

The shaping of a species: the Azorian *Drouetia* Gude (Pulmonata: Zonitidae: *Oxychilus*) as a model

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Abstract – The endemic Azorian zonitid subgenus *Drouetia* Gude, 1911 is spread throughout the archipelago and shows a wide range of variability. The spatial distribution of the Azores islands and their geomorphological history provide a privileged research ground to assess the interactions of isolation and time on the emergence of those variability patterns and thus to infer on the speciation process. Special attention is given to volcanic activity as a disturbing factor of the populational integrity.

From the observed patterns of distribution of intra- and interspecific shell morphology and anatomical variability in *Drouetia* the following speciation scenario may be advanced: Stage one: demic (allotopic) variability; it is postulated that volcanic instability (frequent cyclic eruptions) and intense erosion (deep ravines) create temporary isolates that (?200 yrs) later merge into a larger, genetically richer population. Stage two: close syntopic (interspecific) variability; it is postulated that short-term (>2,000 ys) volcanic stability contributes to consummate speciation. Stage three: extreme syntopic (interspecific) variability; it is postulated that long-term volcanic stability (>1 Mys) leads to supra-specific differentiation.

Key words: *Drouetia*, morphology, anatomy, speciation, volcanic activity, Azores.

INTRODUCTION

Speciation is a multifaceted process leading to the separation of a parcel of a previously wider gene pool and culminating in its reproductive isolation (Mayr, 1977; Howard and Berlocher, 1998). From molecules to the individuals that carry them to the populations they live within, variability and selection, isolation and genetic drift, led by the patient hand of time, interact differently at each of these levels to produce the genetic oddities we call species. The road to speciation usually shows different grades of definition of such interaction. Notwithstanding the basic molecular nature of change – the very stuff of evolution – morphology is its readily visible face and may constitute a rough indicator of the speciation event. Consequently, although wanting validation through molecular methods of deeper resolution and being cautious about the plastic/genetic nature of the characters in use, analysis of the patterns of morphology distribution may point first hand to the framework of the process (Jenner, 2004; Wiens, 2004).

Spread throughout the archipelago and apparently free from visible biotic pressure, the endemic zonitids of the subgenus *Drouetia* Gude, 1911 provide a suitable model to study aspects of this process and the Azores constitute an

adequate laboratory to research it (Martins 1993, 1995, 1998, 1999; Riedel, 1997). The geographical position and distribution of the islands, their different ages and the rhythmicity of the volcanic events that originated them provide an adequate scenario in which various stages of speciation can be found, thus allowing for extrapolations about the process.

MATERIALS AND METHODS

Specimens were collected from the islands of Santa Maria, São Miguel, Graciosa and Flores, and 22 stations were selected for this study (Figure 1; Table 1). The shells and reproductive systems were drawn with a camera lucida under a Wild M8 dissecting microscope and the various shell parameters (Figure 2) were quantified from the drawings. At least three specimens in each station were dissected and only a qualitative approach was attempted for the anatomy. The internal morphology of the penial complex was also prepared for SEM (Jeol JSM 5410) with aid of drying using hexamethyldisilane (10 min at each of the following steps: 95% alcohol; absolute alcohol; 1:1 absolute alcohol/acetone; acetone; hexamethyldisilane). Prior to SEM examination, the

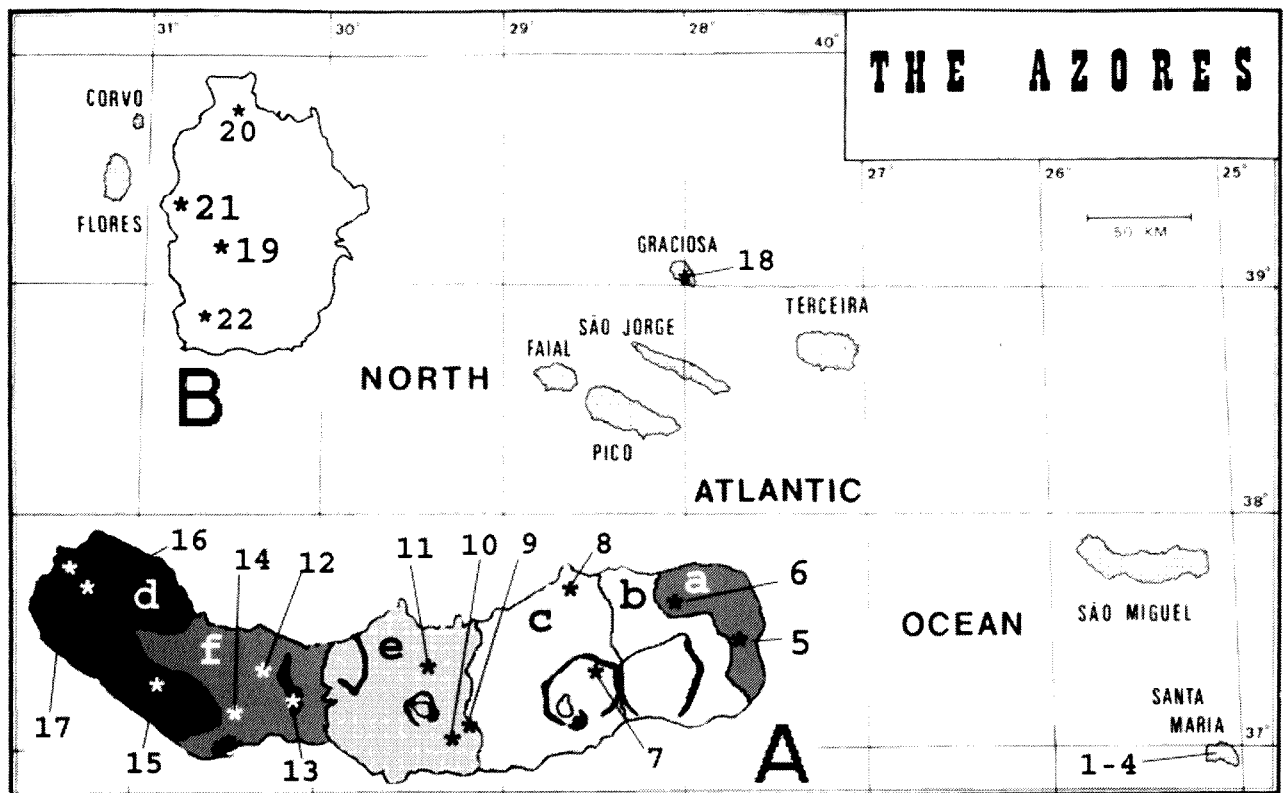


Figure 1 The archipelago of the Azores and the distribution of the collecting stations (* 1-22; see Table 1 for decoding). A. Inset showing São Miguel, representing the location of the stations and the geological history of the island: a, Nordeste; b, Povoação; c, Furnas; d, Sete Cidades; e, Fogo; f, Picos (adapted from Forjaz, 1984 and Nunes *et al.*, 1993). B. Inset showing the location of the stations in Flores. Detail of the location of the stations in Santa Maria was not required for the scope of this study.

Table 1 List of the stations from where the material was collected.

Island/ Station	Observations
Santa Maria	
1	Pico Alto, Santa Maria. 1974; 12-06-1990; 21-10-1993.
2	Terras do Raposo, Santa Maria. 13-06-1990.
3	Santa Bárbara, Santa Maria. 22-10-1993.
4	Panasco, Santa Maria. 16-06-1990.
São Miguel	
5	Ribeira do Tosquiado, S. Miguel. 03-08-1989.
6	Algarvia, S. Miguel. 01-12-1996.
7	Cumieira, Pedras do Galego, Furnas, S. Miguel. 15-03-1989 [7a]; 03-08-1989 [7b].
8	Ribeira dos Carneiros, Ribeira Funda, S. Miguel. 20-03-1989 [8a]; 06-08-1989 [8b].
9	Rosário, Pico do Vento, Vila Franca do Campo, S. Miguel. 23-09-1970.
10	Queimada, Pico do Vento, Água d'Alto, S. Miguel. 21-09-1974.
11	Caldeira Velha, Ribeira Grande, S. Miguel. 06-12-1989.
12	Batalha, S. Vicente Ferreira, S. Miguel. 26-02-2004.
13	Pico do Fogo, Livramento, S. Miguel. 06-03-1984 [13a]; 26-07-1989 [13b].
14	Abelheira, Fajã de Baixo, S. Miguel. 27-02-1988. [14a]; 26-07-1989 [14b].
15	Curral da Achada, Covoada, S. Miguel. 10-04-1988.
16	Túnel, Sete Cidades, São Miguel. 20-06-1989.
17	Ramal dos Mosteiros, S. Miguel. 13-05-1992.
Graciosa	
18	Pedras Brancas, Graciosa. 09-06-1988.
Flores	
19	Laranjeiro, Fajãzinha, Flores. 06-07-1989.
20	Ponta Delgada, Flores. 07-07-1989.
21	Ribeira da Casas, Fajã Grande, Flores. 05-07-1989.
22	Terra Nova, Flores. 05-07-1989.

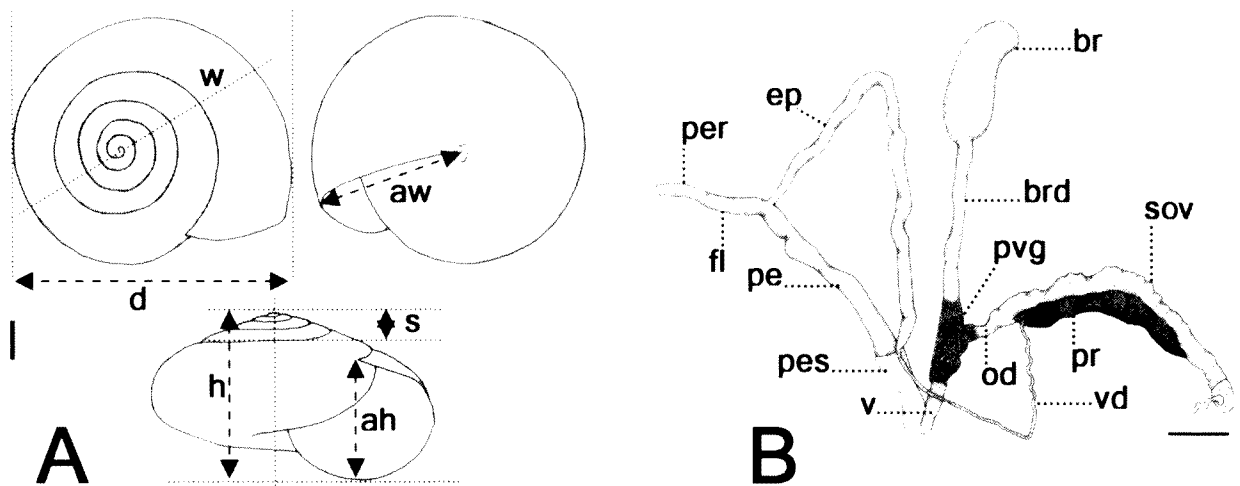


Figure 2 A. Shell of *Oxychilus (Drouetia) atlanticus* showing the morphometric parameters used in the analyses. B. Reproductive system of *Oxychilus (Drouetia) atlanticus* illustrating the terminology used in the text. Scale lines = 1 mm. Abbreviations: ah, height of the aperture; aw, width of the aperture; br, bursa; brd, bursa duct; d, diameter of the shell; ep, epiphallus; fl, flagellum; h, height of the shell; od, oviduct; pe, penis; per, penial retractor muscle; pes, penial sheath; pr, prostate gland; pvg, perivaginal gland; s, spire; sov, spermoviduct; v, vagina; vd, vas deferens; w, number of whorls.

samples were covered with gold/palladium 40/60 in a vacuum evaporator Jeol JEE 400.

Canonical analysis was performed on the shell parameters and dendrograms obtained utilizing cluster analysis based on euclidean distance (UPGMA).

The Azores

The Azores (Figure 1) are subaerial tips of the Mid-Atlantic Ridge and are located about 1,600 km east of continental Europe (Portugal). The archipelago comprises nine volcanic islands spread throughout 600 km along a NW-SE axis, arranged in three groups: Eastern Group (São Miguel and Santa Maria), Central Group (Terceira, Graciosa, São Jorge, Pico and Faial) and Western Group (Flores and Corvo). In this study, only four of the nine islands will be mentioned concerning their volcanic history; their changes after human colonization (about 500 years ago) are assumed to have no bearing in the speciation events here analysed.

Santa Maria (97 km²) is the southernmost and the oldest island (about 8 Mys); subaerial volcanic activity has ceased there since about 3 Mys (Serralheiro and Madeira, 1993). It has fossil beds of marine as well as of terrestrial origin, the latter being very rare.

The island of S. Miguel, eight times larger than Santa Maria and about 60 km north, has a well-studied geo-morphological history (Booth *et al.*, 1977; Moore, 1990; Moore and Rubin, 1991). The eastern tip of the island, Nordeste (a), is now an extinct volcano about 4 Mys old (Nunes *et al.*, 1993; see Figure 1, inset A, for explanation of the lettering

a-f); the complex of Povoação (b), now an extinct and partially eroded caldera, was added to it 2 Mys ago; about 0.75 Mys ago the complex of Furnas (c) was formed. A new island appeared 0.5 Mys ago on what is now the Sete Cidades complex (d). The first island continued to grow westward with the addition of the Fogo complex (e) about 0.25 Mys ago and the two islands were united about 0.05 Mys ago through the Picos region (f). Volcanic activity has been intense in São Miguel; for Furnas alone (c), Queiroz *et al.* (1995) reported 10 eruptions for the period between the years 5000 B.P. and A.D. 1630. On the basis of the events of the last three thousand years, a schedule of cyclic eruptions of the main centers has been proposed by Moore and Rubin (1991): 320 years for Furnas (c), 400 years for Sete Cidades (d), 1,150 years for Fogo (e) and 145 years for Picos region (f). The intensity of the eruptions and the spread of their products have also been recorded. Booth *et al.* (1977) made a quantitative survey of the volcanic activity in São Miguel over the past five thousand years and Walker and Croasdale (1971) presented a detailed history of two main events at the Fogo volcano, 4,600 years B.P. and A.D. 1563, thus allowing for extrapolations on how could it have affected the local biota.

Graciosa, a small (67 km²) and shallow (402 m) island, is probably not older than 600,000 years (Feraud *et al.*, 1980); its volcanic history has been studied by Gaspar (1996). After the highly explosive events of Serra Branca, 350,000 years ago, most of the subsequent volcanism was effusive. Graciosa has had volcanic activity until about 2,000 years ago.

Flores, the westernmost island, is 143 km². Its

Table 2 Basic statistics (N, number of specimens; R, range; SD, standard deviation; X, mean) of shell morphometry of specimens of *Oxychilus (Drouetia)* from São Miguel (Sta 5-17) and from Flores (Sta 19-22) (see also Table 1). Variables: ah, aperture height; aw, aperture width; d, diameter of the shell; h, height of the shell; s, height of the spire; w, number of whorls.

Sta	N	w			d			h			aw			ah			s		
		R	X	SD	R	X	SD	R	X	SD	R	X	SD	R	X	SD	R	X	SD
São Miguel																			
5	16	5.1-5.8	5.5	0.22	6.7-8.1	7.4	0.45	3.7-4.7	4.1	0.35	3.7-4.4	4.1	0.20	2.8-3.5	3.1	0.23	0.4-0.8	0.6	0.11
6	9	5.4-6.1	5.6	0.22	8.1-8.9	8.4	0.29	4.5-5.0	4.7	0.10	4.2-4.9	4.6	0.22	3.1-3.7	3.3	0.19	0.5-0.9	0.7	0.12
7a	5	5.2-5.6	5.4	0.19	6.0-7.0	6.4	0.40	3.4-4.2	3.7	0.30	3.2-3.8	3.4	0.23	2.7-3.1	2.9	0.19	0.4-0.7	0.5	0.12
7b	9	5.2-5.7	5.5	0.17	6.3-9.5	7.6	1.23	3.8-5.2	4.4	0.58	3.4-5.1	4.1	0.67	2.7-3.7	3.2	0.40	0.5-0.8	0.7	0.11
8a	16	5.2-5.9	5.5	0.18	5.7-6.8	6.3	0.27	3.1-3.8	3.5	0.19	3.1-3.9	3.5	0.18	2.3-3.0	2.8	0.16	0.4-0.7	0.5	0.08
8b	9	5.2-5.8	5.6	0.26	6.3-7.8	7.3	0.56	3.3-4.5	4.0	0.39	3.3-4.1	3.8	0.31	2.7-3.2	3.0	0.17	0.4-0.7	0.6	0.11
9	23	5.5-6.0	5.7	0.12	7.4-8.5	8.1	0.29	4.2-5.1	4.7	0.28	3.9-4.6	4.3	0.20	2.9-3.5	3.2	0.14	0.5-1.0	0.8	0.15
10	8	4.7-6.2	5.7	0.48	6.8-10.2	8.7	1.03	3.8-5.7	5.0	0.62	3.9-5.2	4.8	0.43	2.8-3.9	3.4	0.36	0.6-1.0	0.8	0.13
11	11	5.6-6.7	5.9	0.30	7.5-8.7	8.1	0.35	4.2-5.2	4.7	0.33	4.1-4.7	4.4	0.18	2.8-3.4	3.2	0.17	0.6-1.0	0.8	0.15
12	16	5.7-6.3	5.9	0.18	7.0-8.0	7.5	0.25	4.0-4.7	4.3	0.23	3.8-4.4	4.1	0.22	2.7-3.2	2.9	0.16	0.5-0.9	0.7	0.11
13a	16	5.2-5.7	5.5	0.14	5.4-7.2	6.5	0.43	3.2-4.2	3.6	0.27	3.0-4.0	3.5	0.26	2.4-3.0	2.8	0.18	0.3-0.8	0.5	0.13
13b	16	5.5-6.3	5.9	0.21	7.2-9.3	8.2	0.49	4.1-5.4	4.7	0.33	3.8-5.0	4.4	0.28	3.1-3.7	3.4	0.18	0.5-1.0	0.7	0.14
14a	17	5.4-6.3	5.8	0.24	5.7-8.4	7.3	0.88	3.0-4.8	4.0	0.53	3.2-4.6	3.9	0.45	2.3-3.2	2.9	0.26	0.4-1.0	0.7	0.19
14b	17	5.6-6.1	5.8	0.12	7.5-9.2	8.2	0.47	4.2-4.8	4.5	0.25	3.7-4.8	4.4	0.25	2.9-3.7	3.3	0.21	0.5-0.9	0.7	0.13
15	16	5.2-5.8	5.5	0.22	6.5-9.1	7.8	0.82	3.8-5.2	4.5	0.42	3.6-4.8	4.3	0.40	3.0-3.7	3.3	0.22	0.4-1.0	0.7	0.16
16	11	4.5-5.8	5.5	0.37	6.4-8.7	7.6	0.65	3.5-5.1	4.3	0.45	3.7-4.8	4.1	0.32	2.6-3.6	3.2	0.28	0.5-0.8	0.7	0.11
17	18	5.5-6.2	5.8	0.22	8.0-9.4	8.6	0.43	4.5-5.8	5.0	0.32	4.2-5.0	4.6	0.21	3.3-3.9	3.5	0.15	0.6-1.0	0.8	0.12
Flores																			
19	21	5.0-6.3	5.7	0.35	4.9-11.6	9.0	2.22	2.6-6.2	4.6	1.12	2.7-6.5	5.0	1.18	1.9-4.1	3.3	0.74	0.3-0.9	0.6	0.18
20	20	4.7-7.1	6.3	0.61	5.9-8.3	7.4	0.61	3.0-4.5	3.8	0.35	3.0-4.3	3.9	0.33	2.2-2.8	2.6	0.18	0.3-1.0	0.7	0.17
21	13	5.2-5.8	5.5	0.18	7.8-10.1	9.3	0.76	4.1-5.3	4.7	0.37	4.2-5.4	4.9	0.38	3.0-3.8	3.4	0.29	0.5-0.8	0.7	0.11
22	29	4.9-5.9	5.4	0.26	5.8-10.0	7.7	1.23	2.7-5.3	3.9	0.76	3.0-5.3	4.1	0.64	2.1-3.5	2.8	0.48	0.3-0.8	0.5	0.14

oldest rocks are more than 2.5 Mys old and in the island's formation two main complexes can be recognized: the base complex which ended about 0.7 Mys ago, and the upper complex, of which the last eruptive events were date of about 3,000 years ago (Azevedo *et al.*, 1986). Together with tiny Corvo (17 km²), they are the most remote islands of the archipelago.

Drouetia Gude, 1911

Helix atlantica Morelet and Drouët, 1857 was characterized by the absence of umbilical perforation. It was on account of that character that Gude (1911) created the genus *Drouetia* for that

species. Riedel (1964), following Zilch's (1959) tentative proposal, lowered Gude's taxon to the subgeneric level.

In the original description there was no mention to the geographic range of that species nor of its type locality, but Morelet (1860) considered *H. atlantica* as spread throughout most of the islands, being most common in S. Miguel. Acknowledging its variability in shell morphology, he assigned varieties to the populations of Faial and Santa Maria. Riedel (1964), based upon anatomical studies, raised to subspecific rank Morelet's variety *H. a. minor*, from Faial, as well as the large specimens from Santa Maria, which he named

Table 3 Discriminant scores from functions 1 (A) and 2 (B) of canonical analysis of shell morphometry of the populations from São Miguel (stations 5-17). Tukey HSD. Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares. The error term is Mean Square.(Error) = 1,000.

A										
Station	N		1	2	3	4	5	6	7	8
a	8a	16	-2,64394							
ab	13a	16	-2,15592	-2,1559245						
ab	14a	17	-1,66666	-1,6666575						
ab	7a	5	-1,5754	-1,5754004						
bc	8b	9		-0,994286	-0,994286					
bc	12	16		-0,9070718	-0,9070718					
cd	5	16			-0,0446912	-0,044691224				
cde	14b	17			0,23656517	0,236565168	0,236565168			
cde	11	11			0,23743606	0,237436061	0,237436061			
cde	16	11			0,4255534	0,425553402	0,425553402			
def	7b	9				0,5319901	0,5319901	0,53199		
def	13b	16				0,76869205	0,76869205	0,768692		
def	9	23				0,784584902	0,784584902	0,784585		
defg	15	16				1,271782459	1,271782459	1,271782	1,271782	
efg	6	9					1,64867772	1,648678	1,648678	
fg	17	18						1,940986	1,940986	
g	10	8								2,576694
Significance			0,42925	0,1751041	0,05661749	0,115453333	0,059894675	0,061262	0,124334	
B										
a	7a	5	-1,74567							
ab	15	16	-1,35328	-1,3532815						
abc	16	11	-0,97951	-0,9795081	-0,9795081					
abc	5	16	-0,96245	-0,9624464	-0,9624464					
abcd	7b	9	-0,86481	-0,8648134	-0,8648134	-0,864813362				
abcde	8a	16	-0,68093	-0,6809253	-0,6809253	-0,680925263	-0,680925263			
abcde	13a	16	-0,62333	-0,6233334	-0,6233334	-0,623333386	-0,623333386			
bcdef	8b	9		-0,2508469	-0,2508469	-0,250846929	-0,250846929	-0,25085		
bcdef	17	18		-0,1042645	-0,1042645	-0,104264536	-0,104264536	-0,10426		
bcdefg	6	9		0,073036	0,07303599	0,073035987	0,073035987	0,073036	0,073036	
cdefg	13b	16			0,12144338	0,12144338	0,12144338	0,121443	0,121443	
cdefgh	10	8			0,26157933	0,261579327	0,261579327	0,261579	0,261579	0,261579
defgh	9	23				0,557627396	0,557627396	0,557627	0,557627	0,557627
efgh	14b	17					0,73939287	0,739393	0,739393	0,739393
fgh	14a	17						1,028732	1,028732	1,028732
gh	11	11							1,504395	1,504395
h	12	16								1,575966
Significance			0,33998	0,0539891	0,18319477	0,05554915	0,056419566	0,145651	0,052017	0,117018

Oxychilus (Drouetia) atlanticus brincki; he also raised to specific status Morelet's variety *H. a. spectabilis*, from Santa Maria, for which he created the subgenus *Atlantoxychilus*. Martins (1981) described *O. (D.) agostinhoi* from Santa Maria, thus rendering *Drouetia* a polytypic taxon; later (1989) he described two new species from Terceira, (*O. (D.) miceui* and *O. (D.) furtadoi*). In that same year Winter described *O. (D.) batalhana* from São Miguel. Martins *et al.* (1991) and Cunha *et al.* (1993) raised to the specific category Riedel's subspecies from Santa Maria and Faial, respectively.

Taxonomic list of the *Oxychilus* Fitzinger mentioned in the text:

Drouetia Gude, 1911

Oxychilus (Drouetia) atlanticus (Morelet and Drouët, 1857)

[= *O. (D.) batalhana* Winter, 1989]

O. (D.) minor (Morelet, 1860)

O. (D.) brincki Riedel, 1964

O. (D.) agostinhoi Martins, 1981

O. (D.) miceui Martins, 1989

O. (D.) furtadoi Martins, 1989

Atlantoxychilus Riedel, 1964

Oxychilus (Atlantoxychilus) spectabilis (Morelet, 1860)

RESULTS

Analysis of shell morphometry of the specimens from São Miguel showed that, although some stations were significantly different from each other, there was great overlap in shell shape around the island and the similarities did not reveal any definable geographic pattern (Table 2; Figure 3). Replicates of some stations did show significant differences (Table 3). Cluster analysis was performed and confirmed the lack of pattern shown by canonical analysis.

Wide range of sizes was found in the samples of stations 7b and 10 (shell diameter SD 1.23 and 1.03, respectively), revealing stronger heterogeneity.

Inspection of the anatomy of the reproductive system, on the other hand, showed good constancy in morphology within station, but varied somewhat haphazardly between stations (Figure 4). No remarkable differences were detected among the replicates of stations. The length of the epiphallus and of the penial sheath were the characters that showed more disparate differences (see Figure 2 for terminology). Worth mention is the rugose aspect of the epiphallic pore in specimens of station 7 (Figure 4, 7) and to a lesser extent represented also in specimens from station 8 (Figures 4, 8; 10, A).

The specimens from Flores revealed a different

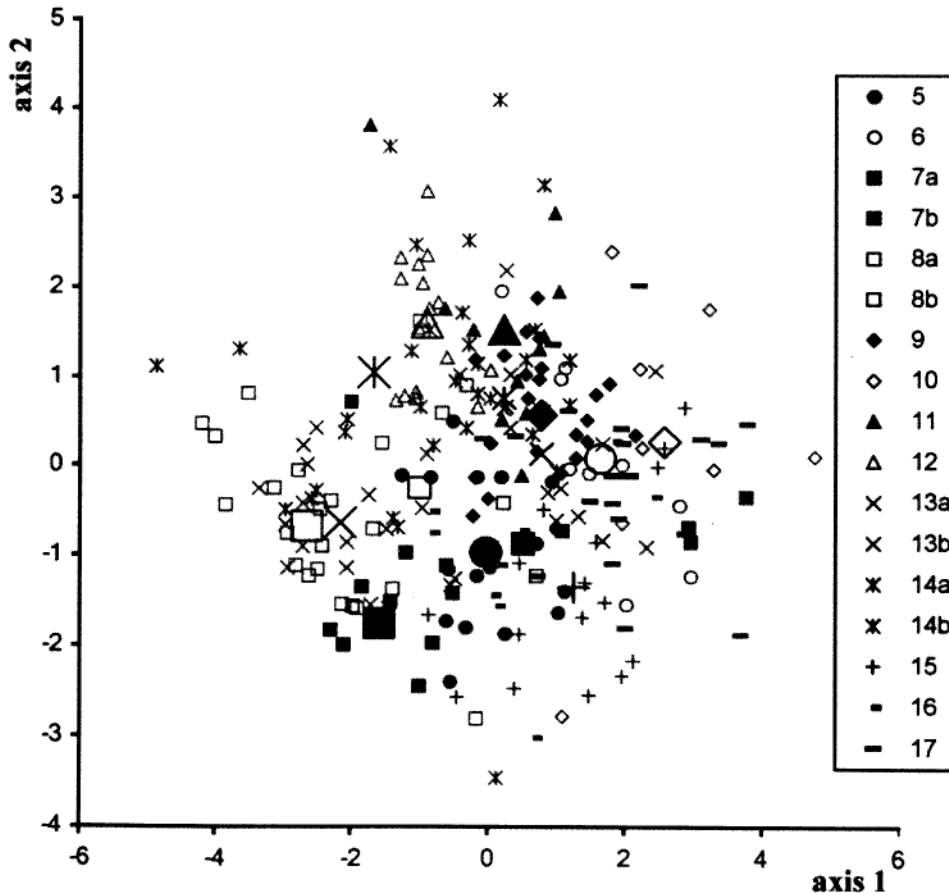


Figure 3 Projection of the specimens from São Miguel (stations 5–17, Table 1), according to the first two canonical functions extracted from shell morphometry (Table 2). The larger symbols represent the centroids for each station. Where there was duplicate sampling (a, b), b is represented by the smaller centroid.

Table 4 Discriminant scores from functions 1 (A) and 2 (B) of canonical analysis of shell morphometry of the populations from Flores (stations 19-22). Tukey HSD. Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares. The error term is Mean Square (Error) = 1.000.

A					
	Station	N	1	Subset 2	3
a	21	13	-1.469514986		
ab	19	21	-0.657685886	-0.6576859	
b	22	29		-0.5377883	
c	20	20			2.425548
Significance			0.065873562	0.9824623	1
B					
a	21	13	-0.594310859		
a	22	29	-0.320505071		
a	20	20	-0.083643016		
b	19	21		0.890169	
Significance			0.396043037	1	

pattern. Basic statistical analysis showed heterogeneity in samples from stations 19 and 22 (SD of shell diameter was 2.22 and 1.23, respectively; Table 2), and canonical analysis of shell morphology showed that station 20 was set apart from the others, thus indicating that it could harbour a different populational unit (Table 4; Figure 5). Cluster analysis revealed that various such units could be identified, and that they are largely syntopic (Figure 6). Inspection of the shell morphology of the specimens thus grouped suggested that four relatively homogeneous units could be made.

The anatomy of those tentatively identified units, however, apart from relative size, deviates little from a basic design. The most obvious, perhaps unique feature shared by all groups from Flores, but absent in *Drouetia* from the other islands, is the smooth interior of the proximal penis (Figures 7; 10, E-H).

Still a different pattern was found in Graciosa. Syntopic specimens, impossible to set apart on the basis of shell morphology alone, are strikingly different in almost every aspect of their reproductive anatomies (Figures 8; 10, C-D).

Santa Maria has four anatomically well-established species. *Oxychilus* (*D.*) *agostinhoi* is the smallest *Drouetia* of the archipelago (Figure 9, A). *Oxychilus* (*D.*) *brincki* and *O.* (*Drouetia*) sp. G, somewhat similar conchologically, are very distinct anatomically (Figure 9, B-C). *Oxychilus* (*Atlantoxychilus*) *spectabilis*, apart from the peculiar groove on the whorls, has a typical *Drouetia*-like shell; however, the internal morphology of the penis is remarkably different (Figures 9, D; 10, L).

DISCUSSION

The patterns

The zonitids are the most speciose pulmonate group in the Azores and are responsible for about one third of its endemic species (Riedel, 1997; Martins, 1999). Such species diversity is expressed not only in the morphology of the shell or, mainly, anatomically but also in the coloration of the animal. Whereas most European zonitids are uniformly dark-blue, the Azorian endemics may exhibit vivid coloration not only on the exposed parts of the body (foot sole and sides) but also on the mantle, visible through the translucent shell. Coloration of the foot may sometimes be related with food sources; some brightly coloured animals were seen feeding on the red wrapping of the seeds of *Accacia* or on the succulent red fruits of *Hedychium*, although that same coloration has been found when such food sources were not available. Therefore, the presence of various foot colorations in a population should not warrant, per se, a taxonomic status, unless consistently linked to other diagnostic characters.

Shell morphology in São Miguel did not show any identifiable pattern of variability, although significant differences were found among some stations (Table 3; Figure 3). However, significant differences were also found between duplicates of the same station (collected months or years apart), thus indicating that variability in shell morphology, besides having some inter-demic expression, could also have associated an intra-demic cohort factor.

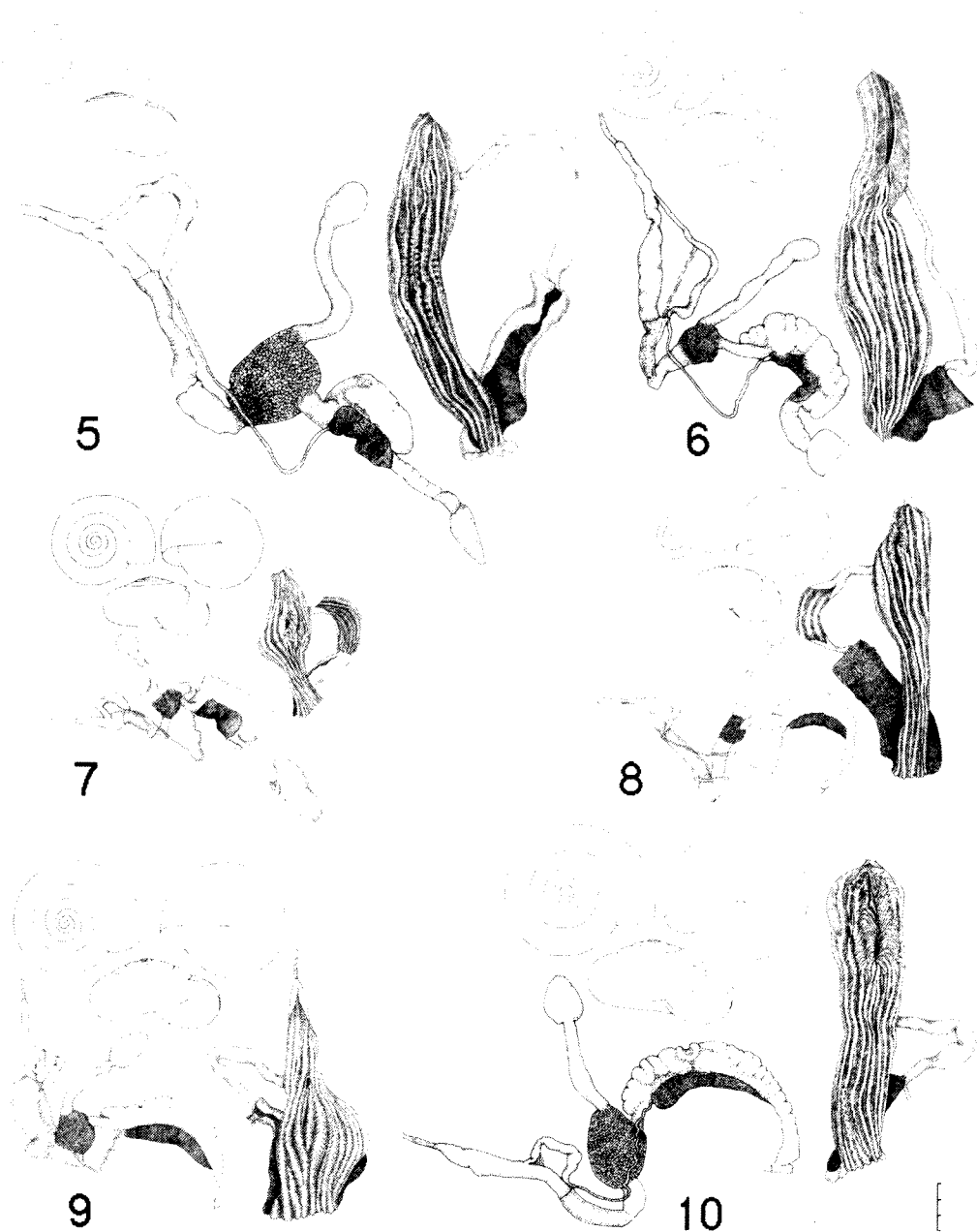


Figure 4 *Oxychilus (Drouetia) atlanticus*, from São Miguel. Pictorial representation of the shell, genitalia and internal morphology of the penis of a specimen from each station (5–17). Scale bar = 1 mm (one division for the shell; two divisions for the genitalia; full length for the penis).

The disparate sizes found in stations 7b and 10 (see also Martins, 1991, figures 4–5) could be related to cohort overlapping; Cunha (1999) has found that *O. (D.) atlanticus* lives to 24–26 months and matures at about 14 months. It appears, then, that on account of shell morphology only one species (*O. (D.) atlanticus*) exists in the island.

Anatomy, on the other hand, maintained a more obvious demic nature, even taking into account that Martins (1991) had cautioned against the variability expressed in the relative dimensions of the reproductive system, often an artifact of different preservation methods. This is clearly the case of *O.*

(D.) batalhana (see Figures 4, 12), described on the basis of the relative proportions of highly variable structures such as the flagellum, but which, for other reasons as well, fits well within the observed variability in São Miguel. *Oxychilus (D.) batalhana* shall, then, be considered a junior synonym of *O. (D.) atlanticus*. Anatomical variability throughout the island does not follow a consistent pattern of relationships: demes closer are sometimes more different than are demes further apart (compare, e.g., in Figure 4, stations 9–10, located side by side or 6–17, at opposite ends of the island). The demic (allotopic) nature of the observed variability

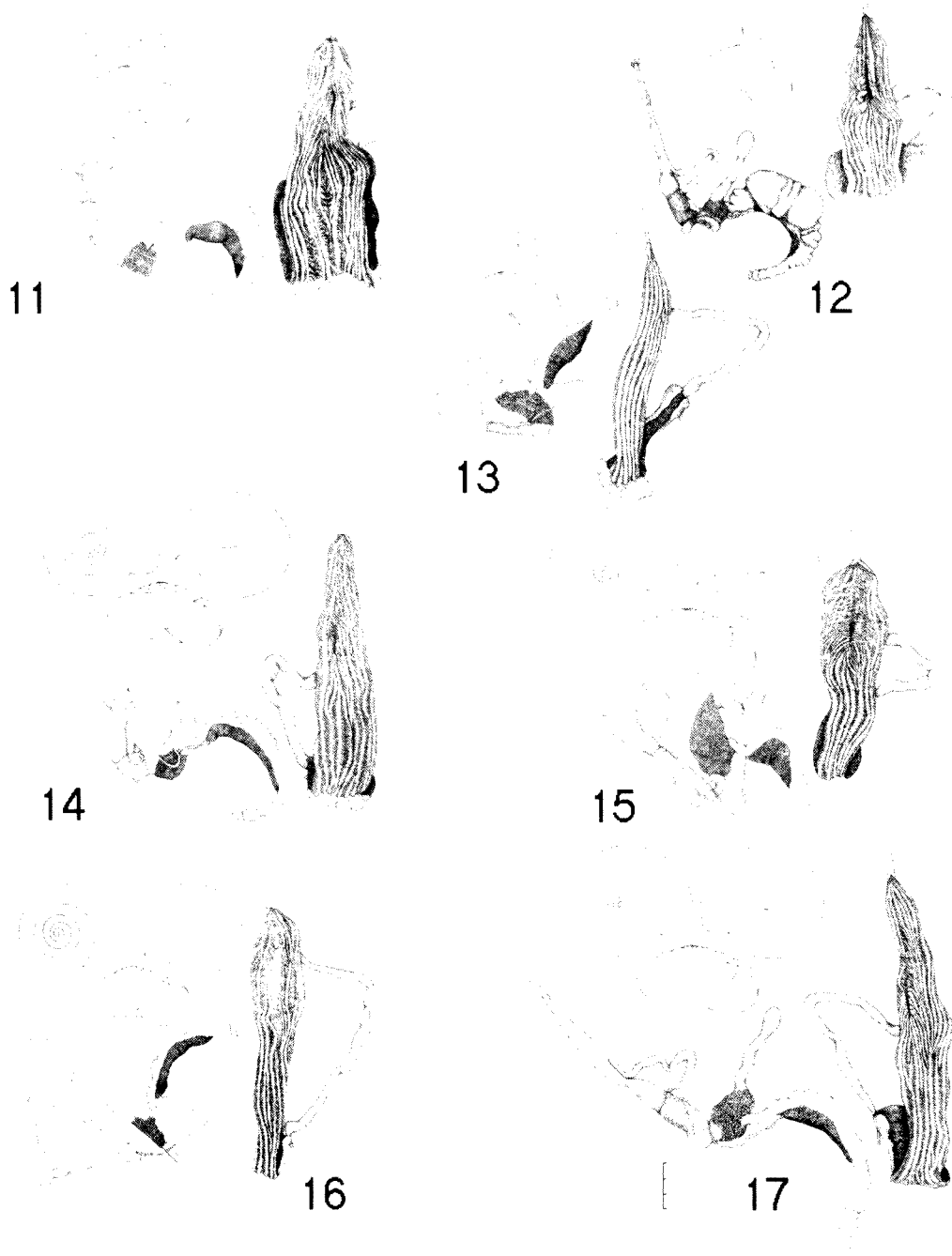


Figure 4 (cont.)

corroborates the findings based on shell morphology that only one species lives in the island.

The situation in Flores, however, is rather different. The anatomical characters, although in some peculiarities (internal ornamentation of the proximal penis) not comparable to those from other islands, exhibit there a rather clear homogeneity. Shell shape, on the other hand, clearly sorts the specimens from the various stations (Table 4; Figure 6). Contrary to the situation in São Miguel, it appears that in Flores various taxonomic units exist, for the various groups occur syntopically.

I previously stated that body coloration, *per se*, is not a reliable character; however, inspection of the coloration of the foot appears to be helpful in this case. Within some variational latitude, sp. A has a yellowish foot, sp. B is whitish, sp. C is black and sp. D is pink. The markings on the mantle are clearly seen in species C and D, less so in the other two. It appears, then, that coloration corroborates shell variability, in spite of some anatomical uniformity.

In Graciosa still another pattern emerges. The shells of syntopic specimens are indistinguishable but the morphology of the reproductive system is

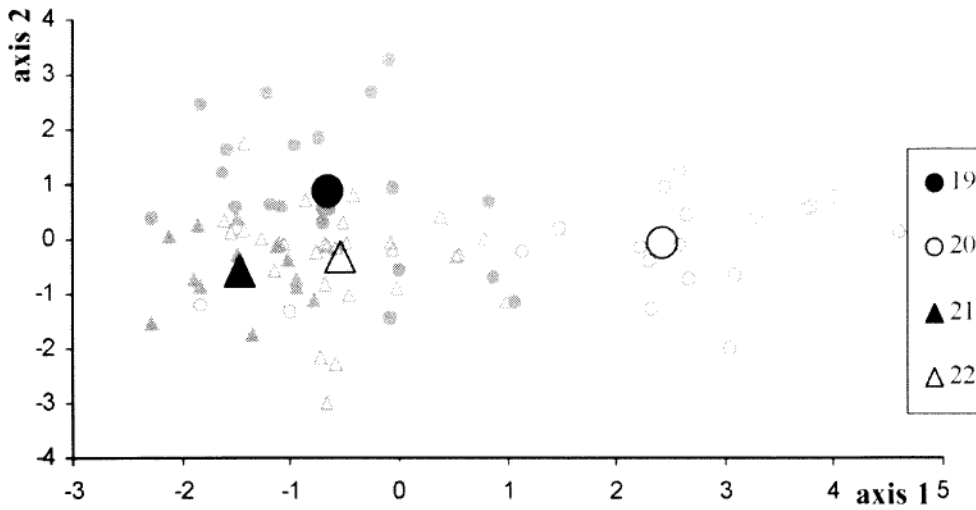


Figure 5 Projection of the specimens from Flores (stations 19–22; Table 1), according to the first two canonical functions extracted from shell morphometry (Table 2). The larger symbols represent the centroids for each station.

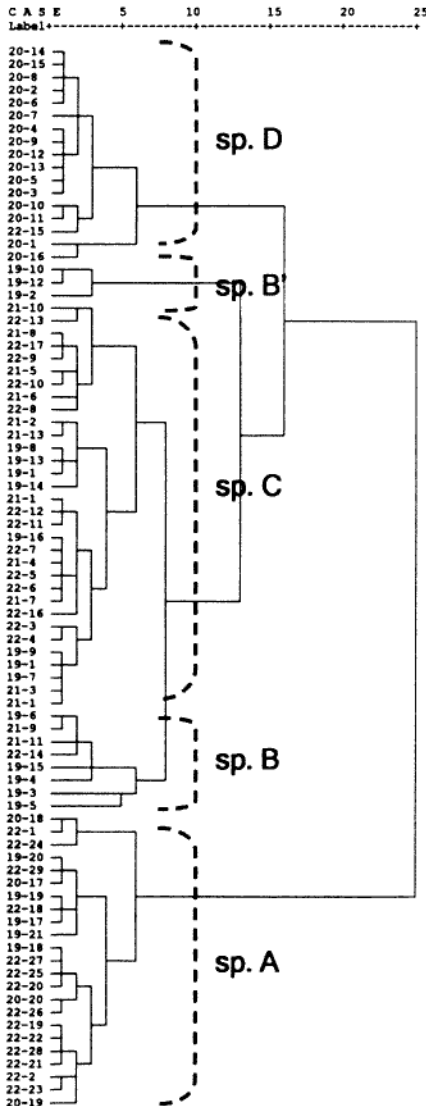


Figure 6 Dendrogram of specimens from Flores (stations 19–22), resulting from cluster analysis (euclidean distance followed by UPGMA) based on shell morphology (Table 2). Several units (sp. A–D) stand out as having possible taxonomic significance.

drastically different (Figure 8, spp. E–F): the relative proportions of the penial sheath, the shape of the epiphallus, the length of the vagina. Inspection of the animals does not yield clearer diagnostic results than the shells: the animals are of a tender pink, much like those of *O. (D.) minor* from Faial and somewhat similar to sp. A from Flores. However, the fact that they live syntopically and that they consistently exhibit such disparate, complex anatomies, without intermediates, is clear indication of the presence of two discrete taxonomic units.

The groups in Santa Maria are markedly separate. *Oxychilus (D.) agostinhoi*, the smallest *Drouetia* of the archipelago, apart from its minute size and flat spire, shows more anatomical similarities with the species of the remaining islands than any of the other species from Santa Maria (Figures 9; 10; see also Martins, 1981). *Oxychilus (D.) brincki* and *O. (Drouetia) sp. G* are somewhat similar conchologically and have been confused since Morelet and Drouët's (1857) description, for both shells appear in the type material (personal observation). However, their anatomies are very distinct and unlike any other species in the Azores (Figures 9, B–C; 10, J, K). *Oxychilus (Atlantoxychilus) spectabilis*, apart from the characteristic furrow around the equator of the last whorl and spiralling up on the spire, has a typical *Drouetia*-like shell; however, the internal morphology of the penis is so remarkably different that it was placed by Riedel (1964) into a separate subgenus (Figures 9, D; 10, L).

The species from Santa Maria exhibit also discrete colour variations. *Oxychilus (D.) agostinhoi* somewhat resembles the European zonitids being dark blue on top and clearing toward the foot, which has a dark rim around it; the mantle markings can hardly be seen through the greenish shell. *Oxychilus (D.) brincki* has bright orange foot and mantle markings similar to some specimens

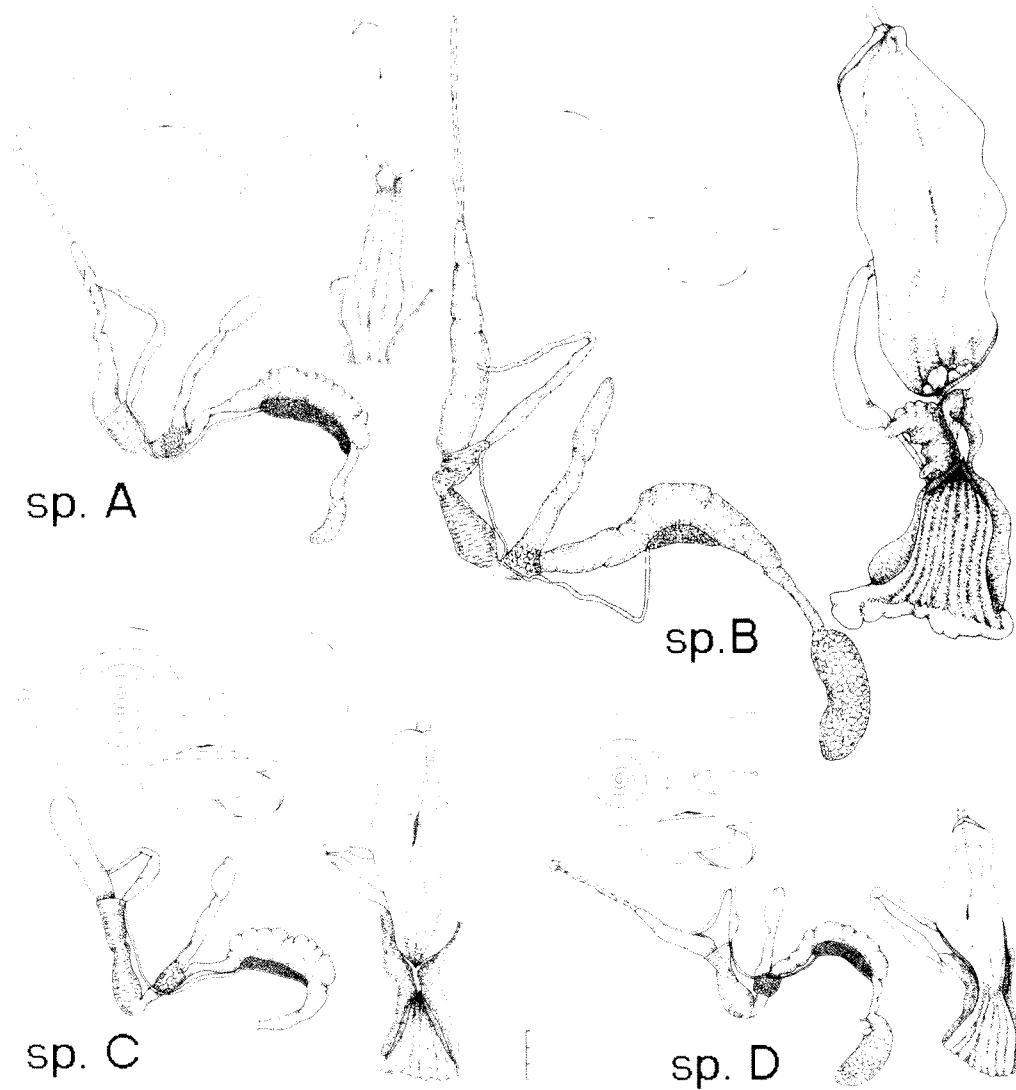


Figure 7 *Oxychilus (Drouetia)* sp. from Flores. Pictorial representation of the shell, genitalia and internal morphology of the penis of a specimen from each (taxonomic) unit (A–D), as expressed in Figure 6. Scale bar = 1 mm (one division for the shell; two divisions for the genitalia; full length for the penis).

from São Miguel. *Oxychilus (D.)* sp. G is greenish with yellowish mantle markings and *O. (A.) spectabilis* is dark yellow with clear mantle markings.

The process

Speciation is usually a time requiring phenomenon that can rarely be followed in its entirety. Apart from direct reading of the fossil record, the process of speciation can be inferred through a comparative and integrative approach.

Several stages of speciation were presented above and various patterns of differentiation exposed. Integrating the various situations may help understanding some elements influencing the process.

Various scenarios are proposed:

a) demic (allotopic) variability in São Miguel. Assuming that *O. (D.) atlanticus* constitutes one (variable) population on the entire island, the

observed differences could perhaps be explained if one admits a strong demic structure in that population, reinforced by gregarious behaviour. Such structure would require an endogenous mechanism of short-term variability (associated with pioneering, i.e., frequent colonization and/or population reduction, and strongly influenced by genetic drift) in order to explain the significant differences observed in the duplicate sampling in some stations. Cunha (1999) observed that *O. (D.) atlanticus* congregates under logs or rocks to lay eggs, a behaviour that may contribute to some dampening of inter-demic gene flow. However, natural expansion of the demes is unavoidable and eventually gene flow is re-established, thus requiring recurrent fragmentation of these larger demes to explain the observed pattern. Cyclic volcanic eruptions could well be an essential part of such fragmenting mechanism, and the volcanological history of São Miguel, presented

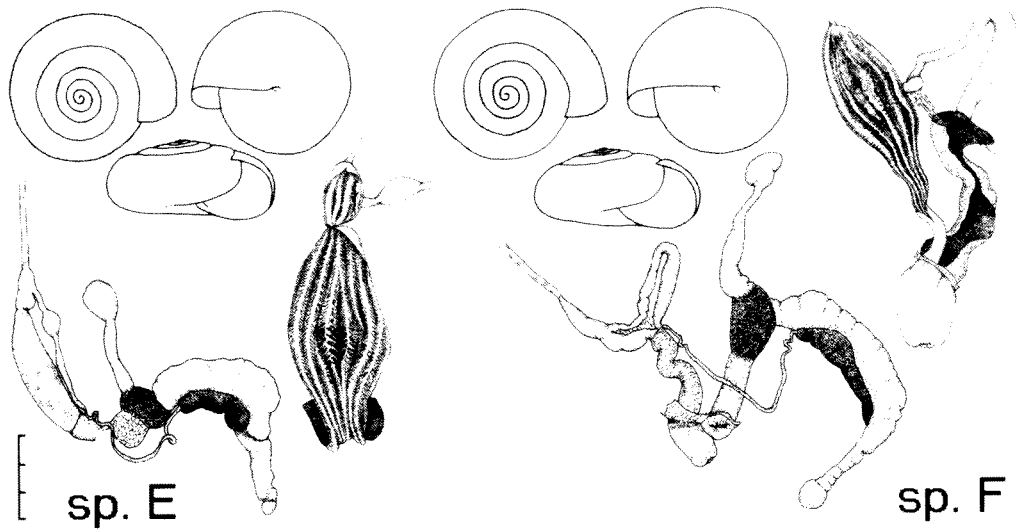


Figure 8 *Oxychilus (Drouetia)* sp. from Graciosa. Pictorial representation of the shell, genitalia and internal morphology of the penis of a specimen from each (taxonomic) unit (sp. E and sp. F). Scale bar = 1 mm (one division for the shell; two divisions for the genitalia; full length for the penis).

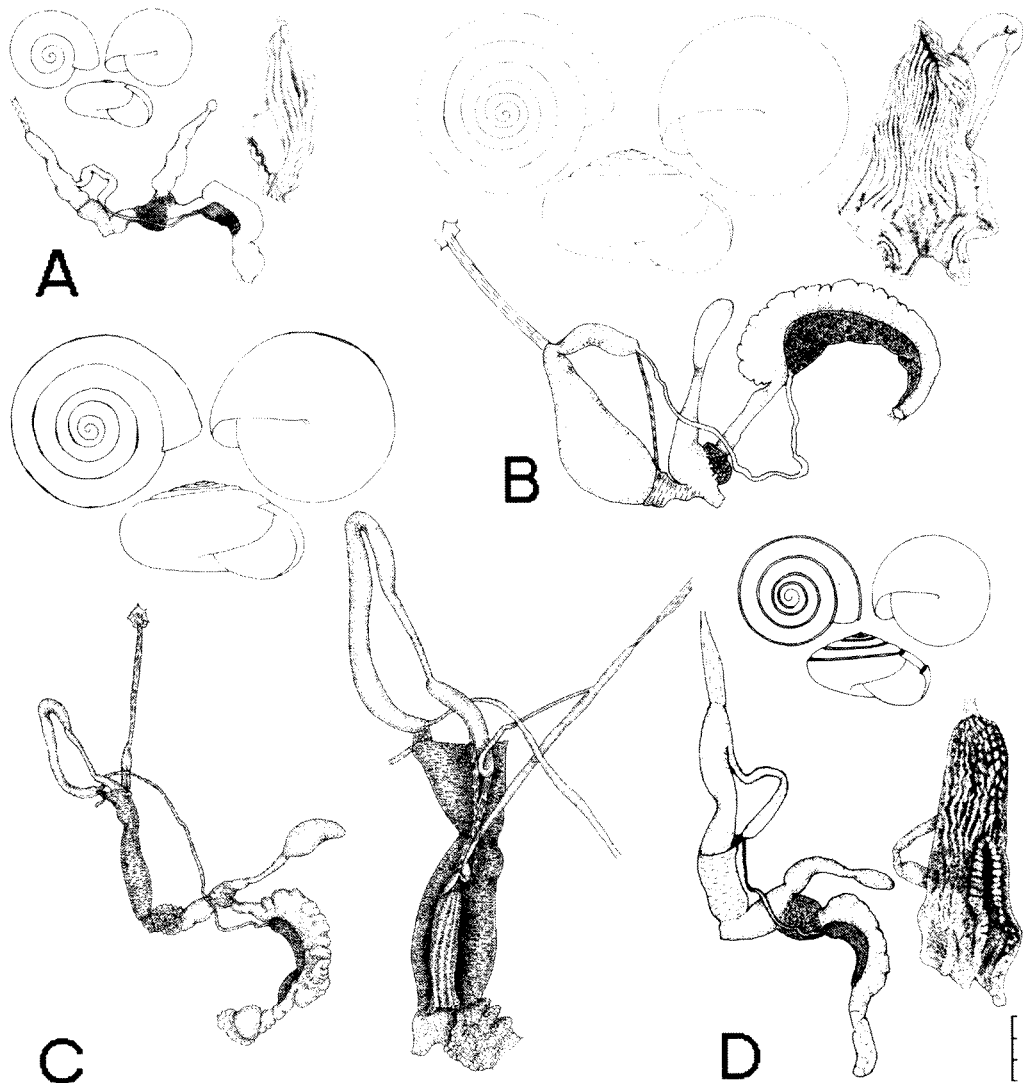


Figure 9 *Oxychilus* from Santa Maria. Pictorial representation of the shell, genitalia and internal morphology of the penis. A, *Oxychilus (Drouetia) agostinhoi*; B, *Oxychilus (Drouetia) brincki*; C, *Oxychilus (Drouetia)* sp. G; D, *Oxychilus (Atlantoxychilus) spectabilis*. Scale bar = 1 mm (one division for the shell; two divisions for the genitalia; full length for the penis).

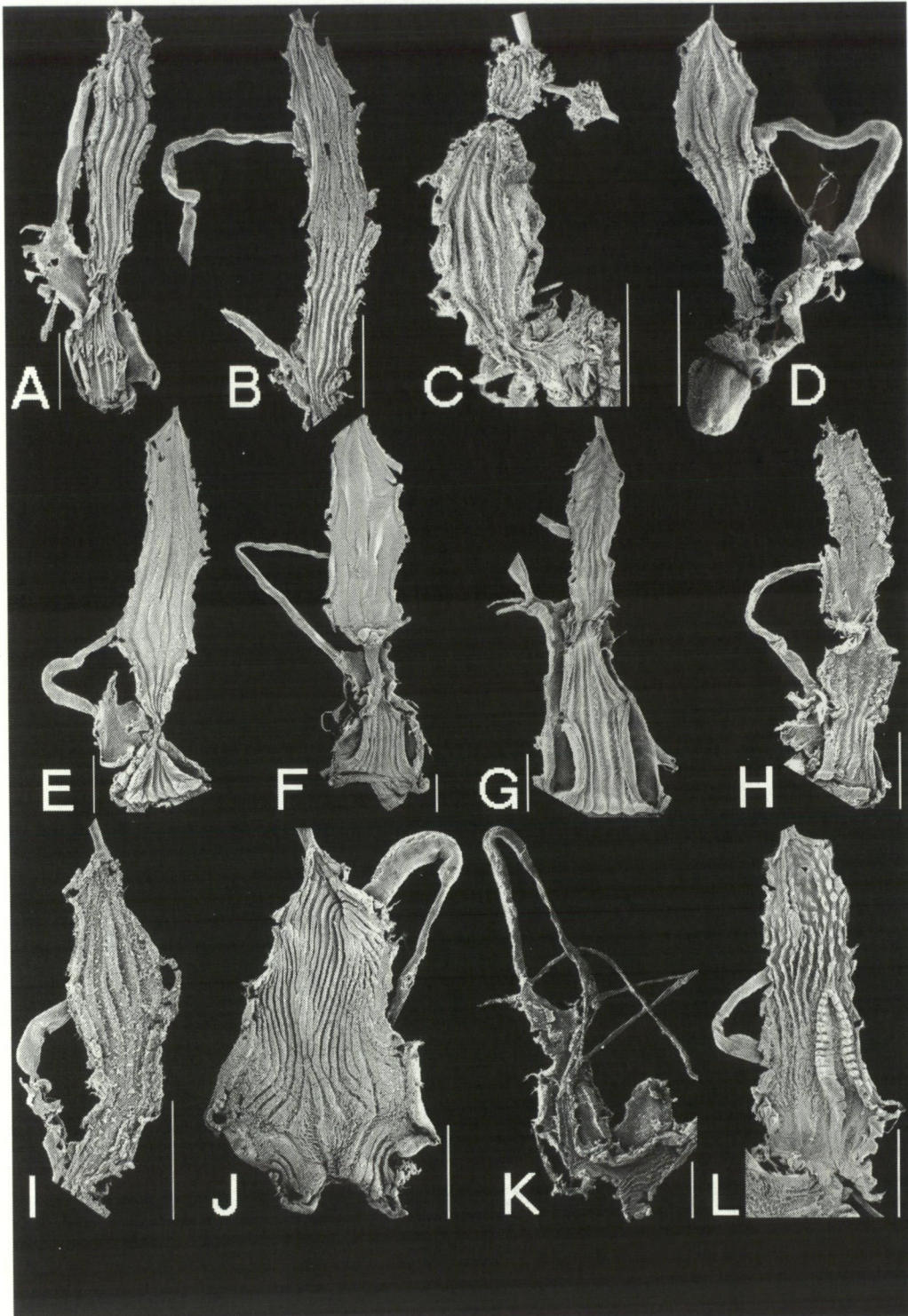


Figure 10 Internal morphology (SEM) of the penis of various species of *Oxychilus*. A–B, *O. (Druetia) atlanticus* (S. Miguel, Sta 8 and Sta 16); C–D, *Oxychilus (D.)* sp. E and sp. F (Graciosa, Sta 18); E–H, *Oxychilus (D.)* sp. A (Sta 20), sp. B (Sta 19), sp. C (Lagoa Seca, 18-10-1993) and sp. D (Sta. 19) (Flores); I–L (Santa Maria): I, *O. (D.) agostinhoi* (Sta 1, 1993); J, *O. (D.) brincki* (Sta 1, 1974); K, *Oxychilus* sp. G (Sta 3); L, *O. (Atlantoxychilus) spectabilis* (Sta 4). Scale bars = 1 mm.

above, meets these requirements. Van Riel *et al.* (2003) have linked genetic differentiation of the endemic *Leptaxis azorica* (Albers) with the geological age of São Miguel. Their study concluded that eastern and western populations differ significantly and that those in the middle are intermediate.

Although the present study progressed as a different approach for it is circumscribed to morphology and anatomy, it takes some legitimacy in Van Riel's findings. How quickly does the biota recover after profound eruptive disturbance can be inferred from Sta 13, Pico do Fogo, which erupted

in 1652; the area is now a rich haven for endemic molluscs. One could, then, assume that within a 200 years span the effects of the eruption had already disappeared and that the colonizers who founded the Pico do Fogo deme would sporadically connect with those nearby. Some eruptions were much stronger than the one just mentioned and affected greater area of the island. Walker and Croasdale (1971) and Booth *et al.* (1978) quantified the deposits of two major eruptions of the Água de Pau volcano (Fogo complex, Figure 1, e), in 4600 B.C. and 1563 A.D. The first one practically cut the island in half and the deposits of the second one reached half a meter as far as Nordeste, at the eastern tip of the island. Curiously, this study corroborated the vivid historical narrative of Gaspar Frutuoso (1522–1591), the first historian of the islands, who reported at length the destruction caused by the eruption. An additional fragmenting mechanism could be the strong erosion that newly formed land is submitted to, with the formation of deeply cut ravines.

Hypothetical as it may seem, the aforementioned mechanism assumes relevance as explanatory when we realize that part of São Miguel is 4 Mys old and *Drouetia* may have reached the (proto)island long enough to have speciated, as it did elsewhere in the archipelago. However, unless a syntopic situation arises where distinct morphologies/anatomies are clearly diagnosed, multiple species will not be detected by the present approach, in spite of the wide range of morphological and anatomical variability herein registered. If such fragmentation mechanism (or another plausible one) is not taken into account, this one species of *Drouetia* living there now had to be a newcomer, thus requiring catastrophic extinction of previous colonizers and a re-colonization event, of unclear origin.

b) close interspecific (syntopic) variability in Flores and in Graciosa. In both cases volcanic activity (perturbation) has been absent for at least 2,000 years. Other mechanisms may have created allopatry and allowed for speciation to progress to the point where, when syntopic, we can say they behave as discrete evolutionary units, perhaps as biological species. The close anatomical resemblance of the specimens from Flores, although conchologically and externally separable, and the conchological and external resemblance of the specimens from Graciosa, although anatomically distinct, illustrate the various ways in which speciation can proceed.

c) extreme interspecific (syntopic) variability in Santa Maria. This is a case of progression in time of scenario b), just mentioned. Volcanism has stopped in Santa Maria more than 3 Mys ago. Here time has been the main factor, allowing for the extreme divergence presented above (see Figure 9). The hypothesis of multiple colonization events to explain the present situation in Santa Maria does

not fit the reality in the remaining islands nor has it found a suitable source of multiple colonizers. *Drouetia* has not left relatives in Europe or Macaronesia, the closest source for more than 95% of the Azorian malacofauna.

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Numbers should be spelled out from one to nine in descriptive text; figures used for 10 or more. For associated groups, figures should be used consistently, e.g. 5 to 10, not five to 10.

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High quality illustrations are required to size (16.8 cm x 25.2 cm) or no larger than 32 cm x 40 cm with sans serif lettering suitable for reduction to size. Photographs must be good quality black and white prints, not exceeding 16.8 cm x 25.2 cm. Scale must be indicated on illustrations. All maps, line drawings, photographs and graphs, should be numbered in sequence and referred to as Figure/s in the text and captions. Each must have a brief, fully explanatory caption. On acceptance a computer disk containing all corrections should be sent with amended manuscript. The disk should be marked with program (e.g. Word, WordPerfect, etc).

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