. UERJ 1658-2, 3, 4 and 6); 17.II.1998, S.B. Santos *leg.* (Col. Mol. UERJ 1659-1, 2, and 3). Vila Dois Rios, Trilha do Caxadaço, 19.X.1995, V.C. Queiroz *leg.* (Col. Mol. UERJ 999-2, 3, 4, 5, and 6); 30.V.1997, S.B. Santos *leg.* (Col. Mol. UERJ 1064-7 and 3); 28.XI.1997, D.P. Monteiro *leg.* (Col. Mol. UERJ 1110-1, 2, 3, 4, and 5); 15.VIII.1996, S.B. Santos *leg.* (Col. Mol. UERJ 1114); 19.X.1995, V.C. Queiroz *leg.* (Col. Mol. UERJ 1144-1, 2, 3, and 4); 08.VIII.1999, M. Sttorti *leg.* (Col. Mol. UERJ 1310); 21.X.2000, D.P. Monteiro *leg.* (Col. Mol. UERJ 2061-1, 2, and 3); 15.III.2001, S.B. Santos *leg.* (Col. Mol. UERJ 2156-2, 3, 4, 5, and 6); 28.X.2001, C.C. Siqueira *leg.* (Col. Mol. UERJ 2225-2, 3, 4, 5, and 6); 2.VIII.2005, A.B Barbosa, Lacerda, L.E.M., T.A. Viana *leg.* (Col. Mol. UERJ 7445-1, 2, and 3). Vila Dois Rios, Trilha da Parnaioca), 28.V.1997, N. Salgado

Table I. Summary of local environmental parameters from three areas (Jararaca, Caxadaço and Parnaioca trails) at Ilha Grande.

| Area	Mean ambient temperature (a) (°C)	Mean ground temperature (a) (°C)	Mean leaf litter depth layer (a) (cm)	Mean relative air humidity (a) (%)	Canopy height (m)	Canopy closure	Elevation	Degree of impact	Time of regeneration
Jararaca Trail	22.46 ± 3.42	20.81 ± 3.42	7.17 ± 2.68	84.81 ± 9.27	33 (c)	Greater	250 m asl	Advanced stage of ecological succession	At least 90 years-old (e)
Caxadaço Trail	29.95 ± 2.09	21.01 ± 2.15	4.11 ± 1.64	83.82 ± 8.98	15-20 (b)	Intermediate	180 m asl	Early stage of ecological succession	At least 50 years-old (c)
Parnaioca Trail	25.29 ± 3.12	22.04 ± 2.16	5.87 ± 2.66	80.93 ± 10.51	15 (c)	Lower	At sea level	Early stage of ecological succession	5 to 25 years-old (c)

a) D.P. Monteiro (unpubl. data), b) ALHO *et al.* (2002), c) VERA-Y-CONDE & ROCHA (2006), d) CALLADO *et al.* (2009), e) SLUYS *et al.* (2012).

leg. (Col. Mol. UERJ 1129-1); 13.VIII.1996, S.B. Santos *leg.* (Col. Mol. UERJ 1139); 13.VIII.1996, S.B. Santos *leg.* (Col. Mol. UERJ 1177-1); 13.VIII.1996, S.B. Santos *leg.* (Col. Mol. UERJ 1175); 13.VIII.1996, S.B. Santos *leg.* (Col. Mol. UERJ 1178-1 and 2); 16.VI.2002, S.B. Santos *leg.* (Col. Mol. UERJ 1827-2); 02.II.2002, D.P. Monteiro *leg.* (Col. Mol. UERJ 2989-1); 01.II.2000, D.P. Monteiro *leg.* (Col. Mol. UERJ 3005-1, 2, and 3); 31.I.2000, D.P. Monteiro *leg.* (Col. Mol. UERJ 3006-1, 2, 3, 4, 5, 6, 7, 8, 10, and 11); 31.I.2000, S.B. Santos *leg.* (Col. Mol. UERJ 3007-1, 2, 3, 4, 5, 6, 7, and 8); 31.I.2000, D.P. Monteiro *leg.* (Col. Mol. UERJ 3288-2); 28.I.2000, P. Coelho *leg.* (Col. Mol. UERJ 3289); 3.VIII.2005, A.B. Barbosa, Lacerda, L.E.M., T.A. Viana *leg.* (Col. Mol. UERJ 7444-1, 2, and 3).

Drawings of the shells in apical, umbilical, and lateral views were made with the aid of a camera lucida under an Olympus SZH10 stereomicroscope. The drawings were used to obtain angular and linear measurements, establish the number of whorls, and calculate the ratios between measurements, according to the criteria proposed by DIVER (1931), PARODIZ (1951), SOLEM & CLIMO (1985) and FONSECA & THOMÉ (1994). The following angular measurements were considered: maximum angle (MA), columellar angle (CA), sutural angle (SA), lower sutural angle (SS'), and spire angle (SPA) (Figs 2 and 3). The linear measurements taken were: shell height (h), aperture height (ah), aperture width (aw), spire height (sh) (Fig. 2), first whorl diameter (1wd), maximum diameter (D), smaller diameter (d), first whorl width (1ww), and second whorl width (2ww) (Fig. 4), diameter umbilical (ud) (Fig. 5). The following ratios were calculated: shell height/maximum diameter (h/D), maximum diameter/umbilical diameter (D/ud), umbilical diameter/shell height (ud/h), aperture height/aperture width (ah/aw), aperture height/smaller diameter (ah/d), first whorl diameter/maximum diameter (1wd/D), maximum diameter/total number of whorls (D/NW), and first whorl width/second whorl width (1ww/2ww) (SOLEM & CLIMO 1985, FONSECA & THOMÉ 1994, EMBERTON 1995a). The method proposed by DIVER (1931) was applied to obtain the number of protoconch whorls (pW), total number of whorls (NW), and total number of teleoconch whorls (TW) (Fig. 4).

The analysis of shell morphological variation followed VALORVITA & VÄISÄNEN (1986), with some modifications. Descriptive statistics were performed for each variable in each group, and normality was tested using the skewness test. In cases when a given variable had asymmetrical distribution, the following transformation procedures were applied to normalize it as appropriate: e-base logarithm of X (Neperian logarithm) [1wd/D, NW], square root of X [D/ud], sin X [MA, ah], cos X [SA, 1ww/2ww], tan X [ah/aw, ah/d], and reciprocal of X (i.e., 1/X) [1ww, 2ww, h/D, D/NW] (KREBS 1998, ZAR 1999), where X is the variable considered.

After normalization, each variable was standardized by reduction (SPIEGEL 1993) and compared using analysis of variance (ANOVA) followed by the Bonferroni's test. Differences
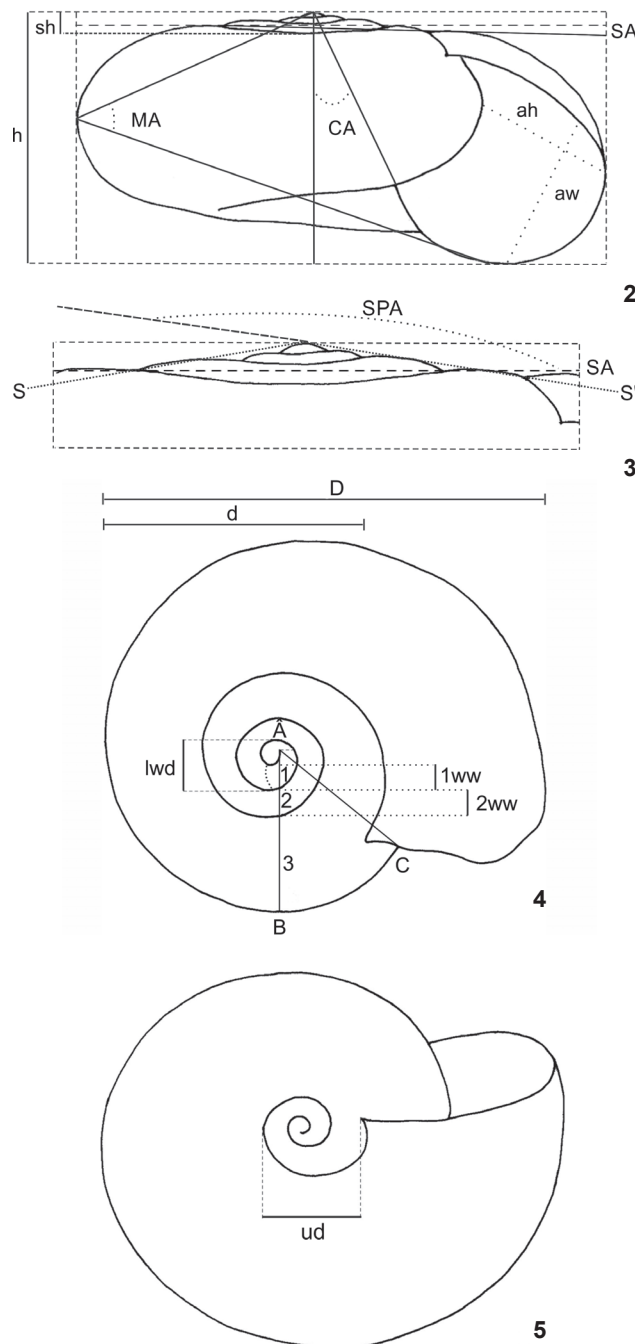
Figures 2-5. *Happiella* cf. *insularis*. Schematic depiction of shell, with angular, linear measurements, and number of whole whorls: (2) CA, columellar angle; MA, maximum angle; ah, aperture height; SA, sutural angle; sh, spire height; aw, aperture width. (3) SPA, spire angle; SA, sutural angle; SS', lower sutural angle. (4) D = maximum diameter; d, smaller diameter; 1ww, first whorl width; 2ww, second whorl width; 1wd, first whorl diameter; 1, 2, 3, number of whole whorls. (5) umbilical view: ud, umbilical diameter.

at p < 0.05 were considered statistically significant. The third decimal place was dropped and differences at p ≤ 0.05 were considered statistically significant.

After the exclusion of the highly correlated variables (KLECK 1982, ENGELMAN 1997), discriminant analysis was performed on 15 variables: MA, CA, SA, SS, SPA, 1wd,h, ah,aw,sh, 1ww, 2ww, pW, NW, and TW. Preliminary Pearson's correlation matrix revealed a high correlation (r ≥ 0.90) between the variables D, d, ud, and ah. These were removed from the analysis, and the variable h, representing all correlated variables excluded, was kept. Upon analysis, the variables MA, SA, sh, and 1ww were also removed, owing to their low contribution to group discrimination, as shown by the discriminant function coefficients. Statistical analyses were performed with the aid of the SYSTAT 7.0 statistical package (ENGELMAN 1997).

RESULTS

Shell morphology

Happiella cf. *insularis* (Fig. 6) has thin, translucent, depressed, shiny shells; periostracum color varies from yellow amber (when alive) to whitish yellow (when fixed in alcohol or in cases when only the shell is found *in situ*); spire slightly elevated (BOËTTGER 1889) (Fig. 9); under the optical microscope, the texture is smooth, with mildly marked growth lines (BOËTTGER 1889) (Fig. 7); under scanning microscopy, protoconch, more granular, and teleoconch, of rougher aspect (Fig. 10), umbilicus opened (Fig. 8) (BOËTTGER 1889); aperture wide, crescent-shaped, slightly oblique; peristome simple (BOËTTGER 1889), thin sharp edges, without teeth (Fig. 9) (THIELE 1931, ZILCH 1959, MONTEIRO & SANTOS 2001); suture not impressed (Fig. 7) (R.L. RAMÍREZ unpubl.

Figures 6-10. *Happiella* cf. *insularis*. (6) habitus; (7-10) specimen Col. Mol. UERJ 1653-2: (7) apical view; (8) umbilical view; (9) apertural view; (10) scanning electron microscopy of view's apical shell. Scale bar: 6-9 = 1 mm, 10 = 100 µm. Photos: 6 = Antônio C. de Freitas, 7-9 = Amilcar B. Barbosa, 10 = Alan C.N. de Moraes, LABMEL/UERJ.

data); body whorl rounded (Fig. 10) (R.L. Ramírez unpubl. data); rapid increment's shell growth (Fig. 7) (Emberton 1995a).

Shell morphometry

Table II shows the morphometric and meristic data of the 102 shells examined. The mean values of these features were lowest in specimens from the Parnaioca Trail.

The results of the ANOVA, distinguished among the three samples collected from Jararaca, Caxadaço, and Parnaioca, revealed significant differences in all linear and angular measure-

ments, except for the mean maximum angle. Specimens from the Jararaca and Parnaioca trails differed significantly in mean columellar and mean spire angles, but the differences in these measurements between samples from Jararaca and Caxadaço and from Parnaioca and Caxadaço were not statistically significant. The shells from Jararaca differed from those from the Caxadaço and Parnaioca trails in the mean sutural and lower sutural angles; shells from Caxadaço and Parnaioca, however, were statistically similar with regards to these two variables. The Bonferroni's test revealed differences between samples from Parnaioca and

Table II. Descriptive statistics of morphometric and meristic variables and ratios for *Happiella* cf. *insularis* collected from three areas on Ilha Grande. Linear measurements (cm): (D) maximum diameter, (d) smaller diameter, (ud) umbilical diameter, (1wd) first whorl diameter, (ah) aperture height, (sh) spire height, (h) shell height, (aw) aperture width, (1ww) first whorl width, (2ww) second whorl width. Angular measurements (degrees): (CA) columellar angle, (MA) maximum angle, (SPA) spire angle, (SA) sutural angle, (SS') lower sutural angle. Ratios: (D/ud) maximum diameter/umbilical diameter, (D/NW) maximum diameter/total number of whorls, (ud/h) maximum diameter/umbilical diameter, (1wd/D) first whorl diameter/maximum diameter, (h/D) shell height/maximum diameter, (aw/d) aperture height/smaller diameter, (ah/aw) aperture height/aperture width. Number of whorls: (pW) number of protoconch whorls, (TW) number of teleoconch whorls, (NW) total number of whorls. (N) sample size, (SD) standard deviation, (VAR) variance.

	Jararaca (N = 33)					Caxadaço (N = 34)					Parnaioca (N = 35)				
	Min.	Mean	Max.	SD	VAR	Min.	Mean	Max.	SD	VAR	Min.	Mean	Max.	SD	VAR
Linear measurements															
D	0.500	0.739	0.875	0.090	0.008	0.371	0.591	0.806	0.169	0.028	0.319	0.476	0.833	0.125	0.015
d	0.325	0.494	0.606	0.071	0.005	0.227	0.405	0.575	0.110	0.012	0.221	0.331	0.558	0.079	0.006
ud	0.090	0.161	0.206	0.030	0.000	0.059	0.124	0.241	0.054	0.002	0.044	0.089	0.193	0.037	0.001
1wd	0.038	0.068	0.086	0.011	0.000	0.031	0.056	0.080	0.012	0.000	0.030	0.056	0.083	0.012	0.000
sh	0.011	0.019	0.033	0.004	0.000	0.015	0.021	0.027	0.003	0.000	0.010	0.019	0.027	0.004	0.000
h	0.245	0.355	0.413	0.037	0.001	0.189	0.284	0.400	0.076	0.005	0.157	0.228	0.366	0.056	0.003
ah	0.127	0.191	0.240	0.026	0.000	0.100	0.161	0.225	0.046	0.002	0.082	0.127	0.233	0.031	0.000
aw	0.066	0.179	0.300	0.045	0.002	0.098	0.167	0.253	0.046	0.002	0.072	0.125	0.220	0.036	0.001
1ww	0.025	0.052	0.433	0.068	0.047	0.018	0.032	0.046	0.007	0.000	0.022	0.033	0.052	0.007	0.000
2ww	0.033	0.051	0.104	0.012	0.000	0.029	0.055	0.073	0.078	0.006	0.026	0.043	0.080	0.011	0.000
Angular measurements															
MA	44.0	47.772	55	2.446	5.985	42	46.426	51	2.074	4.305	42	47.200	59	3.595	12.924
CA	15.0	20.803	28	3.107	9.655	10	19.970	29	4.344	18.877	10	17.942	25	4.129	17.055
SA	0.5	1.590	5	0.930	0.866	1	2.500	4	0.904	0.818	1	2.242	5	1.017	1.034
SS'	151.0	161.742	171	5.026	25.267	147	156.264	167	5.029	25.291	147	154.742	166	4.767	22.726
SPA	158.0	168.151	175	3.700	13.757	160	166.588	172	3.210	10.310	160	165.200	172	3.332	11.105
Ratios															
h/D	0.445	0.481	0.533	0.021	0.000	0.428	0.483	0.581	0.029	0.000	0.439	0.480	0.519	0.020	0.000
D/ud	3.718	4.656	5.500	0.418	0.175	3.282	5.075	7.000	0.858	0.737	3.266	5.657	7.900	0.985	0.970
ud/h	0.361	0.450	0.583	0.054	0.003	0.238	0.419	0.658	0.085	0.007	0.276	0.380	0.632	0.079	0.006
ah/aw	0.643	1.129	2.606	0.340	0.115	0.735	0.965	1.234	0.132	0.017	0.650	1.037	1.388	0.165	0.027
ah/d	0.300	0.396	0.612	0.062	0.003	0.346	0.394	0.471	0.030	0.000	0.034	0.380	0.504	0.071	0.005
1wd/D	0.057	0.094	0.172	0.020	0.000	0.063	0.100	0.152	0.025	0.000	0.069	0.122	0.219	0.035	0.001
D/NW	0.115	0.192	0.224	0.020	0.000	0.103	0.163	0.573	0.081	0.006	0.098	0.128	0.215	0.029	0.000
1ww/2ww	0.521	0.826	1.200	0.154	0.023	0.066	0.758	1.000	0.178	0.031	0.519	0.781	1.000	0.104	0.010
Number of whorls															
NW	2.909	3.823	4.413	0.275	0.076	3.380	3.908	4.472	0.287	0.082	2.908	3.671	4.188	0.322	0.103
TW	1.433	2.538	3.587	0.410	0.168	1.794	2.376	3.166	0.316	0.099	1.720	2.191	2.936	0.334	0.112
pW	0.118	1.284	2.844	0.588	0.239	0.950	1.531	2.105	0.287	0.082	0.955	1.499	2.119	0.307	0.094

Jararaca in nine morphological features, between Jararaca and Caxadaço samples in eight features, and between samples from Caxadaço and Parnaioca in seven features – i.e., samples from Caxadaço and Parnaioca were less dissimilar to each other than to the sample from Jararaca (Table III).

ANOVA revealed significant differences in the D/ud, ud/h, ah/aw, 1wd/D, and D/NW ratios across samples. Bonferroni's test showed Parnaioca and Jararaca samples to differ in four of these ratios (D/ud, ud/h, 1wd/D, and D/NW), Caxadaço and Parnaioca samples to differ in three (D/ud, 1wd/D, and D/NW), and Jararaca and Caxadaço samples to differ on two of these ratios (ah/aw and D/NW) – i.e., differences in measurement

Table III. Results of ANOVA followed by Bonferroni's multiple comparison test, applied to linear and angular measurements, ratios, and number of whorls of *Happiella* cf. *insularis* specimens collected from the Jararaca (Jar), Caxadaço (Cax), and Parnaioca (Par) trails, Ilha Grande. Differences were considered statistically significant* at $p \leqslant 0.05$. For abreviations see Table II.

	p	Jar x Cax	Jar x Par	Cax x Par
Linear measurements				
D	0.000*	0.000*	0.000*	0.001*
d	0.000*	0.000*	0.000*	0.002*
ud	0.000*	0.002*	0.000*	0.002*
1wd	0.000*	0.000*	0.000*	1.000
sh	0.036*	0.099	1.000	0.061*
h	0.000*	0.000*	0.000*	0.000*
ah	0.000*	0.002*	0.000*	0.000*
aw	0.000*	0.753	0.000*	0.000*
1ww	0.000*	0.000*	0.001*	1.000
2ww	0.008*	0.033*	0.012*	1.000
Angular measurements				
MA	0.470	1.000	1.000	0.751
CA	0.010*	1.000	0.010*	0.101
SA	0.000*	0.000*	0.002*	0.187
SS'	0.000*	0.000*	0.000*	0.611
SPA	0.003*	0.193	0.002*	0.285
Ratios				
h/D	0.947	1.000	1.000	1.000
D/ud	0.000*	0.106	0.000*	0.012*
ud/h	0.000*	0.212	0.000*	0.082
ah/aw	0.024*	0.020*	0.559	0.427
ah/d	0.490	1.000	0.757	1.000
1wd/D	0.000*	1.000	0.000*	0.004*
D/NW	0.000*	0.000*	0.000*	0.003*
1ww/2ww	0.175	0.238	0.450	1.000
Number of whorls				
NW	0.005*	0.807	0.107	0.004*
TW	0.001*	0.192	0.000*	0.099
pW	0.015*	0.022*	0.056*	0.929

ratios were most pronounced between samples collected from the Jararaca and Parnaioca trails, as were differences in linear measurements (Table III).

The mean total number of whorls (NW) differed significantly between the samples from Caxadaço (greater mean) and Parnaioca (Table III). The mean total number of teleoconch whorls (TW) differed significantly between the Jararaca and Parnaioca samples. The mean number of protoconch whorls (pW) differed significantly not only between samples from Jararaca and Caxadaço, but also between samples from Jararaca and Parnaioca (Table III).

Discriminant analysis

The discriminant analysis (Fig. 11) allowed the distinction of all three samples (Wilks's Lambda = 0.300, F = 6.689, df = 22, p = 0.000), particularly with respect to the sample from Jararaca, which differed the most from the others. The samples from Caxadaço and Parnaioca were more similar to each other than each was to the sample from Jararaca (Fig. 11). This analysis correctly classified 67% of the specimens (Fig. 11), with 34 out of 102 being incorrectly classified.
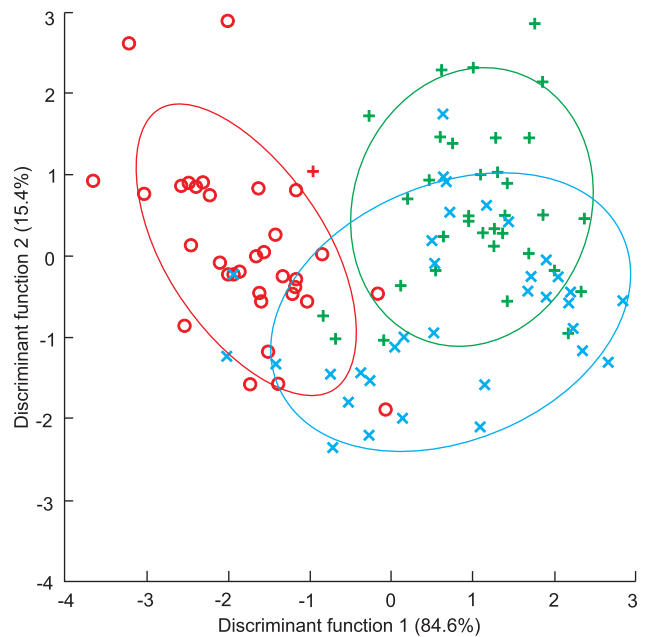

Figure 11. Graphic depiction of the discriminant analysis of morphometric variables of three *Happiella* cf. *insularis* samples collected from the Jararaca (○), Caxadaço (✕), and Parnaioca (✚) trail areas, Ilha Grande, RJ, Brazil.

The proportions of explanation were of 84.6% and 15.4% for the first and second discriminant functions, respectively. The coefficients also revealed the following variables to be major contributors to the degree of differentiation achieved

with the first function: shell height (h), lower sutural angle (SS'), spire angle (SPA), aperture height (ah), number of protoconch whorls (pW) e number of teleoconch whorls (TW).

Discriminant function 1 = -2.263 h – 1.077 SS' + 0,964 SPA + 0.877 ah + 0.736 pW + 0.674 TW – 0.411 2ww + 0.296 aw + 0.294 + 0.113 1wd + 0.038 CA.

Discriminant function 2 = -1.046 NW + 0.992 SS' – 0.941 SPA + 0.900TW – 0.870 ah + 0.799 pW – 0.780 aw + 0.465 h + 0.396 CA + 0.249 1wd + 0.035 2ww.

DISCUSSION

The morphology of the shell of *H.* cf. *insularis* fits the description for Systrophiidae perfectly: shell thin, translucent, polished, generally smooth, spire apex slightly prominent, discoid, simple peristomatic edge. According to BAKER (1925), *Happiella* shells are characterized by a very low spire and an umbilicus normally reduced to a small perforation. In his original description of *H.* cf. *insularis*, BOËTTGER (1889) reported a maximum diameter of 5.25 mm, very close to the mean values found for the Caxadaço and Parnaioca samples, and shell height of 2 mm, very close to the mean obtained for the sample from Parnaioca. Aperture height and aperture width were originally described as measuring 2 mm and 2.25 mm, respectively, with the latter measurement falling within the confidence interval of the sample from Jararaca. The original description of the number of whorls (3.5) is also within the confidence intervals of both Jararaca and Parnaioca samples. According to the original description, the umbilicus size is one-fourth the maximum diameter of the shell, a similar ratio to that found in our specimens.

Our results revealed significant differences among the three populations of *H.* cf. *insularis* examined, which may be explained by differences in the degree of forest conservation in each area surveyed. The original vegetation in the Caxadaço and Parnaioca areas has been disturbed, more dramatically so in the latter, where the vegetation was entirely slashed down in some areas to make way for the now disabled Vila Dois Rios-Jararaca Dam road and for a number of plantations that served the now defunct Cândido Mendes Penal Colony. Along the Caxadaço Trail, inhabited until the construction of the penal colony by local native fishermen, the environmental changes were less pronounced. The Jararaca Trail region, by contrast, is better preserved. It has a relatively undisturbed secondary forest in lower-altitude areas and primary forest in higher areas. This translates into a deeper leaf litter layer, a more closed canopy, and lower temperature (Table I). As in other instances (SHIMEK 1930, BOYCOTT 1934, CAIN 1977, CLARKE *et al*. 1978, TILLIER 1981, EMBERTON 1995b, COOK 1997, WELTER-SCHULTES 2000, TESHIMA *et al*. 2003), better conservation certainly influences the environmental conditions overall, including leaf litter quality, structure, humidity, and depth, which in turn influence mollusk morphology (GOULD 1968, PEAKE 1978, CIPRIANI 2007). Investi-

gating *Ainohelix editha* (Adams, 1868) in Hokkaido, Japan, TESHIMA *et al*. (2003) demonstrated that shell size and growth rates are adaptations to the environmental conditions; CHIBA (2004), investigating the genus *Mandarina* Pilsbry, 1894 on the Bonin Islands, found that differentiation in shell shape and dimensions are accelerated in degraded environments.

In the present study, the smallest mean values were obtained for specimens collected from the Parnaioca Trail area (Table II), the most disturbed of the three areas (shallower leaf litter, more open canopy, higher temperature). The lower capacity of the local leaf litter to retain water is likely responsible for the smaller size of the snails. The smaller mean aperture height of the shell may represent a strategy to prevent excessive water loss (GOODFRIEND 1986). MACHIN (1967), PEAKE (1978), EMBERTON (1982), and GOODFRIEND (1986), along with other investigators, have reported that smaller specimens are found in terrestrial gastropod populations living in dry areas with a strong incidence of sunlight.

The mean shell diameter and number of whorls were greatest in snails collected from Jararaca (Table II), consistent with the hypothesis that higher humidity and lower temperatures promote increased rates of shell growth in terrestrial gastropods (GOULD 1984, GOODFRIEND 1986, EMBERTON 1994). BAUR (1988) concluded that the size of the shell of *Chondrina clienta* (Westerlund, 1883) increases in higher temperatures and lower population densities, as verified by ANDERSON *et al*. (2007) for *Oreohelix cooperi* (Binney, 1838). BAUR (1988) commented that the phenotypic plasticity found in *C. clienta* may be adaptive, as the genetic makeup of snails allows for different shell growth patterns under different environmental conditions.

The populations of the most disturbed areas – the Caxadaço and Parnaioca trails – are more similar to each other than to the population of the Jararaca Trail, which is the best-preserved area (Fig. 11). The greater similarity observed between the Parnaioca and Caxadaço samples can be explained by the intermediate degree of conservation of the Caxadaço region, which is closer to the degree of conservation of the Parnaioca area than to that of the Jararaca region.

The findings of this study corroborate investigations conducted in other countries showing that morphological differentiation is a result of the isolation of populations in areas that are distinct in vegetation cover, dominant plant species, maximum altitude, and soil type – i.e., areas that offer different microhabitats. As CHIBA (2004) pointed out, degraded environments accelerate differentiation by eliciting new ecological interactions and new habitat conditions, thus subjecting species to a number of selective pressures.

We believe that a similar process has occurred in the areas investigated in the present study, where the different degrees of forest degradation, added to different degrees of moisture, contributed to the morphological differentiation of the three studied populations of *H.* cf. *insularis*. However, as Brazilian species of Systrophiidae are not yet well defined, we

have decided not to treat the three populations as separate species, and we recommend that they continue to be identified as *H.* cf. *insularis* until ongoing anatomical studies are concluded, and a decision on their taxonimical status is reached.

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