J. Moll. Stud. (1999), **65**, 99–109

MORPHOLOGICAL AND MOLECULAR RESOLUTION OF A PUTATIVE CRYPTIC SPECIES COMPLEX: A CASE STUDY OF *NOTOACMEA FASCICULARIS* (MENKE, 1851) (GASTROPODA: PATELLOGASTROPODA)

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(Received 28 August 1997; accepted 6 May 1998)

ABSTRACT

Evidence that *Notoacmea fascicularis* (Menke, 1851) is a complex of at least two distinct taxa of species rank is ambiguous. A discriminant function analysis of conchological data shows a weak geographic effect, while radular morphology clearly delineates two sympatric groups with rare intermediates. Lastly, molecular data (mt cytochrome *c* oxidase subunit I) suggests a single species and a geographic effect. We consider *N. fascicularis* to be a single taxon, variable for radular lateral tooth morphology. In the past these two different radular morphologies would be indicative of generic rank. Our knowledge of the intraspecific variability of most gastropod characters is poor, and this makes specific identifications or groupings based on single character systems such as the radula precarious. Adequate sampling and evaluation of population-level character states (conchological, anatomical and molecular) is needed to identify as well as falsify cryptic species complexes.

INTRODUCTION

Cryptic or sibling species are taxa that are difficult or impossible to distinguish on morphological characters alone (Mayr & Ashlock, 1993), and they have confused and troubled systematists since the late 1800's (Mayr, 1963). In the marine realm they have been noted in almost every major phylum; Knowlton (1993) lists over 130 marine invertebrate examples of sibling species, including 21 molluscan cases. Since Knowlton's listing, numerous additional gastropod examples have been investigated, for example by Crossland *et al*. (1993), Rolan-Alvarez, *et al*. (1995), Kool (1995), Zaslavskaya (1995) and Gofas & Jabaud (1997). Within the Patellogastropods sibling species have been discussed in Patellidae (Acuna & Munoz, 1995) and Lottiidae (Test, 1946; Lindberg & McLean, 1981).

While Mayr (1948) used the existence of sibling species to argue against the morphological species concept, he also contended that additional data sets (morphometric, genetic, ecological) could successfully resolve sibling species complexes. Like Mayr, Knowlton (1993) emphasized that many of the taxonomic problems associated with sibling species resulted from a failure to use all available characters. However, she also pointed out that acquisition of comprehensive data sets is often difficult for many marine invertebrate groups. This is particularly true for molluscan studies where the bulk of specimens in research collections are shells (Brusca & Brusca, 1990).

The development of molecular techniques for sampling genomic characters has substantially increased the power of discovery and resolution of sibling species complexes, and may ultimately increase the number of marine species by an order of magnitude (Knowlton, 1993). However, these same techniques also furnish data sets that can refute putative sibling species and instead reveal cases of polymorphism or plasticity within taxa.

Here we examine a putative example of cryptic species in the patellogastropod limpet *Notoacmea fascicularis* (Menke, 1851). Like most gastropods, this species was initially recognized and delineated on conchological characters. Although it was a variable species with respect to shell colour and pattern (Carpenter, 1857), the range of variation within these characters did not substantially overlap with other tropical eastern Pacific taxa, and thus the identity of *N. fascicularis* was never viewed as problematic (Dall, 1871; Pilsbry, 1891; Keen, 1958). During the 1960's McLean (pers. comm.) examined radulae of more than 25 specimens of *N. fascicularis* from 11 localities between Cabo Pulmo, Baja California Sur,

Mexico and Costa Rica (Table 1). Within these specimen lots he discovered two distinct radular morphologies (Table 1, Fig 1). Based on this discovery McLean (1971:327) concluded that 'There is some indication that this is a complex involving more than one species; some populations have the three lateral teeth of nearly equal size, while in others the outer lateral is reduced. As yet no shell characters have been found that correlate with the radular differences.'

McLean's suggestion that *N. fascicularis* was a cryptic species complex of at least two species demonstrates the taxonomic weight afforded the patellogastropod radula. Heavy reliance on radular characters in the diagnosis of patellogastropod species has a long and noteworthy history in gastropod taxonomy (Thiele, 1866–1893; Dall, 1871; Oliver, 1926; Koch, 1949; McLean, 1966; Powell, 1973; Christiaens, 1973, 1975a,b; Lindberg & McLean, 1981; Lindberg, 1988). For over 25 years McLean's suspicion of a cryptic species complex within the taxon *N. fascicularis* has remained untested. With the advent of molecular techniques it is now possible to produce a third data set which may resolve this question. We used partial sequences of cytochrome *c* oxidase subunit I (COI) to examine the correlations between phylogeny, conchology and radular morphology in *N. fascicularis*.

MATERIALS AND METHODS

We examined 59 specimens of *N. fascicularis*; 29 of these specimens were from collections available to McLean when he observed radular variation in this species. WBS collected the remaining 30 specimens between 1995–1997 for this study (Table 1). Institutional abbreviations used herein are as follows: LACM—Natural History Museum of Los Angeles County, Los Angeles, CA; UCMP—Museum of Paleontology, University of California, Berkeley, CA. Localities along the west coast of Mexico are abbreviated as: CSL—Cabo San Lucas, Baja California Sur; COL—Colima; MAN—Manzanillo; MAZ—Mazatlan; PVA—Puerto Vallarta.

For on-line data, see
http://www.ucmp.berkeley.du/collections/archdat.html.

Shell morphology

Digital images of the ventral, dorsal, and profile of each shell were captured with a digital camera connected to a Scion LG-3 Scientific Frame Grabber system. Taking the maximum shell length obtained with dial calipers and converting this measurement to pixels scaled a baseline for each image. Once the images were scaled for size, measurements of projected shell area, shell length, shell width (at widest point), apex position (from anterior and posterior ends), projected shell muscle scar area, and shell height were determined (Fig. 2).

Five ratios were also calculated: (1) distance from anterior edge to apex/distance from posterior edge to apex, (2) projected shell muscle scar area/projected shell area, (3) shell height/shell width, (4) shell height/shell length, and (5) shell width/shell length (Fig. 2).

Data for localities with $\geqslant 5$ specimens were partitioned into three data treatments for discriminant function analysis (DFA). The first data treatment consisted of all linear measurements, area measurements, and ratios (DFA ALL). The second data treatment consisted just of ratios (DFA RATIOS), while the third data treatment contained only log linear and area measurements [DFA LOG (x)].

Table 1. Specimen lots of *Notoacmea fascicularis*. 1–11 examined by McLean and 1–14 this study. Conchological and radular data sets were available for lots 1–14; molecular data sets for lots 12–14 only. Unless otherwise noted all localities are within Mexico.

	Locality Nos.	Locality & Date	Lateral Tooth Morphology
1	LACM 66–41	Acapulco, Guerrero. 1966	3 unequal
2	LACM A.7079.70	Bahia Santiago, Colima. 1967	7 unequal
3	LACM 65–13	Cleotus, Tres Marias Ids. 1965	1 unequal
4	LACM 63–10	Manzanillo, Colima. 1963	2 equal; 2 unequal
5	LACM 152423	Manzanillo, Colima. 1965	1 equal
6	LACM 63–11	Mazatlan, Sinaloa. 1963	2 unequal
7	LACM A.8981.68	Mazatlan, Sinaloa. 1966	2 equal
8	LACM 65–20	Zihuantenejo, Guerrero. 1965	1 unequal; 1 intermediate
9	LACM 70–65	Playas de Coco, Costa Rica. 1970	1 equal
10	LACM 66–7	Pulmo Reef, Baja CA Sur. 1966	1 unequal
11	LACM 67–97	Salina Cruz, Oaxaca. 1967	2 unequal
12	UCMP 15201	Cabo San Lucas, Baja CA Sur. 1995–97	2 equal; 1 intermediate; 7 unequal
13	UCMP 15202	Mazatlan, Sinaloa. 1995–97	5 equal; 1 intermediate; 11 unequal
14	UCMP 15203	Puerta Vallarta, Jalisco. 1995–97	3 equal

These treatments were individually submitted to DFA (Systat 7.0®) to identify morphological attributes that would distinguish specimens relative to an independent classification variable. Previously, DFA has been used to separate members of a sibling species complexes when morphological characters show considerable overlap (Knowlton, 1993). Two independent classification variables were used: (1) locality and (2) tooth morphology. Thus, there are a total of six DFA results; two classifications by three data treatments (see Appendix).

Radular morphology

An anterior portion of the radular ribbon from each specimen was dissected from the head region posterior to the odontophore and placed in a 10% sodium hypochlorite solution for ≤ 5 minutes and rinsed in distilled water. The radular ribbon was placed on an ElectroScan Model E3 Environmental Scanning Electron Microscope (ESEM) stub with cusps oriented upwards. The stubs were then placed in the ESEM vacuum chamber where 150× to 265× digital images of each radula were captured. Assignment to equal, unequal, or intermediate radular categories was based on the criteria illustrated in Fig. 1.

Molecular sequence data

To detect the presence of molecular divergence between individuals with equal and unequal radulae, Cytochrome c Oxidase subunit I (COI) was partially sequenced and compared among 30 individuals from three localities. COI was chosen for this study based on its interspecific and intraspecific levels of variation found among sequences of eastern Pacific patellogastropods (Wray; Simison; Clabaugh; Lindberg; all unpubl. data). All material was initially preserved in 70% ethanol.

Extraction. Two equally successful DNA isolation protocols were used: (1) saturated salt/chloroform extraction, and (2) CTAB/phenolchloroform extraction. For each extraction, pedal tissue was cut from the foot margin approximately 3–5 mm along the margin and 3–5 mm towards the center of the foot. The tissue was soaked in deionized water to remove any residual ethanol and finely diced. For the saturated salt technique, the diced tissue was placed in a 1.5 ml tube containing 250 μl isolation buffer (100 mM TRIS, 10m M EDTA and 400 mM NaCl), 60 μl 10% SDS, and 10 μl proteinase K. The mixture was then vortexed and stored on a shaker at 37°C overnight. Following tissue digestion, 175 μl of saturated NaCl solution was added. The samples were inverted for 5 minutes and spun at 13k for 30 minutes. The supernatant was washed with chloroform using 2 times supernatant volume and mixed by inversion for 2 minutes. The supernatant DNA was precipitated using two volumes of ice cold 100% ethanol and spun at 13k for 15 minutes. The DNA pellet was washed twice with two volumes of 70% ethanol, and dried for five minutes in a speed vac. The DNA was eluted in 100 μl of double-distilled water and stored at −20°C.

For the CTAB technique, diced tissue was added to a 1,5 ml tube containing 600 μl 2XCTAB and 9 μl of proteinase K then incubated at 37°C overnight. 600 μl of phenol:chloroform:isoamyl alcohol (25:24:1) was added to the tissue mixture and mixed via inversion for 5 minutes. The solution was then centrifuged at 13k for 15 minutes. The supernatant was added to 600 μl of chloroform:isoamyl alcohol (24:1), mixed for 5 minutes and centrifuged at 13k for 15 minutes. DNA was precipitated using 600 μl isopropanol and stored at −20°C for 2 hours. The precipitate was centrifuged at 13k for 30 minutes at 4°C, and then the pellet washed twice with two volumes of 70% ethanol. It was then centrifuged at 13k for 20 minutes before being dried by speed vac for 5 minutes and eluted in 100 μl of deionized water.

Amplification. Amplification of a section of the coding region of COI was achieved with the HCO-2193 and LCO-1490 primers described by Folmer *et al.* (1994). In a 0.5 ml gene amp tube, on ice, 36.45 μl double-distilled water, 5 μl 10x PCR buffer (Perkin Elmer), 2.5 μl 10 μM dNTP's (Pharmacia), 2.5 μl 25μM $MgCl_2$ (Perkin Elmer), 1 μl each of the 10 μM HCO and LCO primers, 1 μl of template, and 0.25 μl of taq (Perkin Elmer) were combined. A negative control containing all reagents except the template was run in parallel. The tube was then transferred to a Perkin Elmer 9600 geneamp. The cycling parameters began with an initial denaturation at 95°C for 2 minutes followed by 36 cycles with three temperature plateaus of 95°C for 50 seconds, 45°C for 50 seconds, and 72°C for 90 seconds, ending with a 7 minute extension at 72°C. PCR products were purified using Wizard® PCR preps DNA Purification System.

Sequencing. Direct double stranded cycle sequencing of 20 to 30 ng of COI PCR product was performed in both directions using the ABI® cycle sequencing kit following a half reaction ABI® cycle sequencing protocol. Cycle sequencing was performed using a Perkin Elmer 9600 geneamp. The cycling parameters were 25 cycles at 96°C for 10 seconds, 50°C for 5 seconds, and 60°C for 4 minutes. Cycle sequencing product was purified using Princeton Separations Centrisep spin columns, then dried down with a speed vac. The dried, purified cycle sequencing product was resuspended with a 2.5 μl loading solution of 5:1 deionized Formamide:25mM EDTA with 50mg/ml Blue Dextran. 1.5 μl of sample and loading solution was loaded on a 36 cm 4% acrylaminde gel. The gel was run and analysed on an ABI Prism® 377 DNA sequencer.

Alignment and Analysis. All sequences were aligned by hand using ABI® Sequence Navigator. Because COI is a coding region, alignments were not problamatic; all of the intraspecific substitutions were synonomous. Intraspecific sequences were compared

among 30 *N. fascicularis* individuals. Intraspecific distances were calculated for three species of Chilean lottiid limpets [*Scurria ceciliana* (Orbigny, 1841), *Scurria boehmita* (Ramirez, 1974), and *Lottia viridula* (Lamarck, 1819)] and interspecific distances calculated for seven Chilean limpet species.

A pair-wise distance matrix and parsimony-based phylogeny of the *N. fascicularis* alignment data was produced using PAUP 3.1.1 (Swofford, 1993). The phylogeny was then used to map the distribution of radular types to detect possible correlations between equal and unequal radular groups or localities.

RESULTS

Shell morphology

The results of the DFA support McLean's (1971) assertion that shell characters are not correlated with the radular differences. Wilks' lambda for the three treatments with tooth type as the classification variable range between $p = 0.043$ and 0.084 (Table 2) and only 61%–68% of the shells can be correctly assigned to their respective radular group based on shell parameters (Table 2). The results of the second series of DFA using locality as the classifying variable appear to provide better discrimination of the taxa (Fig. 3). Wilks' lambda for the three treatments was less than $p = 0.0010$ (Table 2).

The different treatments in the DFA analysis [DFA ALL, DFA RATIOS, and DFA LOG (x)] produced different patterns of discrimination. DFA ALL provided the greatest discrimination by locality, but also had the highest correlation between sample size and the percentage of specimens correctly classified (Table 2). The distribution of group centroids for DFA ALL and DFA LOG (x) were similar to one another, and these treatments also had the greatest range of canonical functions, primarily in the size variables (see Appendix). The range of canonical functions from the DFA RATIOS treatment were substantially smaller than those from the DFA ALL and DFA LOG (x) treatments. Furthermore, in the DFA ALL treatment only the APEXPOS ratio was found to be significant in the discrimination of the groups, the remaining four ratio-based variables were not significant in the discrimination (Appendix). These results suggests that size is important in discriminating between groups in DFA ALL and DFA LOG (x) (see also Reist, 1985).

Radular morphology

Radular morphologies consisted of 10 equal and 18 unequal specimens with two intermediate forms (Fig. 1). Based on examination of the collections and radular preparations in LACM we estimate that McLean examined 26 specimens of *N. fascicularis* from 11 lots before suggesting the possibility of sibling species. Only one of the 11 lots apparently examined by McLean contained specimens with both radular morphologies (Manzanillo in 1963), although at Zihuantenejo a single specimen with unequal morphology and one with the intermediate condition were present (Table 1). The remaining nine lots exhibited only a single radular morphology, either equal or unequal. Among the new collections both extreme radular forms and intermediates were present at Cabo San Lucas and Mazatlan, while only equal morphologies were present at Puerto Vallarta (Table 1).

Table 2. Statistics for discriminant function analysis and classification based on shell and tooth morphology.

Shell morphology	*n*	DFA ALL Correctly Classified Wilks' lambda $p = 0.000$	DFA Ratios Correctly Classified Wilks' lambda $p = 0.001$	DFA Log(x) Correctly Classified Wilks' lambda $p = 0.000$
Cabo San Lucas	10	70%	50%	70%
Colima	7	86%	57%	86%
Manzanillo	5	100%	40%	60%
Mazatlan	18	44%	17%	44%
Tooth morphology		Wilks' lambda $p = 0.094$	Wilks' lambda $p = 0.043$	Wilks' lambda $p = 0.053$
Equal	18	67%	61%	67%
Unequal	31	68%	65%	68%

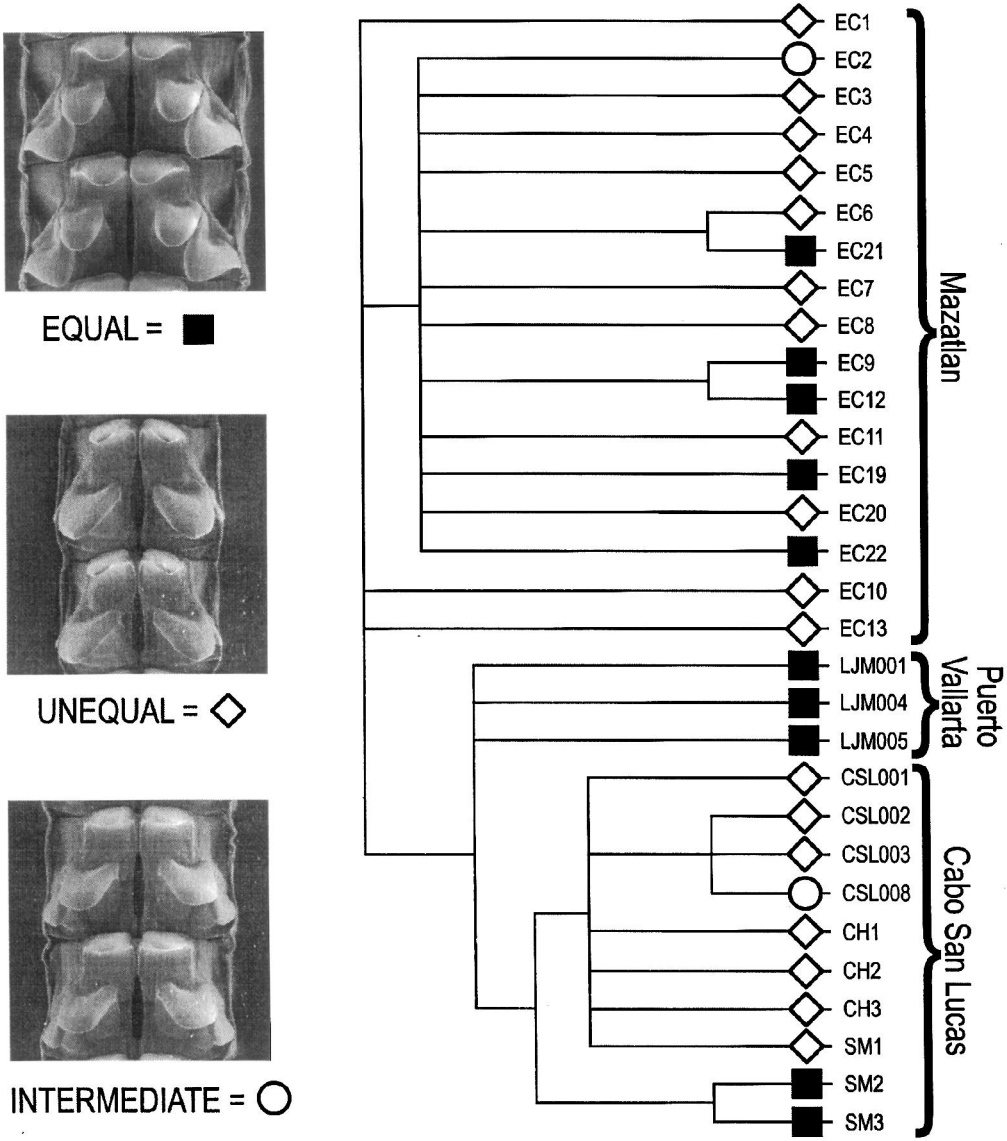

Figure 1. Cladogram of *Notoacmea fascicularis* (Menke, 1851) based on parsimony analysis of COI molecular data. Geographic distributions and radular variability are mapped on to tree.

Molecular sequence data

Without suggesting that species be defined by some arbitrary molecular distance, we observe that distances found among *N. fascicularis* individuals fall well within the range of distances found among individuals of three Chilean *Scurria* species, and well outside the range of distances found between species of the Chilean clade. Differences range between 0–14 base pairs per 710 positions for *N. fascicularis* (0–2.0%) (Table 3) and between 5–9 base pairs per 639 positions within Chilean species (0.8–1.4%). In contrast, interspecific distances calculated amongst the seven Chilean species ranged from 40–157 base pairs per 632 positions (6.3–24.8%).

The molecular phylogeny of *N. fascicularis* (Fig. 1) reveals clear geographic groupings with

a random distribution of radular types across the groups. Although further populational level studies are needed, our analysis suggests that the radular differences found in *N. fascicularis* are intraspecific variation and are not sequestered in independent evolutionary lineages.

DISCUSSION

Evidence that *N. fascicularis* is a complex involving two separate taxa of species rank is ambiguous. Conchological data subjected to a discriminant function analysis (DFA) show a weak geographic discrimination by shell characters. However, the discrimination appears to be primarily size-based and sample size further confounds this result. Radular morphology clearly delineates two distinct groups with rare intermediates, while molecular data suggests a single species and clear geographical associations. Thus, shell morphology and molecular data support the presence of a single species rank taxon, while radular data is consistent with the presence of a cryptic species complex (for a similar example in the hydrobiid *Tatea* see Ponder *et al*., 1991). Based on these results we suggest that the taxon *N. fascicularis* is variable for radular lateral tooth morphology.

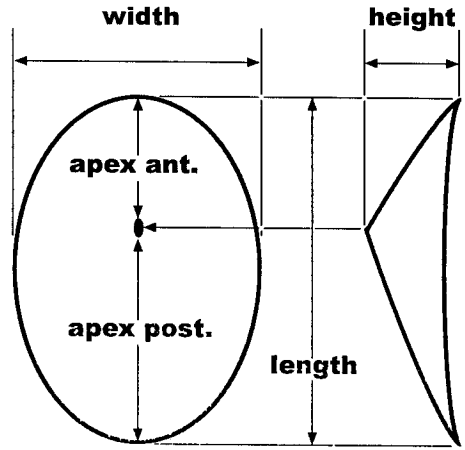

Figure 2. Shell measurements used in discriminant function analysis.

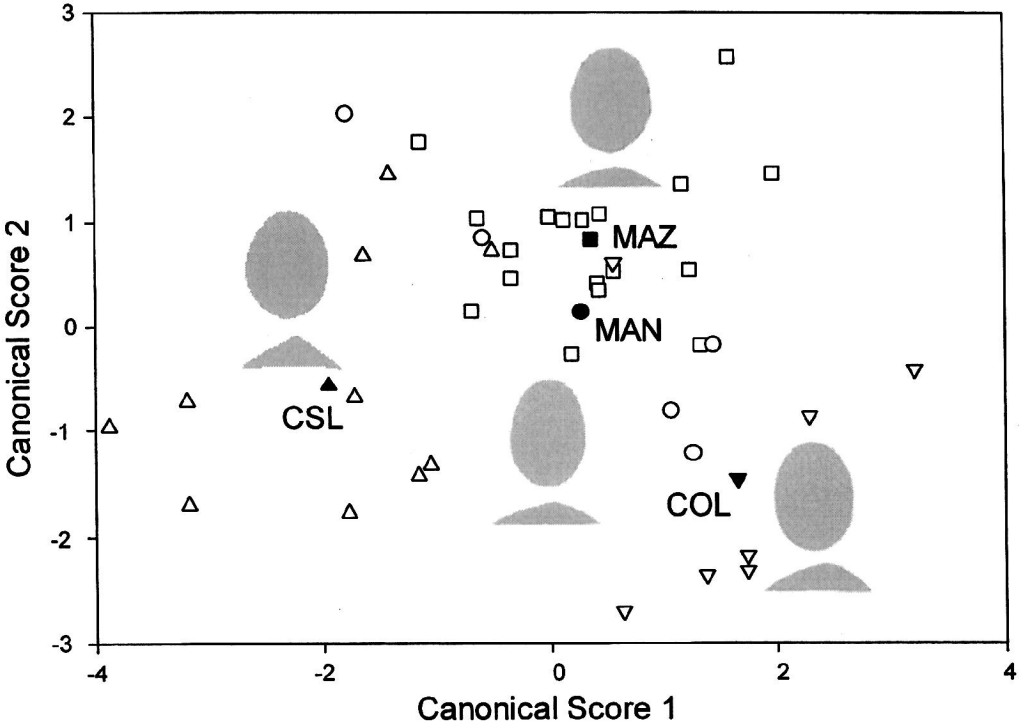

Figure 3. Scatterplot of first and second canonical scores from discriminant function analysis of *Notoacmea fascicularis* shell measurements. Open symbols are individual scores; solid symbols represent group means; shell silhouettes from arbitrarily chosen specimens from each locality. ▲ = Cabo San Lucas, ■ = Mazatlan, ● = Manzanillo, ▼ = Colima.

Historically, malacologists have placed substantial emphasis on radular characters in taxonomic studies, and McLean's suggestion that *N. fascicularis* was a cryptic species complex is consistent with this emphasis, as well as the then known range of variation in patellogastropod radular characters. While radular teratologies and variation in tooth cusp number have long been known (Jackson, 1907; Pelseneer, 1928; Peile, 1922; Eslick, 1940), the frequency and strongly bimodal distribution of radular morphologies seen in *N. fascicularis* were unprecedented.

Heavy reliance on radular characters in the diagnosis of patellogastropods can be traced back at least to the work of Dall (1871). With few exceptions (e.g. Grant, 1937; Eslick, 1940; Sasaki & Okutani, 1993, 1994), most workers have viewed the patellogastropod radula as invariant at the species level and thus an important and conservative character in determining and grouping species-level taxa (Dall, 1871; Oliver, 1926; McLean, 1966; Lindberg, 1981; Ponder & Creese, 1982; Lindberg & McLean, 1981). Moreover, the differences in the size of the third lateral teeth, such as documented here in a single putative species, were often regarded as characters indicative of generic rank (McLean, 1966; Christiaens, 1975a, b; Lindberg, 1981).

Grant's (1937:38) aversion to the use of radular tooth morphology in patellogastropod systematics did not originate from insights into possible variation in tooth shape, but rather her perception that unequal wear, abrasion and injuries associated with feeding were likely to deform individual teeth. Instead of tooth morphology she proposed that characters of the radular membrane were more diagnostic at specific and higher rankings. Sasaki & Okutani's (1993) example of variation in the patellogastropod radula is ontogenetic; uncini initially present at the edges of the radular membrane disappeared with growth in *Nipponacmea nigrans* (Kira, 1961) and *Nipponacmea teramachii* (Kira, 1961). Eslick (1940) also observed ontogenetic change in lateral tooth morphology of *Patella* species in England. Lastly, Sasaki & Okutani (1994) documented north-south clinal differences in second lateral tooth cusp morphology in *Nipponacmea habei* Sasaki & Okutani, 1994. However, none of these examples are of the magnitude of differences observed in the radula of *N. fascicularis*.

This, and a recent example in the littorinid *Lacuna* (Padilla, 1998), strongly suggest that our knowledge of the range of intraspecific variability of the gastropod radula is rudimentary at best, and that rigorous population level evaluation of radular morphology should be undertaken prior to its use in any phylogenetic analysis or species definition. It also shows the danger of basing identifications or groupings on single character systems. As Knowlton (1993) cautioned, reliance on a single character to distinguish sibling species is precarious, because single characters that delimited species in some taxa represented intraspecific variability in others. This insightful statement precisely describes the condition in *N. fascicularis*. Without the additional data sets and study, *N. fascicularis* might otherwise have been broken into separate species or subspecies based on radular morphology.

The basis of this radular variability remains unexplored in these patellogastropods. However, there is a statistically significant size effect associated with the radular types at Mazatlan (Table 2), where individuals with equal teeth are significantly larger than individuals with unequal teeth ($t = 4.235$, p value $= 0.001$). Sample sizes were not adequate to explore this relationship at other localities and this association is not present in the pooled data. Its absence in the pooled data most likely results from the confounding of different habitat-specific growth rates, a common feature of patellogastropod taxa (Giesel, 1969; Branch, 1974; Lindberg & Wright, 1985; Brown & Quinn, 1988; Liu, 1994). Adequate sampling of additional populations is needed to test further this hypothesis.

The pooled data also show no clinal variation in the occurrence of radular morphologies. Both radular morphologies occur in male and female limpets and individuals with different radulae can occur on the same rock in juxtaposition (WBS, pers. obs.). Gut contents appear identical in specimens with both tooth types, suggesting that unlike the phenotypic plasticity in *Lacuna* (Padilla, 1998), radular morphologies in *N. fascicularis* are neither habitat nor food induced.

This study and its findings calls into question past uncritical use of radular characters and single character taxonomies in patellogastropod classifications, and is likely applicable to other gastropod groups as well. Prior systematic work in the patellogastropods requires re-evaluation. For example, the sibling species pairs described from the Galapagos (Lindberg & McLean, 1981) are certainly suspect and, in the case of the patellogastropods, further

Table 3. Absolute pairwise distances between specimens of *N. fascicularis* from Mazatlan (EC), Cabo San Lucas (CSL, CH, SM) and Puerto Vallarta (LJM, Mexico; cytochrome *c* oxidase subunit I, 639 base pairs.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	EC1	–																													
2	EC2	2	–																												
3	EC3	1	1	–																											
4	EC4	1	1	0	–																										
5	EC5	1	1	0	0	–																									
6	EC6	2	2	1	1	1	–																								
7	EC7	1	1	0	0	0	1	–																							
8	EC8	3	3	2	2	2	3	2	–																						
9	EC9	3	3	2	2	2	3	2	4	–																					
10	EC10	0	2	1	1	1	2	1	3	3	–																				
11	EC11	1	1	0	0	0	1	0	2	2	1	–																			
12	EC12	4	4	3	3	3	4	3	5	1	4	3	–																		
13	EC13	0	2	1	1	1	2	1	3	3	0	1	4	–																	
14	EC19	1	1	0	0	0	1	0	2	2	1	0	3	1	–																
15	EC20	1	1	0	0	0	1	0	2	2	1	0	3	1	0	–															
16	EC21	2	2	1	1	1	0	1	3	3	2	1	4	2	1	1	–														
17	EC22	1	1	0	0	0	1	0	2	2	1	0	3	1	0	0	1	–													
18	LJM001	7	9	8	8	8	9	8	10	10	7	8	9	7	8	8	9	8	–												
19	LJM004	7	9	8	8	8	9	8	10	10	7	8	9	7	8	8	9	8	0	—											
20	LJM005	8	10	9	9	9	10	9	11	11	8	9	10	8	9	9	10	9	1	1	–										
21	CSL001	9	11	10	10	10	11	10	12	12	9	10	11	9	10	10	11	10	2	2	3	–									
22	CSL002	8	10	9	9	9	10	9	11	10	8	9	9	8	9	9	10	9	3	3	4	1	–								
23	CSL003	8	10	9	9	9	10	9	11	10	8	9	9	8	9	9	10	9	3	3	4	1	0	–							
24	CSL008	8	10	9	9	9	10	9	11	11	8	9	10	8	9	9	10	9	3	3	4	1	0	0	–						
25	CH1	9	11	10	10	10	11	10	12	12	9	10	11	9	10	10	11	10	2	2	3	0	1	1	1	–					
26	CH2	10	12	11	11	11	12	11	13	13	10	11	12	10	11	11	12	11	3	3	4	1	2	2	2	1	–				
27	CH3	8	10	9	9	9	10	9	11	11	8	9	10	8	9	9	10	9	3	3	4	1	2	2	2	1	2	–			
28	SM1	10	12	11	11	11	12	11	13	13	10	11	12	10	11	11	12	11	3	3	4	1	1	1	2	1	2	2	–		
29	SM2	11	13	12	12	12	13	12	14	14	11	12	13	10	12	12	13	11	4	4	5	4	5	5	5	4	5	5	5	–	
30	SM3	9	11	10	10	10	11	10	12	12	9	10	11	8	10	10	11	9	2	2	3	2	3	3	3	2	3	3	3	2	–

detailed studies of putative species and complexes will undoubtedly reduce estimates of diversity in some faunas while increasing it in others.

ACKNOWLEDGEMENTS

We thank J.H. McLean for providing specimens of *N. fascicularis* for study, and together with D.K. Padilla, C. Meyer and two anonymous reviewers, for providing helpful criticism of the manuscript. We also thank R. Nehm for thoughtful discussions. This work was supported, in part, by a grant from the Hawaiian Malacological Society and UCMP Field Funding to WBS. This is contribution no. 1669 from the University of California Museum of Paleontology.

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APPENDIX

Standardized canonical discriminant function (CDF) scores for locality and radular morphology classifications. DFA ALL = linear measurements, area measurements, and ratio data, DFA Ratios = ratio data alone, DFA Log (x) = log transformed linear and area measurements. ns = not significant in discrimination, N/A = not applicable.

Locality Classification

Variable	DFA ALL		DFA Ratios		DFA Log(x)	
	CDF 1	CDF 2	CDF 1	CDF 2	CDF 1	CDF 2
SHELLAREA	1.517	0.501	N/A	N/A	1.785	0.021
SHELLLENGTH	−3.498	−3.085	N/A	N/A	1.405	−6.452
SHELLWIDTH	ns	ns	N/A	N/A	ns	ns
APEXANT	5.862	−1.522	N/A	N/A	−1.334	2.559
APEXPOST	ns	ns	N/A	N/A	ns	ns
SCARAREA	−1.267	5.328	N/A	N/A	0.236	5.113
APEXHT	−1.019	−1.791	N/A	N/A	−1.538	−1.413
APEXPOS	−1.830	0.905	0.255	0.735	N/A	N/A
SCARSHELL	ns	ns	0.882	−0.515	N/A	N/A
HTWIDTH	ns	ns	−0.843	−1.571	N/A	N/A
HTLENGTH	ns	ns	0.060	0.770	N/A	N/A
WIDTHLENG	ns	ns	Ns	ns	N/A	N/A

Radular classification

Variable	DFA ALL	DFA Ratios	DFA Log(x)
	CDF 1	CDF 1	CDF 1
SHELLAREA	0.318	N/A	0.670
SHELLENGTH	1.109	N/A	2.204
SHELLWIDTH	ns	N/A	Ns
APEXANT	3.191	N/A	3.149
APEXPOST	ns	N/A	Ns
SCARAREA	−5.593	N/A	−5.572
APEXHT	1.219	N/A	−0.546
APEXPOS	ns	0.769	N/A
SCARSHELL	ns	−0.804	N/A
HTWIDTH	ns	0.599	N/A
HTLENGTH	−0.765	0.873	N/A
WIDTHLENG	ns	ns	N/A