

**AN ELECTROPHORETIC STUDY OF TWO SIBLING SPECIES
OF THE GENUS *Gymnophthalmus* AND ITS BEARING ON
THE ORIGIN OF THE PARTHENOGENETIC *G. underwoodi*
(SAURIA: TEIIDAE)**

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ABSTRACT

The average heterozygosity of a population of the unisexual lizard *Gymnophthalmus underwoodi* and of an undescribed bisexual species *Gymnophthalmus* sp were estimated from horizontal starch gel and vertical polyacrylamide gel electrophoresis for allozymes of 14 different loci. The heterozygosity levels for both species were very low (about 0.06). The origin of the unisexual *G. underwoodi* is discussed, considering the new data and previous studies. Probably, two species have been confused under the name *G. underwoodi*, both parthenogenetic, but the data suggest that they differ with regard to mechanism of origin.

INTRODUCTION

About 34 species of unisexual reptiles are known at present: most of these (about 32) are lizards (Darevsky *et al.*, 1985; Skalka and Vozenilek, 1986; Moritz, 1987; Vrijenhoek *et al.*, 1989). Although a high proportion of these species are obligate parthenogens, it has been suggested that some of them have both bisexual and unisexual populations (Vanzolini, 1970).

A great amount of evidence has been accumulated supporting a hybrid origin for parthenogenesis in lizards (see reviews by Darevsky *et al.*, 1985 and by Cole *et al.*, 1988). Such evidence, based mainly on color pattern, pholidosis, karyotypes, al-

lozymes, and more recently on mitochondrial DNA analysis (Brown and Wright, 1979; Wright *et al.*, 1983; Densmore *et al.*, 1985, 1989a,b; Moritz *et al.*, 1989a,b), supports a hybrid origin for exclusively unisexual species. This model is, however, far from universally applicable; some authors have proposed a nonhybrid origin for some parthenogenetic lizards, based on the absence of the presumed parental species, on the presence of occasional males or on low levels of allozyme heterozygosity. The classic case is the Amazonian teiid lizard *Cnemidophorus lemniscatus* (Vanzolini, 1970). There is no evidence, neither morphological nor geographical, to distinguish the bisexual from the parthenogenetic populations; so both are considered as belonging to the same species (Vanzolini, 1970; Peccinini-Seale and Frota-Pessoa, 1974). Although Peccinini-Seale (1989) shows sharp differences in karyotypes and protein electrophoresis among the populations of *Cnemidophorus lemniscatus* of the Amazon valley, the mechanisms of origin have remained unsolved. Based on mitochondrial DNA studies, it appears now that the lizard originated via hybridization (Sites *et al.*, 1989 and Vyes *et al.*, 1989 *apud* Moritz *et al.*, 1989a,b). The recent discovery of triploid populations of this lizard (Serena, 1985; Dessauer and Cole, 1989), the several cytotypes described (Peccinini, 1971; Peccinini-Seale and Frota-Pessoa, 1974; Peccinini-Seale, 1989), and the electrophoretic studies (Peccinini-Seale, 1989; Dessauer and Cole, 1989), give support to the idea that *C. lemniscatus* is a complex of sibling or cryptic species. Much more morphological and geographical data are necessary to confirm whether hybridization is the only mechanism involved in the origin of this complex of species. Until then, a nonhybrid origin can not be discarded.

The small microteiid lizards of the *Gymnophthalmus underwoodi* complex (Vanzolini, 1976; Hoogmoed, 1973), some species of *Lacerta* (Cuellar, 1977) and *Lepidodactylus lugubris* (Pasteur *et al.*, 1987) are other related examples of parthenogenetic lizards for which a spontaneous origin remains possible.

Gymnophthalmus underwoodi was originally recognized as parthenogenetic by Thomas (1965). It occurs on Trinidad, Tobago, Barbados, St. Vincent, Surinam, and in some areas of Brazilian Amazonia. Hoogmoed (1973), investigating the presence of femoral pores in a large series of this lizard concluded that this species was parthenogenetic. Vanzolini (1976), reported the presence of males in some populations and suggested a nonhybrid origin for the lizard. Later, Hardy *et al.* (1989), studying the parthenogenetic reproduction of *G. underwoodi* suggested that the males found by Vanzolini (1976) were in reality "members of a closely related, cryptic or sibling, bisexual species that may be one of the ancestors of *G. underwoodi* (Cole *et al.*, unpublished observations)". Cole *et al.* (1983) analyzed electrophoretic patterns of 21 structural protein gene loci of *G. underwoodi* from Trinidad and Surinam and found a heterozygosity index of 0.38 with apparently fixed polymorphism at eight loci. This high level of heterozygosity led them to propose a hybrid origin for

G. underwoodi. After their studies, Cole *et al.* (1983) proposed that the parental species involved in the origin of *G. underwoodi* were *G. speciosus* and *G. pleei*. Recently, Cole *et al.* (1989) added new data to the problem. Through karyotypic and electrophoretic studies, they deduced the existence of a putative *Gymnophthalmus* species, that may have hybridized with *G. speciosus* to originate *G. underwoodi*. They eliminated the hypothesis that *G. pleei* could be one of the parental forms of *G. underwoodi*, because these two species do not share several genetic markers. For Dessauer and Cole (1989), such hybridization may have occurred, as proposed for the *Cnemidophorus lemniscatus* complex, in a savanna and/or in a forest edge ecotone as a consequence of habitat shifts during the recent pleistocene.

Four years ago, Vanzolini and Carvalho, during a herpetological survey of the Brazilian state of Roraima, initiated systematic work on the *G. underwoodi* complex. They determined that at least two forms were involved and occurred sympatrically (Vanzolini and Carvalho, personal communication). One of them, an all female population, was attributed to *G. underwoodi*. The other, a bisexual form, easily distinguished by morphological characters, is being described as a new species (Vanzolini and Carvalho, 1991). P.E. Vanzolini and C.M. Carvalho kindly supplied us with specimens of these two species, referred to here as *G. underwoodi* and *Gymnophthalmus* sp n.

The electrophoretic patterns of allozymes were studied in order to evaluate the magnitude of the protein differences among these two forms and to clarify the origin of *G. underwoodi*.

MATERIALS AND METHODS

A total of 28 specimens were utilized to analyze electrophoretically the gene products of 14 loci: 8 females of *G. underwoodi* and 7 males and 13 females of *G. sp n*. The samples of *G. underwoodi* were obtained in a forest on Ilha de Maracá in the Rio Uraricoera (03°24'N 61°38'W), Roraima, Brasil. Specimens of *G. sp n*. were collected at Fazenda Salvamento, an open area on the right bank of Rio Uraricoera (03°20'N 61°24'W), Roraima. They were shipped to the laboratory alive. The livers were removed from the lizards and frozen immediately in liquid nitrogen. Voucher specimens were deposited in the herpetological collection of the Museu de Zoologia, University of São Paulo (see appendix). Distribution and ecology of these two species are discussed by Vanzolini and Carvalho (1991).

Livers were ground, according to Selander *et al.* (1971). Homogenates were then centrifuged at 3,000 rpm for 30 minutes, and the supernatants refrozen at -20°C prior to use. Standard procedures of horizontal starch gel electrophoresis were used as described by Malavasi and Morgante (1982). The following buffer systems were

used (see Table I). 1 - TC 8.5. Electrode: 0.135 M tris - 0.08 M citric acid, pH 8.0. Gel: 0.08 M tris - 0.05 M citric acid, pH 8.5. 2 - TC 8.0. Electrode: 0.687 M tris - 0.157 M citric acid, pH 8.0. Gel: 0.023 M tris - 0.005 M citric acid, pH 8.0. 3 - LiOH 8.2. Electrode: 0.05 M LiOH - 0.19 M boric acid, pH 8.1. Gel: 0.005 M LiOH - 0.0019 boric acid - 0.045 M tris - 0.0072 M citric acid, pH 8.2.

Table I - Loci examined, abbreviations used, Enzymes Commission (E.C.) numbers (Commission on biochemical nomenclature, 1974) and respective buffer systems.

Locus	No. loci scored	Abbreviation	E.C.	Buffer system
Esterase	2	EST	3.1.1.1	LiOH
Fumarate hydratase	1	FUM	4.2.1.2	TC 8.5
Glucose dehydrogenase	1	GDH	1.4.1.2	TC 8.5
Glutamate-oxaloacetate transaminase	2	GOT	2.6.1.1	LiOH
3-Hydroxyisobutyrate dehydrogenase	1	HBDH	1.1.1.30	TC 8.5
Isocitrate dehydrogenase	1	IDH	1.1.1.42	TC 8.5
Lactate dehydrogenase	2	LDH	1.1.1.27	TC 8.0
Malate dehydrogenase	2	MDH	1.1.1.37	TC 8.0
Phosphoglucumutase	1	PGM	2.7.5.1	TC 8.0
Superoxide dismutase	1	SOD	1.15.1.1	TC 8.5

Gels were prepared with a 12% starch blend (6% Electrostarch - Electros-tarch Co., Madison, Wisconsin, USA and 6% potato starch - Sigma Chemical Co., St. Louis, Missouri, USA). Electrophoresis conditions were 4°C; buffer system 1 - 7.8 V/cm for 7 hr; buffer system 2 - 5.6 V/cm for 25 hr; and buffer system 3 - 27.8 V/cm for 7 hr. The loci examined and buffer conditions used are listed in Table I. The staining techniques were basically those of Shaw and Koen (1968), Shaw and Prasad (1970) and Harris and Hopkinson (1976).

Vertical polyacrylamide gel electrophoresis was also used to obtain better resolution according to: *Polyacrylamide Gel Electrophoresis* (1983, page 19), with the following modifications: a "separating gel" concentration of 5% was used; the gel and the electrode buffer did not contain SDS; and 20% sucrose was added to the gel to reduce convection currents.

Multiple loci were numbered from cathode to anode and letters were assigned to the electromorphs according to their mobility, beginning with the fastest one. The electromorphs were identified by comparison with standards included in each gel (internal controls) and by critical side-by-side comparisons (line-ups -

Richardson *et al.*, 1986). The average heterozygosity per loci was calculated by Nei's (1978) method for small populations.

RESULTS

Table II summarizes the results obtained. Selected zymograms of all enzymatic systems studied are presented in Figure 1. After study of 14 loci, 21 electromorphs were identified.

Table II - Frequencies of electromorphs observed among *G. underwoodi* and *G. sp n.*, and their respective values of average heterozygosity per locus. The minus sign (-) indicates that the electromorph migrated cathodically. The number of samples analysed are in brackets.

Locus	Electromorph	<i>Gymnophthalmus</i>	
		<i>sp n.</i>	<i>underwoodi</i>
EST-1	a	null (19)	1.00 (8)
EST-2	a	0.53 (20)	0.50 (8)
	b	0.47	0.50
FUM	a	1.00 (7)	1.00 (8)
GDH	a	1.00 (15)	1.00 (8)
GOT-1	a	1.00 (7)	- (8)
	b	-	1.00
GOT-2	a	- 1.00 (7)	- 1.00 (8)
HBDH	a	- (7)	0.94 (8)
	b	1.00	0.06
IDH	a	1.00 (15)	1.00 (8)
LDH-1	a	1.00 (15)	1.00 (8)

Continued

Table II - Continued.

Locus	Electromorph	<i>Gymnophthalmus</i>	
		sp n.	<i>underwoodi</i>
LDH-2	a	1.00 (15)	1.00 (8)
MDH-1	a	1.00 (15)	1.00 (8)
MDH-2	a	1.00 (15)	1.00 (8)
PGM	a	0.08	0.06
	b	0.05	0.88
	c	- (19)	0.06 (8)
	d	0.84	-
	e	0.03	-
SOD	a	1.00 (15)	1.00 (8)
H _L	=	0.057 ± 0.004	0.064 ± 0.024

The only problem of interpretation is related to the Esterase system. There are two ways of interpreting the EST-zymogram. Four electromorphs were found in *G. underwoodi* and two in *G. sp*, the latter shared with *underwoodi*. The faster electromorph of Figure 1A was considered to be coded by one locus (EST-1) and was only found in *G. underwoodi*. Because electromorph "b" was absent in one specimen of the sample of *G. sp*, the three lower and equidistant electromorphs were considered as an independent system (EST-2). In this case, *G. underwoodi* would be monomorphic for EST-1 and have a fixed heterozygosity for EST-2; *G. sp* would be polymorphic for EST-2 and have no EST-1 activity. The alternative view is to accept that each of the two faster and two lower electromorphs were coded by two different loci. Under this alternative, *G. underwoodi* would have a fixed heterozygosity at both loci and *G. sp* would be monomorphic at one locus and polymorphic, with a "null" allele, at the other. Figure 1A shows the first, and more probable hypothesis, because it considers the electromorph equidistance and does not accept the presence of a "null" allele at one locus already having another expressed allele in the same species.

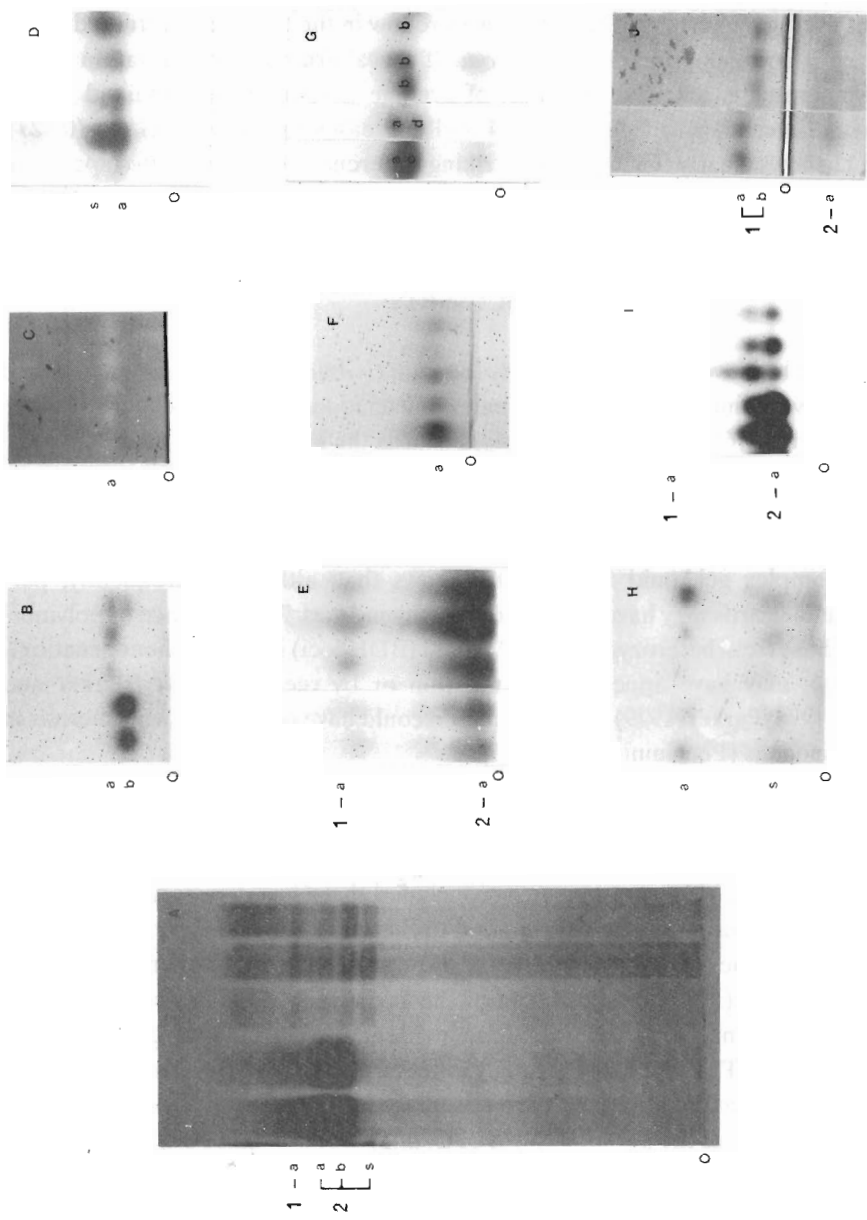


Figure 1 - Electrophoretic patterns of some allozymes. In all zymograms, the first two individuals are *Gymnophthalmus* sp. n. and the following three are *G. underwoodi*. A - EST-1 and EST-2 in vertical polyacrylamide gel. All the others were done in horizontal starch gels: B - HBDH. C - SOD. D - IDH. E - MDH-1 and MDH-2. F - FUM. G - PGM. H - GDH. I - LDH-1 and LDH-2. J - GOT-1 and GOT-2. The letter O shows the site of sample application, and the letter S indicates secondary bands. The cathode is below

Only three loci (EST-2, HBDH, and PGM) were polymorphic in *G. underwoodi*. In *G. sp n.* polymorphism was detected at two loci: EST-2 and PGM. An apparently fixed polymorphism at the locus EST-2 occurs in *G. underwoodi*. The levels of average heterozygosity per locus were low in the two species studied: 0.057 for *G. sp n.* and 0.064 for *G. underwoodi*. If the alternative interpretation of the EST-zymograms is adopted, the level of average heterozygosity obtained for *G. underwoodi* increases to 0.101 ± 0.024 and decreases for bisexual *G. sp* (0.027). Figure 1J shows clearly that there is a striking difference between the two species in terms of mobility of the electromorphs at locus GOT-1: *G. sp* is characterized by electromorph "a" and *G. underwoodi* by electromorph "b".

DISCUSSION

The low levels of heterozygosity found in *Gymnophthalmus underwoodi* and *G. sp n.* are very similar to the average index of 0.05 found for bisexual teiids (Parker and Selander, 1976). For unisexual diploid lizards the heterozygosity indexes range from 0.27 to 0.57 (Dessauer and Cole, 1984). Pasteur *et al.* (1987), found levels of heterozygosity for parthenogenetic diploid populations ranging from 0.036 to 0.055, and an index of 0.108 for a triploid parthenogenetic population in *Lepidodactylus lugubris* complex gekkonid lizards. This shows that, although infrequently, parthenogenetic lizards may have low heterozygosity indexes. The presence of polymorphism without fixed heterozygotes (PGM and HBDH loci) in the parthenogenetic *G. underwoodi*, may have appeared by mutation or by recombination (Parker and Selander, 1976; Parker, 1979). Recombination could have occurred during meiosis in the parthenogens (Peccinini-Seale, 1989).

The low heterozygosity found in *G. underwoodi* (0.064), and the fact that there is only one locus (EST-2) with apparently fixed polymorphism, suggest the possibility of a nonhybrid origin for this species. Although Cole *et al.* (1983), studying the electrophoretic patterns of proteins concluded that *G. underwoodi* had a hybrid origin, their conclusions cannot be applied to the population studied in this paper: the indexes obtained are sharply different (0.38 against 0.064). Yonenaga-Yassuda and collaborators (in preparation) studied the karyotypes of the same sample of *G. underwoodi* we analysed and found no evidence of structural chromosomal heteromorphism. This result also strongly differs from that of Cole *et al.* (1989), who demonstrated a marked structural chromosomal heteromorphism and, on this basis, suggested that one of the parental forms of *G. underwoodi* might be *G. speciosus*.

The differences between these two populations of *G. underwoodi* are strong, and clearly suggest that there are two parthenogenetic species involved under the name *underwoodi*. In fact, it is very possible that the forms that we and Cole *et al.*

(1983) studied are sibling species that have had different origins. The electrophoretic results presented here, especially the low heterozygosity level, and the preliminary karyotypic data mentioned above (obtained by Yonenaga and co-workers), suggest that the *G. underwoodi* form from Ilha de Maracá, Roraima, could have had a nonhybrid origin as Vanzolini (1976) already postulated for this species. On the other hand, the electrophoretic and the karyotypic data published by Cole *et al.* suggest that they have found another form of *underwoodi*, for which data are compatible with an origin via hybridization. The size of the differences among the respective studied reinforces the hypothesis that *G. underwoodi* is a complex of sibling species, and can thus explain the disparity in the results obtained.

This problem has become even more interesting after the recent discovery of the sympatric *G. sp* (Vanzolini and Carvalho, 1991). The differences between these species, besides mode of reproduction, include pholidosis and caudal color pattern. We detected, in *G. sp*, at least one more difference from *G. underwoodi*: GOT-1 presented only the "a" allele (Figure 1), supporting specific rank. The discovery of *G. sp* in open plant formations resulted in new ecological and evolutionary perspectives on the origin of the form of *G. underwoodi* by Cole *et al.* (1983). *G. sp* may be one of the forms responsible for the hybrid origin of *G. underwoodi*. Therefore, biochemical and karyotypic studies of other populations of *Gymnophthalmus*, together with a taxonomic revision of the group, have become necessary to understand the phylogenetic relationships and the evolutionary implications of parthenogenesis in this group.

ADDS TO PROOF

Cole *et al.* (1990) recently performed extensive morphological, karyotypic and biochemical genetics studies on *G. underwoodi*. Based on morphological analysis it was suggested that the population from Ilha de Maracá is a distinctive clone of *G. underwoodi*, though it could be a different species. These new data reinforce the hypothesis proposed in this paper that at least two species have been confused under the name *G. underwoodi*.

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RESUMO

A heterozigiosidade média de uma população do lagarto unissexual *Gymnophthalmus underwoodi* e do bissexual, ainda não descrito *Gymnophthalmus* sp, foi estimada a partir de eletroforese em gel de amido horizontal e em gel de poliácridamida vertical para alozimas de 14 locos diferentes. O nível de heterozigiosidade para ambas as espécies foi muito baixo (por volta de 0,06). A origem do lagarto unissexual *G. underwoodi* é discutida, considerando este novo dado e estudos prévios feitos nesta espécie. Sugere-se que sob o nome *Gymnophthalmus underwoodi* estão sendo indiscriminadamente tratadas duas boas espécies com mecanismos de origem diferentes.

APPENDIX

All *Gymnophthalmus* used in these analyses have been deposited as voucher specimens in the herpetological collection of the Museu de Zoologia, Universidade de São Paulo, São Paulo, Brasil. Locality data and field numbers are presented in the table below:

Taxon	Locality	N	Voucher specimens
<i>G. underwoodi</i>	Ilha de Maracá,	8	887133,34,39,40
	Roraima, Brasil		887339-42
<i>G. sp n.</i>	Fazenda Salvamento,	20	887131,32,37,38
	Roraima, Brasil		887301-03, 05-11, 16-20, 38

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