

***Euraphia eastropacensis* (Cirripedia, Chthamaloidea), a New Species of Barnacle from the Tropical Eastern Pacific: Morphological and Electrophoretic Comparisons with *Euraphia rhizophorae* (deOliveira) from the Tropical Western Atlantic and Molecular Evolutionary Implications.¹**

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ABSTRACT: *Euraphia eastropacensis* sp. nov., of the tropical Eastern Pacific, is distinguished from its tropical Western Atlantic congener, *E. rhizophorae*, by morphological and electrophoretic evidence. Because of the apparent recent radiation of high intertidal chthamaloids and the recent closure of the Isthmus of Panama, one would expect that these two species of *Euraphia* were geminates. However, utilizing electrophoretic data, a large genetic distance value (0.95) was found, and this creates difficulties when explaining speciation between the two in terms of the molecular clock. A molecular evolutionary interpretation of the data suggests that the two species may have speciated before the closure of the Isthmus of Panama, probably as early as the Upper Miocene.

THE MARINE COMMUNITIES common to the proto-Caribbean and the tropical Eastern Pacific were separated by the tectonic closure of the Isthmus of Panama 3 million years ago. Morphologically similar remnants of this separation were presently found on both sides of the Isthmus of Panama. Among the barnacles we have members of the genus *Conopea*, *Megabalanus*, and *Euraphia* (Laguna 1985).

The population representing *Euraphia eastropacensis* sp. nov. was first reported from the Pacific coast of Panama by Southward and Newman (1977), who thought they both represented populations of *E. rhizophorae*, previously known from the tropical Western Atlantic. They noted that the morphologically similar Pacific and Caribbean populations occupy ecologically similar environments. Subsequent studies suggested that the two populations were sibling species (Newman, pers. comm. 1983; Henry, pers. comm. 1984). The purposes of this paper are to compare both populations, to describe the new tropical Eastern Pacific species, to provide electrophoretic evidence concerning their distinct-

ness, and to explain the evolutionary implications raised with respect to the molecular clock hypothesis.

MATERIALS AND METHODS

Specimens of *Euraphia* were collected on mangrove roots from the high intertidal on both coasts of Panama, from July to August of 1984. The Caribbean populations were from Corazon (San Blas) and Galeta (Colon), and the Pacific populations from Don Bosco (Panama) and Las Lajas (Chiriqui). To avoid a collecting bias (genetically similar specimens), samples were taken randomly from different mangrove roots at each site. This is important because barnacles are usually clumped together, and it is possible that most of the individuals within a clump could be more closely related than those between clumps, even after considering the barnacles' larval dispersal part of their life cycle.

Some specimens were preserved and dissected for morphological studies; others were frozen at -20°C and later transferred to -70°C , for electrophoretic essays. Forty-four individuals from two populations (22 from Galeta; 22 from Don Bosco) were analyzed

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electrophoretically. Electrophoretic techniques were those described by Hedgecock (1979) and Dando and Southward (1980), as modified by Huber (1985). All reagents used were from SIGMA Chemical Company, San Diego, California. Scoring and nomenclature follows the conventions of Ayala et al. (1973). Genetic distance (D) values were calculated by the method of Nei (1978). Genetic identity (I) values were later obtained from the genetic distance ($D = -\log_e I$).

SYSTEMATICS
ORDER THORACICA
FAMILY CHTHAMALIDAE
SUBFAMILY EURAPHIINAE
Genus *Euraphia* Conrad, 1837

Euraphia rhizophorae (deOliveira 1940); deOliveira 1941; Stubbings 1967; Bacon 1976; Newman and Ross 1976. (Figures 1A,C; 2A,C; 3A,C).

Distribution: Inhabiting the uppermost reaches of the intertidal; usually attached to *Rhizophora* (mangrove) roots; known from Brazil to Panama and the Antilles.

Diagnosis: Shell low conic, dark brown. Scutum as high as wide, articular ridge about 2/3 length of tergal margin, articular furrow elongated, shallow lateral depressor muscle pit, and a deep elongated adductor muscle scar. Tergum high with an open, shallow elongate, articular furrow.

Euraphia eastropacensis new species. (*Euraphia rhizophorae*, Southward and Newman 1977, in part) (Figures. 1B,D; 2B,D; 3B,D).

Holotype: United States National Museum (USNM) cat. No 222502 (Don Bosco, Panama).

Paratypes: USNM cat. No 222503 (Las Lajas, Panama); British Museum of Natural History (BMNH) 1985. 137–138 (Las Lajas, Panama).

Etymology: Eastern (“eas”) Tropical (“tro”) Pacific (“pac”).

Diagnosis: Shell high conic; grayish with purple-pink areas apically. Scutum wider than high, articular ridge about 3/4 length of tergal margin. Deep lateral depressor muscle pit, and a shallow elongated adductor muscle

scar. Tergum with very deep articular furrow. Standard genetic distance from the population of *Euraphia rhizophorae* of the Caribbean coast of Panama is 0.95 ± 0.31 (based on data for 17 presumptive gene loci).

Distribution: High intertidal, on mangroves and volcanic rock; Mazatlan (Mexico) to Panama; may range as far south as northern Ecuador.

Description: Scutum wider than high, purple; articular ridge long, about 3/4 length of tergal margin; articular furrow deep and narrow; lateral depressor muscle pit faint, adductor muscle scar shallow. Tergum same color, wide and “bilobed” above (Figure 2,B); articular furrow very deep; moderately deep depressor muscle crests. Mandible tridentoid with setae on upper and lower margins, lower angle with many spines (Figure 3B). Cutting edge of maxillae divided into three regions, two outer of same breadth and height, but narrower than the sunken middle one.

ELECTROPHORETIC CONFIRMATION

Seventeen presumptive gene loci were resolved for both populations (Table 1). Five were found to be monomorphic in all individuals (*AcpH*, *Ao*, *Aph*, *Me*, *Pgm*), and 12 (*Ao-2*, *Est-1*, α *Gpdh*, *Gdh*, *Got*, *Gpi*, *Hk-1*, *Hk-2*, *Idh-1*, *Idh-2*, *Prot*, *6-Pgdh*) were scored as polymorphic (those with any degree of variation in mobility). The presense of heterozygote bands on the gels suggests that these are possibly gene loci. In addition to the monomorphic loci, a common allele was found in both populations at three of the polymorphic loci (*Ao-2*, *Hk-1*, *Hk-2*). All other nine polymorphic loci showed fixed differences.

Table 2 shows allelic frequencies for each population at each polymorphic locus. The minimum genetic distance between *Euraphia eastropacensis* and *E. rhizophorae* is 0.60 (standard error 0.12), and the standard genetic distance (D) between them is 0.95 (standard error 0.31). The standard genetic distance with the error will approximately equal a range in time from 6 to 28 million years, using the calibration of Yang et al. (1974).

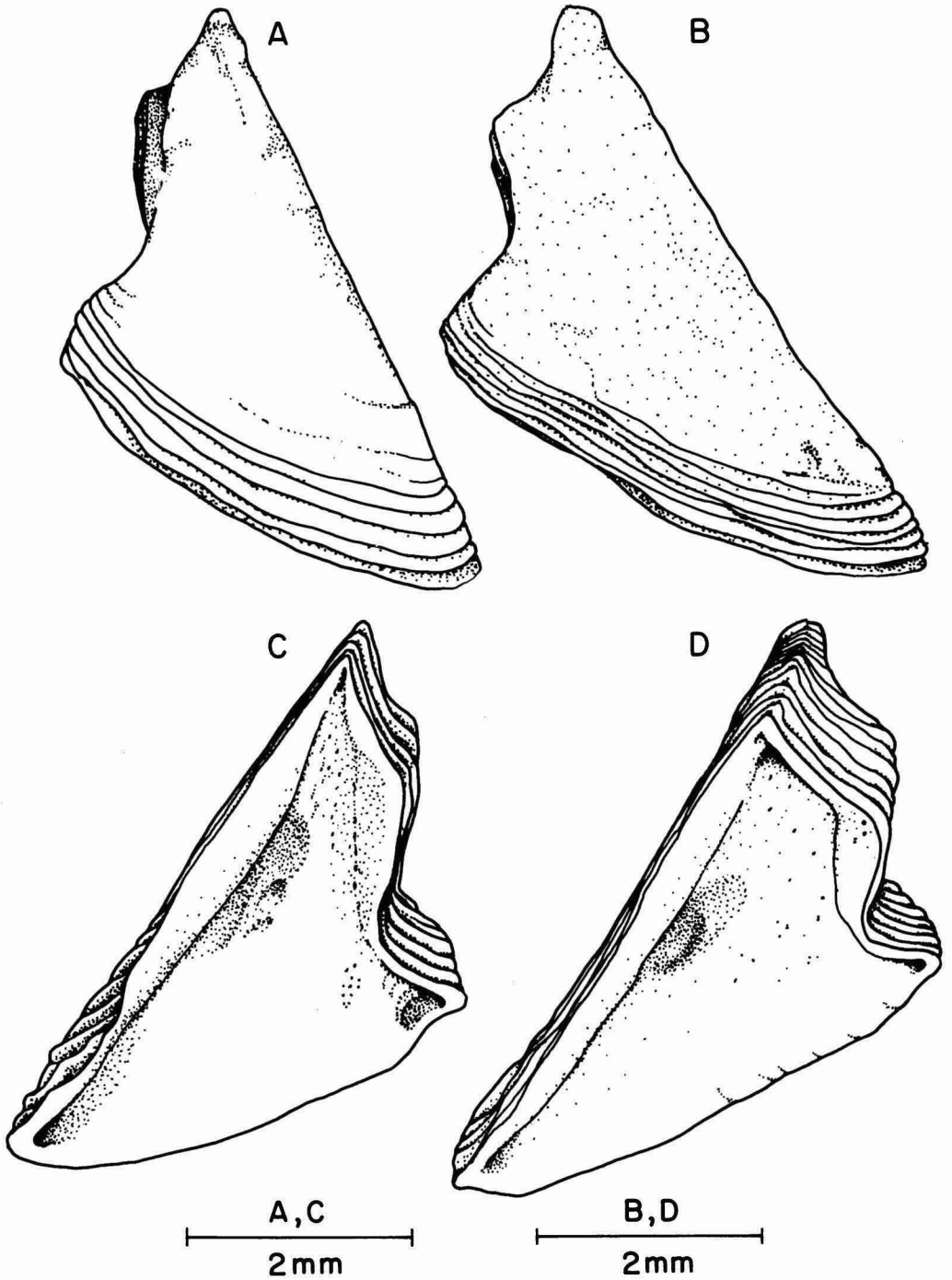


FIGURE 1. *Euraphia rhizophorae* s.s. (deOliveira 1940): external (A) and internal (C) views of left scutum; *Euraphia eastropacensis* n.sp., external (B) and internal (D) views of left scutum.

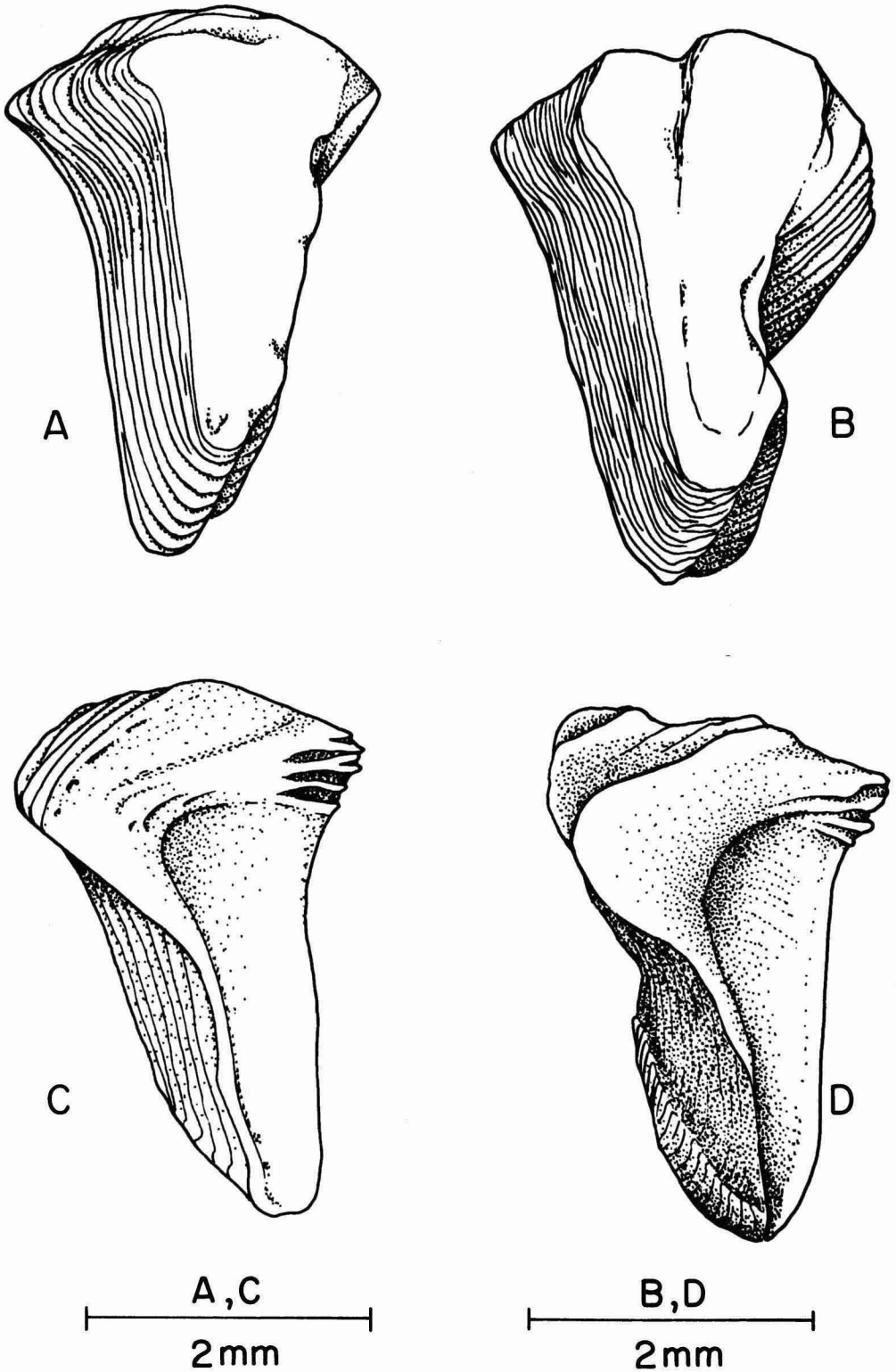


FIGURE 2. *Euraphia rhizophorae* s.s. (deOliveira 1940), external (A) and internal (C) views of left tergum; *Euraphia eastropacensis* n.sp., external (B) and internal (D) views of left tergum.

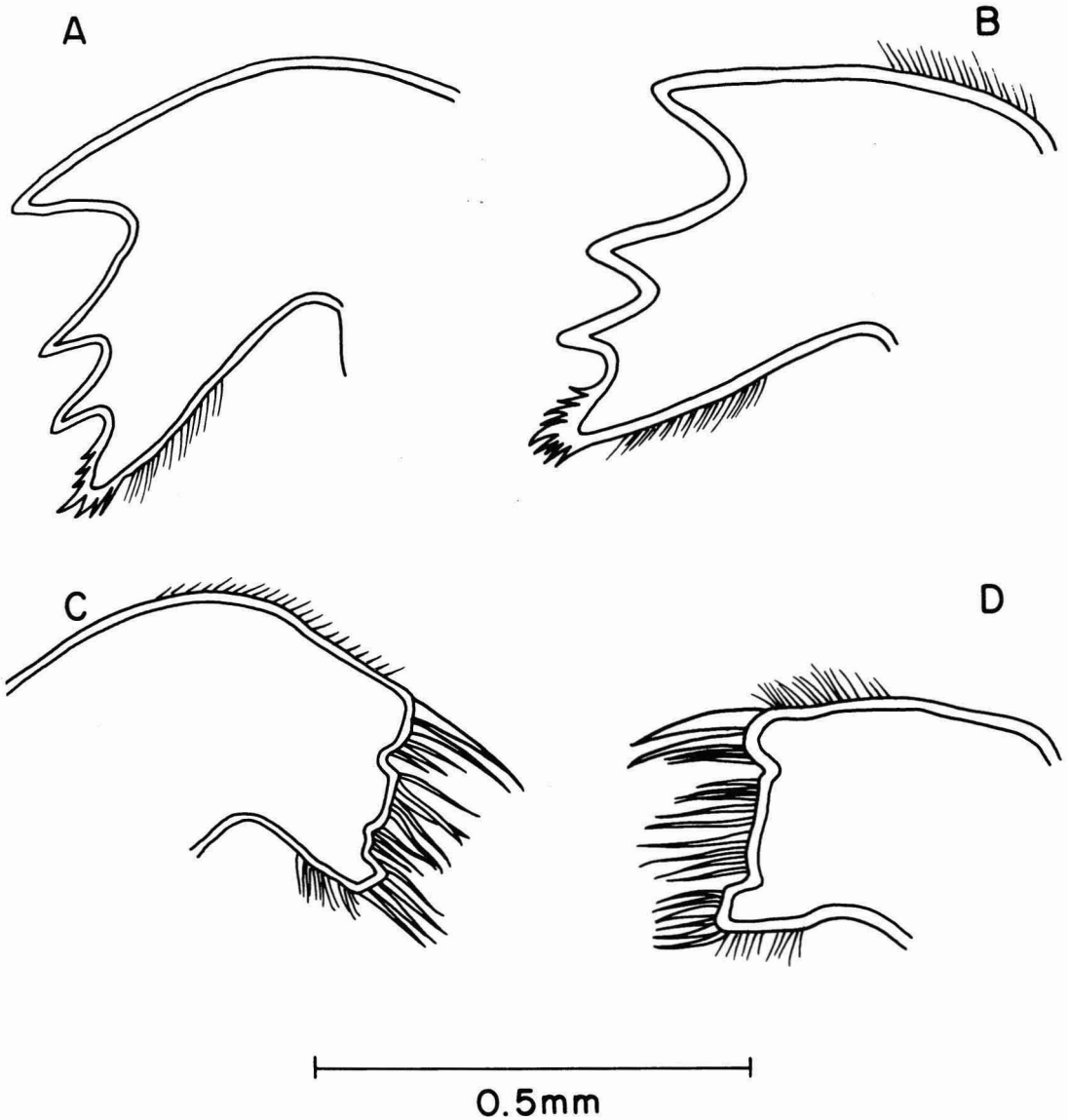


FIGURE 3. *Euraphia rhizophorae* s.s. (deOliveira 1940), lateral views of the mandible (A) and maxilla (C); *Euraphia eastropacensis* n.sp., lateral views of the mandible (B) and maxilla (D).

DISCUSSION AND CONCLUSIONS

The genetic identity (I) between the Caribbean and Pacific populations of *Euraphia* was 0.38 (D = 0.95). For most intraspecific populations the I value ranges from 0.8 to 1 (Thorpe 1982). This is much higher than the value derived for *Euraphia* (0.38). Avise

(1976) and Thorpe (1982) report standard genetic distance values of >0.6 for populations differing at the specific level. The presence of fixed differences in nine of the polymorphic loci studied is convincing evidence that the Caribbean and Pacific populations of *Euraphia* are different at the species level.

With the tectonic closure of the Isthmus of

TABLE 1
ENZYMES AND PROTEINS ASSAYED IN *Euraphia* spp., AND BUFFER SYSTEM USED

EC NO.	LOCI	ABBREVIATION	BUFFER SYSTEM
3.1.3.2	Acid phosphatase	Acph	E
?	Aldehyde oxydase	Ao	A
3.1.3.1	Alkaline phosphatase	Aph	A
NONSPECIFIC	Esterases	Est	E
2.6.1.1	Glutamate-oxaloacetic transaminase	Got	B
1.4.1.2	Glutamate dehydrogenase	Gdh	B
1.1.1.8	α -Glycerophosphate-dehydrogenase	α Gpdh	C
2.7.1.1	Hexokinase	Hk	B
1.1.1.42	Isocitrate dehydrogenase	Idh	C
1.1.1.37	Malate dehydrogenase	Mdh	C
1.1.1.40	Malic enzyme	Me	C
5.3.1.9	Phosphoglucose isomerase	Pgi	A
1.1.1.44	6-Phosphogluconate dehydrogenase	6Pgdh	C
2.7.5.1	Phosphoglucomutase	Pgm	C
NONSPECIFIC	General Protein	Prot	E

A = Poulick, B = DH buffer, C = JRP buffer (Ayala, et. al., 1973). E = LiOH-buffer (Selander, et. al., 1971).

TABLE 2
ALLELIC FREQUENCIES FOR POLYMORPHIC LOCI IN TWO POPULATIONS OF *Euraphia*

LOCUS	ALLELE	PACIFIC		ATLANTIC	
		NO ALLELES	FREQUENCY	NO ALLELES	FREQUENCY
Ao-2	114	1	0.062	0	0
	100	15	0.938	15	1.000
Est-1	100	0	0	16	1.000
	95	16	1.000	0	0
Gdh	100	0	0	16	1.000
	60	16	1.000	0	0
Got	110	2	1.000	0	0
	100	0	0	2	1.000
α Gpdh	100	0	0	16	1.000
	82	16	1.000	0	0
Hk-1	120	16	1.000	5	.312
	100	0	0	11	.688
Hk-2	100	10	1.000	1	.100
	80	0	0	9	.900
Idh-1	120	16	1.000	0	0
	100	0	0	16	1.000
Idh-2	100	0	0	16	1.000
	66	16	1.000	0	0
Pgi	100	0	0	16	1.000
	86	16	1.000	0	0
6-Pgdh	100	0	0	16	1.000
	60	16	1.000	0	0
Prot	100	0	0	26	1.000
	85	26	1.000	0	0

Panama (3 million years ago) the marine biotas of the Caribbean and Pacific Oceans were physically separated. Morphologically similar geminate species presently found on

either side of the Panamanian Isthmus, are thought likely to be the result of this vicariant event. Low genetic distance values (<0.4) would be expected for the sibling species

formed by this vicariant event (Lessios, 1979; Vawter et al, 1980). However, an extremely high genetic distance (0.95) has been found between these two species of *Euraphia*.

There are two hypotheses that could explain the high genetic distance found for *Euraphia*. The one generally accepted is that the molecular clock is constant, and in that event, the species of *Euraphia* may have speciated before the closure of the Isthmus of Panama. The other is that the molecular clock may run at different rates for different organisms, due to differential mutation rates and different selective pressures.

When dealing with electrophoretic studies, the molecular clock cannot be thought of as a "true clock with substitutions taking place at regular time intervals" (Thorpe, 1982). The clock is based on probability of genetic substitutions which are not expected to occur at regular intervals (Ayala, pers. comm. 1985). This would enable us to understand the genetic distance discrepancies found for some species.

The deployment of living chthamaloid species suggests that their number, in general, has been declining since their early radiation in the Cenozoic. Only the high intertidal group of *Chthamalus* has been very successful (Stanley and Newman, 1980). Based on the lack of a fossil record and the low D-values for most species of *Chthamalus* studied (Hedgecock, 1979), it has been proposed that most extant species of the genus have radiated recently (Stanley and Newman, 1980; Newman and Zullo, pers. comm. 1985).

Therefore, it is possible that the species of *Euraphia* studied here may have evolved recently. In this case, the genetic distances would have to be <0.6 , younger than 6 m.y., in order to fall outside the ranges expected from the results, 6–28 m.y. (including the standard error; 16 m.y. without the error). This is not the case, and unless we can explain the high genetic distance by assuming a higher mutation rate for *Euraphia*, the two species separated well before the closure of the Isthmus of Panama.

The genetic distance between the two species of *Euraphia* can be compared with those of other barnacles and other organisms. Hed-

gecock (1979) found high (0.46–0.77) genetic distance values between four closely-related species of *Chthamalus* from the tropical Eastern Pacific. This genus is closely related to *Euraphia*, and the genetic distance values for transisthmian species of the latter (this report) are not substantially different from some *Chthamalus* (0.77).

Some "transisthmian" electrophoretic work shows similar, but lower genetic distances for "siblings". Lessios (1979) found a mean genetic distance of 0.64 (0.82) (the numbers in parentheses were obtained from analyses restricted to 12 loci common to both genera) between the sea urchins, *Echinometra viridis* and *E. lucunter*, and of 0.55 (0.66) between *E. lucunter* and *E. vabruni*, from the Atlantic and the Pacific respectively. He also found that the mean genetic distance between *Diadema antillarum* (Caribbean) and *D. mexicanum* (Pacific) was 0.026(0.033). Nevertheless, Vawter et al. (1980) suggested that in the case of the genus *Echinometra*, Lessios may have been looking at species that already existed before the closure of the Isthmus of Panama.

Woodring (1966) suggested that the fauna in both oceans were very similar until the late Miocene. However, even though the complete closure of the "Panamic Strait" took place in the late Pliocene, the physical conditions on the proto-Caribbean and Pacific had begun to differ by about the late Miocene (Keigwin 1982). Therefore, it is very likely that, being under different selective pressures, *E. rhizophorae* and *E. eastropacensis* may have diverged by then. This would be in agreement with the constant "Clock Hypothesis". However, it will not explain the values found for *Diadema* (Lessios, 1979).

Assuming that the molecular clock is running slowly for *Diadema* (Lessios, 1979), it is also possible that it may be running faster for the species of *Euraphia*. Species of the latter genus live high on the intertidal possibly under greater selective pressure (heat, desiccation, terrestrial, and aquatic predators) than the subtidal species of *Diadema*.

However, the fossil record, biogeography, and morphology suggest that euraphian chthamaloids are relatively old at the generic level, dating at least to the Upper Miocene if

not the Middle Eocene (Newman et al. 1969, pers. comm. 1985). Therefore it is possible that the morphologically stable species of *Euraphia rhizophorae* and *Euraphia eastropacensis*, existed before the rise of the Panamic Isthmus. This hypothesis is supported by the high genetic distance values that were found.

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