Molecular analysis of the taxonomic and distributional status for the hermit crab genera *Loxopagurus* Forest, 1964 and *Isocheles* Stimpson, 1858 (Decapoda, Anomura, Diogenidae)

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ABSTRACT
Recent studies of adult and larval morphology have revealed characters that could justify reassignment of *Loxopagurus loxochelis* to the genus *Isocheles*. Current taxonomy of these two similar genera is based only on adult morphology and disregards morphological characters of the first zoeal stage. There has been no previous attempt to resolve evolutionary relationships among the species of these two genera using molecular tools. Herein, we include these species in a phylogenetic analysis of selected anomuran decapods based on sequences of the 16S ribosomal gene. Our present molecular analysis does not support reassignment of *Loxopagurus loxochelis* to the genus *Isocheles*. In contradiction to previous suggestions, we show that *L. loxochelis* and *I. sawayai* are indeed different species. We also corroborate the previous report of *I. sawayai* in Venezuelan waters.

KEY WORDS
Crustacea,
Decapoda,
Anomura,
Diogenidae,
Isocheles,
Loxopagurus,
molecular systematics,
16S rRNA,
South Atlantic.

RÉSUMÉ

Statut taxonomique et distribution des genres Loxopagurus *Forest, 1964 et* Isocheles *Stimpson, 1858 (Decapoda, Anomura, Diogenidae) sur la base de données moléculaires.*

Des études récentes concernant la morphologie de l'adulte et des larves ont révélé des caractères qui pourraient justifier un transfert de *Loxopagurus loxochelis* au genre *Isocheles*. La taxonomie classique de ces deux genres similaires est basée uniquement sur l'adulte et ne tient aucun compte des caractères morphologiques du premier stade de la zoé. Il n'y a eu jusqu'à présent aucun essai de résoudre les affinités entre les espèces de ces deux genres au moyen de l'analyse moléculaire. Dans ce travail, nous incluons ces espèces dans une analyse phylogénique de décapodes anomoures choisis, basée sur les séquences du gène ribosomique 16S. Notre analyse moléculaire ne justifie pas le transfert de *Loxopagurus loxochelis* dans le genre *Isocheles*. En contradiction avec les suggestions précédentes, nous montrons que *L. loxochelis* et *I. sawayai* sont bien des espèces différentes. Nous confirmons également la présence d'*I. sawayai* dans les eaux vénézuéliennes.

MOTS CLÉS
Crustacea,
Decapoda,
Anomura,
Diogenidae,
Isocheles,
Loxopagurus,
systématique moléculaire,
16S rRNA,
Atlantique Sud.

INTRODUCTION

Loxopagurus loxochelis (Moreira, 1901) is a monotypic hermit crab genus endemic to the southwestern Atlantic coast, occurring in Brazil (from Bahia to Rio Grande do Sul states), Uruguay, and Argentina (Melo 1999). *Isocheles* Stimpson, 1858 is poorly known, with only a few of the constituent species treated in studies concerning biology or ecology (unpublished data). The biogeographical distribution of this genus is restricted to shallow waters of the tropical and subtropical American coasts, where five species occur: *I. pilosus* (Holmes, 1900), *I. pacificus* (Bouvier, 1907) and *I. aequimanus* (Dana, 1852), in the eastern Pacific Ocean; and, *I. sawayai* Forest & de Saint Laurent, 1968, and *I. wurdemanni* Stimpson, 1859, in the western Atlantic Ocean (Forest & de Saint Laurent 1968). Species of *Loxopagurus* and *Isocheles* are similar in general somatic morphology, and their current taxonomy is based solely on adult morphology. A critical study of the relationship between these genera and between constituent species of *Isocheles* has been long called for (Provenzano 1959; Forest & de Saint Laurent 1968). A recent, unpublished systematic review on the basis of adult and larval morphology (Nucci 2002) reported characters that potentially justify reassignment of *L. loxochelis* to *Isocheles*. Nucci examined *L. loxochelis* and *I. sawayai* and, on the basis of morphological characters of adults, and published information on larvae (Forest & de Saint Laurent 1968; Negreiros-Fransozo & Hebling 1983; Bernardi 1986; Hebling *et al.* 2000), proposed that *L. loxochelis* be placed into synonymy with *I. sawayai*. To date, most systematic studies have been based solely on morphology and molecular tools have been rarely applied to solve questions of species status or to determine lower level phylogenetic relationships (Morrison *et al.* 2002). On the basis of partial sequences of the large ribosomal subunit 16S, we test morphologically based reassignment of *Loxopagurus* to *Isocheles*, and consequently whether *L. loxochelis* is a synonym of *I. sawayai*.

MATERIAL AND METHODS

Our phylogenetic analysis was based exclusively on a partial fragment of the 16S rDNA gene. Mitochondrial DNA (mtDNA) is maternally inherited, easy to isolate, and abundant; even so, absence of

TABLE 1. — Hermit crab species used for the phylogenetic reconstructions with respective date and site of collection, museum catalog number, and genetic database accession numbers (GenBank). Notes: **a**, Morrison *et al.* (2002); **b**, Zaklan & Cunningham (unpublished); **c**, Pérez-Losada *et al.* (2002); **d**, Schubart *et al.* (2000a). *, This species was referred to as "*Pachycheles haigae*" in Pérez-Losada *et al.* (2002); according to Harvey & De Santo (1996), *P. haigae* is a junior synonym of *P. laevidactylus*.

Species	Collection site, date	Catalog No.	GenBank accession No.
Calcinus obscurus Stimpson, 1859	Panama City (Panama), 2001	–	AF436058 a
Calcinus tibicen (Herbst, 1791)	Ubatuba (Brazil), X.2001	CCDB 769	DQ369940
Clibanarius albidigitus Nobili, 1901	Panama City (Panama), 2001	–	AF425323 b
Clibanarius antillensis Stimpson, 1859	Florida (USA), VII.1998	ULLZ 4683	DQ369941
Coenobita compressus (H. Milne Edwards, 1837)	Amador Causeway (Panama), 2001	–	AF436059 a
Dardanus insignis (de Saussure, 1858)	Caraguatatuba (Brazil), IV.2001	CCDB 774	DQ369943
Dardanus venosus (H. Milne Edwards, 1848)	Ubatuba (Brazil), XI.2001	CCDB 766	DQ369944
Isocheles pilosus (Holmes, 1900)	California (USA), 2001	–	AF436057 a
Isocheles sawayai Forest & de Saint Laurent, 1968	Ubatuba (Brazil), XII.2000	CCDB 302	DQ369938
Isocheles sawayai Forest & de Saint Laurent, 1968	Margarita Is. (Venezuela), I.2002	CCDB 308	DQ369937
Isocheles wurdemanni Stimpson, 1859	Texas (USA), X.1997	ULLZ 3890	DQ369936
Lithodes aequispinus Benedict, 1895	Abashiri (Japan), 2001	–	AF425329 b
Lithodes maja (Linnaeus, 1758)	Nova Scotia (Canada), 2001	–	AF425330 b
Lithodes santolla (Molina, 1782)	Beagle Channel (Argentina), 2001	–	AF425331 b
Loxopagurus loxochelis (Moreira, 1901)	Ubatuba (Brazil), XI.2000	CCDB 765	DQ369939
Pachycheles laevidactylus Ortmann, 1892*	Tramandaí (Brazil), 2001	–	AY050076 c
Paguristes calliopsis Forest & de Saint Laurent, 1968	Ubatuba (Brazil), IX.1999	CCDB 768	DQ369932
Paguristes erythrops Holthuis, 1959	Paraty (Brazil), X.1996	CCDB 773	DQ369935
Paguristes robustus Forest & de Saint Laurent, 1968	Ubatuba (Brazil), III.2001	CCDB 1252	DQ369934
Paguristes tortugae complex Schmitt, 1933	Ubatuba (Brazil), XI.1999	CCDB 1251	DQ369933
Pagurus brevidactylus (Stimpson, 1859)	Ubatuba (Brazil), XI.2001	CCDB 771	DQ369945
Pagurus criniticornis (Dana, 1852)	Ubatuba (Brazil), XI. 2001	CCDB 779	DQ369947
Pagurus leptonyx Forest & de Saint Laurent, 1968	Ubatuba (Brazil), VIII.2001	CCDB 1257	DQ369946
Petrolisthes armatus (Gibbes, 1850)	Louisiana (USA), 1998	ULLZ 3779	AJ130802 d
Petrochirus diogenes (Linnaeus, 1758)	Ubatuba (Brazil), III.2001	CCDB 776	DQ369942

recombination is a limitation because phylogeny will reflect only the maternal lineage (Hartl & Clark 1997). This gene has nonetheless shown its utility in both phylogenetic and population studies for over a decade (Bucklin *et al.* 1995; Schubart *et al.* 2000a, 2002; Stillman & Reeb 2001; Tudge & Cunningham 2002; Young *et al.* 2002; Harrison 2004; Morrison *et al.* 2004; Machordom & Macpherson 2004; Mantelatto *et al.* in press; Robles *et al.* in press), and it is a common choice for use in phylogenetic studies on decapods (Schubart *et al.* 2000a; Mathews *et al.* 2002; Harrison 2004; Morrison *et al.* 2004; Machordom & Macpherson 2004).

Hermit crabs used in our analyses were collected between 1996 and 2002 (Table 1). Specimens selected for DNA analysis were preserved directly in 75 to 90% ethanol. Species identifications were confirmed on the basis of morphological characters from available references (Moreira 1901; Costa 1962; Forest & de Saint Laurent 1968; Melo 1999; McLaughlin 2003). Genetic vouchers from which tissue subsamples were obtained have been deposited at the University of Louisiana at Lafayette, Zoological Collection (ULLZ) or at the University of São Paulo, Crustacean Collection of Department of Biology (CCDB) of Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto (FFCLRP) (Table 1).

We included three of the five known species of *Isocheles* in our analysis. We were unable to obtain two species, *I. aequimanus* and *I. pacificus*. Besides the three species of *Isocheles*, and *L. loxochelis*, we included other hermit crabs from the family Diogenidae for comparison, along with representatives of other selected anomuran groups to more broadly root the analysis. Some of the comparative sequences included in the analysis were retrieved

from GenBank (Table 1). In addition to *Isocheles* and *Loxopagurus*, we used from the Diogenidae two species of *Calcinus*, two of *Clibanarius*, two of *Dardanus*, four species of *Paguristes*, and one of *Petrochirus*; from the Coenobitidae we used a species of *Coenobita*; from the Lithodidae, we used three species of *Lithodes*; and from the Paguridae we used three species of *Pagurus*. A species of *Pachycheles*, and one of *Petrolisthes* were used as representatives of the Porcellanidae (Table 1).

DNA extraction, amplification and sequencing protocols follow Schubart *et al.* (2000a) with modifications as in Mantelatto *et al.* (in press) and Robles *et al.* (in press). Total genomic DNA was extracted from muscle tissue of walking legs or the chelipeds. Muscle was ground and incubated for 1-12h in 600 µl of lysis buffer at 65°C; protein was separated by addition of 200 µl 7.5M of ammonium acetate prior to centrifugation. DNA was precipitated by addition of 600 µl of cold isopropanol followed by centrifugation; the resultant pellet was washed with 70% ethanol, dried and resuspended in 10-20 µl of TE buffer.

An approximately 550-basepair region of the 16S rRNA gene was amplified from diluted DNA by means of polymerase-chain-reaction (PCR) (thermal cycles: initial denaturizing for 10 min at 94°C; annealing for 38-42 cycles: 1 min at 94°C, 1 min at 45-48°C, 2 min at 72°C; final extension of 10 min at 72°C) with the primers designated as follows: 16SH2 (5'-AGATAGAAACCAACCTGG-3'), 16SL2 (5'-TGCCTGTTTATCAAAAACAT-3'), and 16Sar (5'-CGCCTGTTTATCAAAAACAT-3') (for references on the primers see Schubart *et al.* 2000a, b).

PCR products were purified using Microcon 100® filters (Millipore Corp.) and sequenced with the ABI Big Dye® Terminator Mix (PE Biosystems) in an ABI Prism 3100 Genetic Analyzer® (Applied Biosystems automated sequencer). All sequences were confirmed by sequencing both strands.

Sequences were edited with the Sequencher software program (version 4.1, Genecodes, Ann Arbor, MI) and aligned on Bioedit (Hall 1999) with the following settings: pairwise parameters = gap opening 6.0, gap extension 0.2, multialignment parameters = gap opening 6.0, gap extension 0.2. Ambiguous regions of the alignment were removed. Base composition, pattern of substitution for pairwise comparison, and analysis of variability along the 16Smt DNA was performed as implemented in PAUP 4.0 beta 10 (Swofford 2003). Homogeneity of nucleotide frequency among taxa was also assessed with a χ^2 test as implemented in PAUP.

Phylogenetic analyses were conducted using MRBAYES for Bayesian analysis (BAY), and PAUP 4.0 beta 10 (Swofford 2003) for both Maximum Parsimony (MP) and Neighbor Joining (NJ) analyses. These three methods, plus Maximum Likelihood, are widely used in reconstructing phylogenies. Though they have different approaches, usually they result in the same phylogenetic tree (Hedges 2002). By using different methods we are able to evaluate the robustness of our results (Hedges 2002). Prior to conducting the BAY or NJ analyses, the model of evolution that best fits the data was determined with the software MODELTEST (Posada & Crandall 1998). The Bayesian analysis was performed by sampling one tree every 100 generations for 2 000 000 generations, starting with a random tree. A preliminary analysis showed that stasis was reached at approximately 7000 generations. Thus, we discarded 201 trees corresponding to 20 000 generations and obtained a 50% majority rule consensus trees from the remaining 19 800 saved trees. Neighbor-joining analysis was carried out with a maximum likelihood distance correction set with the parameters obtained from MODELTEST (Posada & Crandall 1998). Maximum parsimony analysis was performed as a heuristic search with gaps treated as a fifth character state, multistate characters interpreted as uncertain, and all characters considered as unordered. The search was conducted with a random sequence addition of 1000 random trees, including tree bisection and reconnection (TBR) as a branch swapping option. One tree was stored at every repetition, and branch swapping was performed on the best trees only. To determine confidence values for the resulting trees, for both NJ and MP, we ran 1000 bootstrap replicates based on the same parameters as above. On the molecular trees, confidence values > 50% were reported for both NJ (1000 bootstraps) and MP (1000 bootstraps) analyses while for the BAY

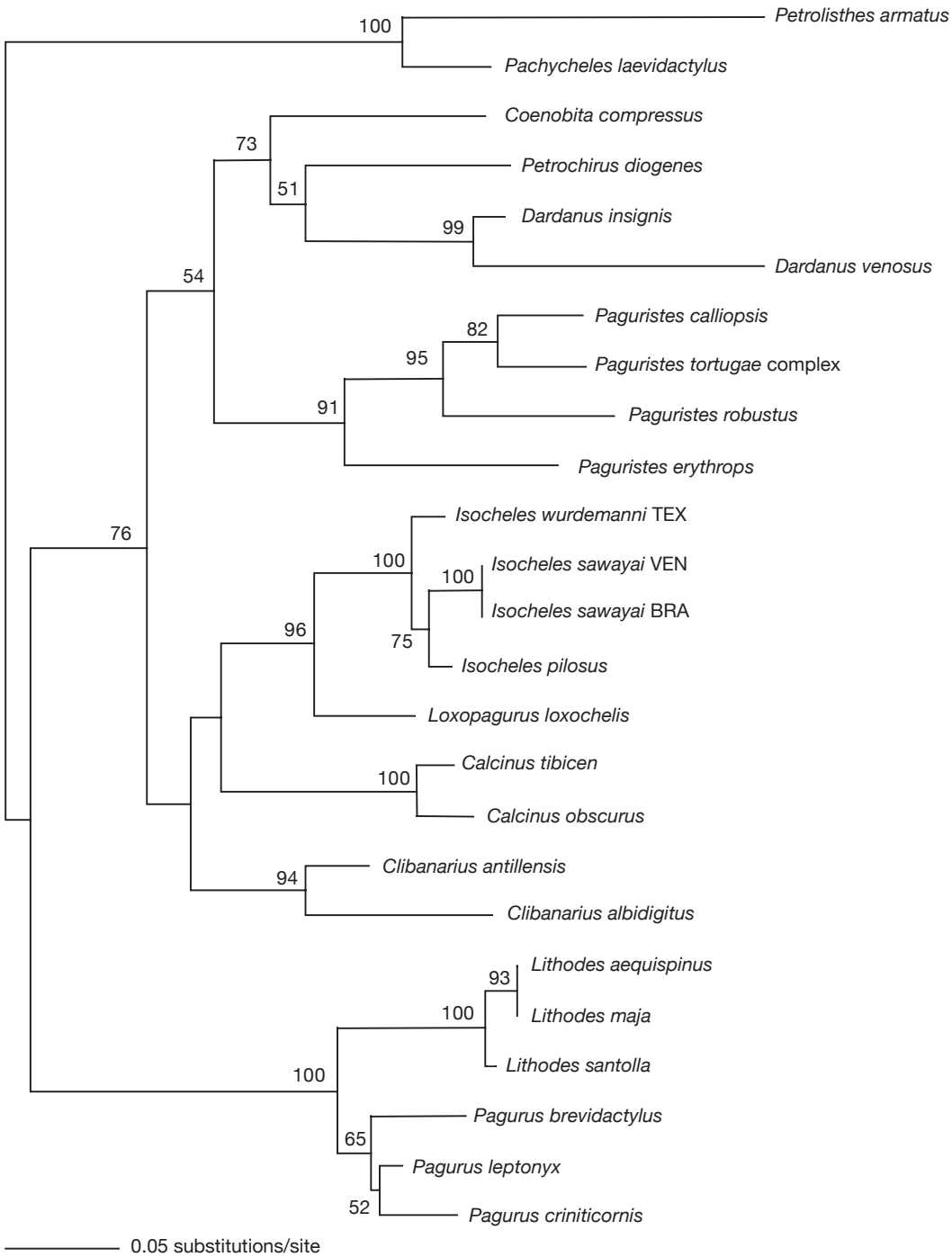

FIG. 1. — Single phylogenetic tree obtained from NJ analysis of 16S sequences for *Loxopagurus*, *Isocheles*, and other selected anomurans. Numbers are significance values for 1000 bootstraps; values ≤ 50% are not shown. Abbreviations: **BRA**, Brazil; **TEX**, Texas; **VEN**, Venezuela.

analysis values were reported for posterior probabilities of the respective nodes among all the saved trees. Sequences have been deposited in GenBank (Table 1).

RESULTS

Our analysis included 25 individuals representing 24 species of anomurans (Table 1). The 16S rDNA alignment contained a total of 538 basepairs excluding primer regions. From these, 84 characters were excluded (38-47; 229-285; 350-366) from the analysis because of uncertain alignment. The complete alignment has been submitted to GenBank as a popset. The fragment used for the analysis contained 233 non-variable and 221 variable characters, of which 175 were parsimony informative. The nucleotide composition of the 16S mtDNA of this dataset can be considered homogeneous (χ^2 = 26.38, df = 78, p = 0.99), with a larger percentage of A-T (0.340%; 0.337%). The best-fitting model of substitution, selected with the Akaike information criterion (AIC; Akaike 1974) as implemented in MODELTEST (Posada & Crandall 1998), was the transversional model with invariable sites and a gamma distribution (TVM+I+G) (Rodríguez *et al.* 1990) and with the following parameters: assumed nucleotide frequencies: A = 0.3769, C = 0.1155, G = 0.1474, T = 0.3603; substitution rates A-C = 0. 9031, A-G = 9.5000, A-T = 2.4516, C-G = 0.2223, C-T = 9.5000, G-T = 1.0000; proportion of invariable sites I = 0.2896; variable sites followed a gamma distribution with shape parameter = 0.4762. These values were used to obtain both BAY and NJ trees.

MP analysis yielded a single tree of length 680, with consistency index (CI) = 0.519 and retention index (RI) = 0.683. Overall, the distance, Bayesian and parsimony methods resulted in similar tree topologies, though differences were observed (Figs 1-3). In the three analyses, all representatives of the Diogenidae, *Calcinus*, *Clibanarius*, *Dardanus*, *Paguristes*, *Petrochirus*, *Loxopagurus*, and *Isocheles*, were clustered in a single clade along with *Coenobita compressus*, the only member of the Coenobitidae included in our analysis. Selected species representing each of the diogenid genera were clustered together as expected. The three species of *Pagurus*, representing the Paguridae, were also grouped in a single clade with another clade representating the Lithodidae as a sister group (Figs 1-3).

Within the Diogenidae, all three analyses place *Loxopagurus* as a sister group to a clade with the species of *Isocheles*, but on an independent and basal branch. Differences between *L. loxochelis* and *I. sawayai* were substantial, accounting for 9.8% of the gene fragment analyzed (27 transitions, 18 transversions, and 19 indels). Among species of *Isocheles* in the analysis, samples from Brazilian and Venezuelan populations of *I. sawayai* proved to be identical. *Isocheles sawayai* was positioned closest to *I. pilosus* among its congeners in the analyses.

DISCUSSION

Our partial fragment analysis of 16S DNA shows that *L. loxochelis* is diverged at a level of genetic difference that clearly justifies its continued recognition as a separate species from *I. sawayai*. As result, we find no genetic justification for synonymy of *Loxopagurus* with *Isocheles*. Species of these two taxa are highly divergent in mtDNA-gene sequences (9.8% of difference, with 27 transitions, 18 transversions and 19 indels on basepairs sequences), at levels comparable to or exceeding divergence typically measured between other decapod genera (Schubart *et al.* 2000b). This is reflected in the three molecularly based phylogenetic trees, where members of *Loxopagurus* and *Isocheles* occur on a common branch within the Diogenidae. In all three analyses, species of *Isocheles* are grouped into a well supported clade with *Loxopagurus* as the sister group. Therefore, there is sound genetic evidence to support the morphological bases for the separation of these two genera.

Forest (1964) described the new genus, *Loxopagurus*, on the basis of specimens of *Pagurus loxochelis* from Bahia (Brazil) originally described by Moreira (1901). Forest reported several similarities in general body morphology and color between the new genus *Loxopagurus* and the genus *Isocheles*, particularly in the shape of the shield and peduncles,

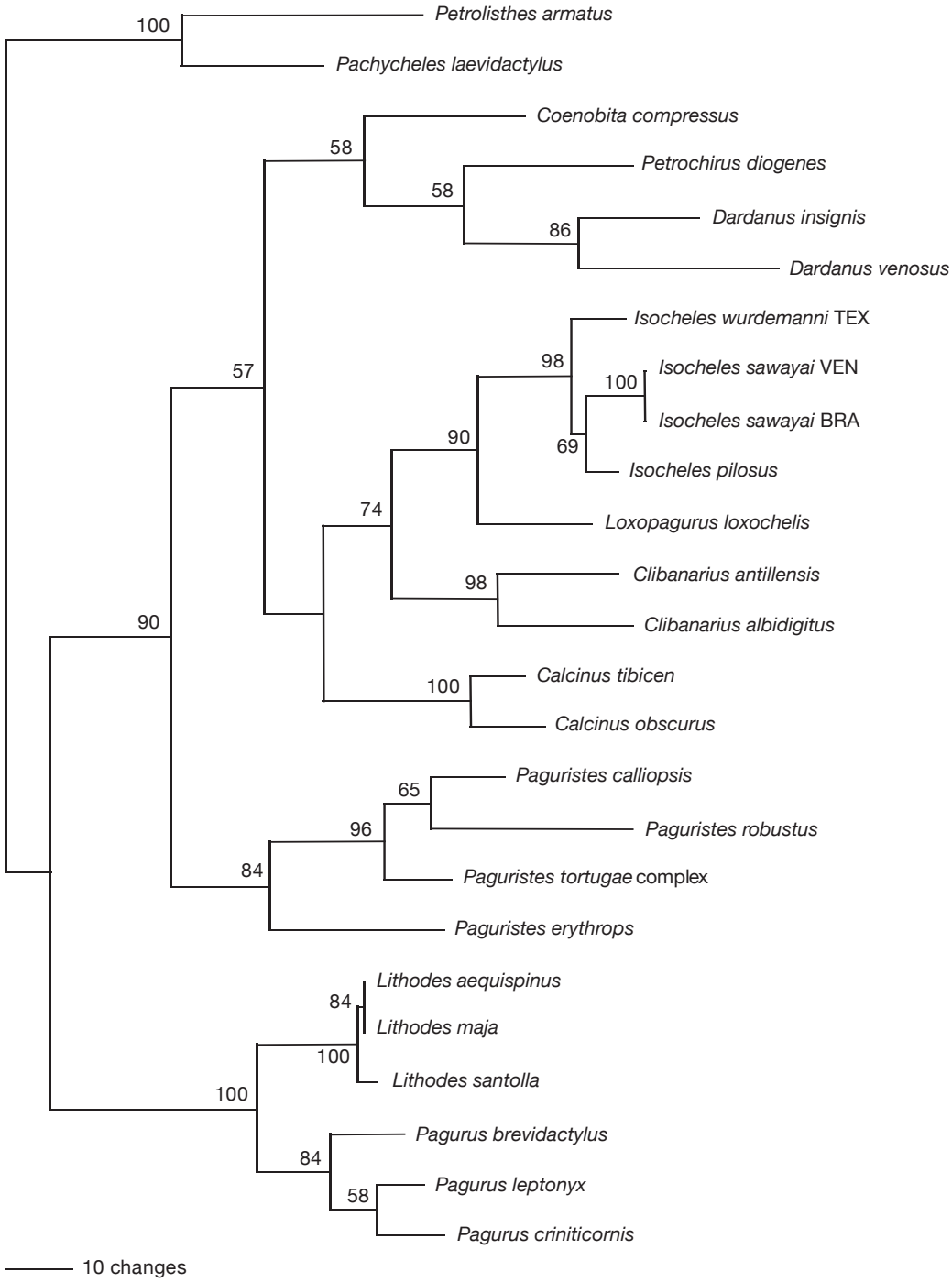

FIG. 2. — Single phylogenetic tree obtained from MP analysis of 16S sequences for *Loxopagurus*, *Isocheles*, and other selected anomurans. Numbers are significance values for 1000 bootstraps; values ≤ 50% are not shown. Abbreviations: **BRA**, Brazil; **TEX**, Texas; **VEN**, Venezuela.

antennal characters (pilosity of the flagellae and aciculae), and features of the mouthparts. According to Forest, the only difference was found in the shape and sculpture of the chelipeds (heterochely in *Loxopagurus* and isochely in *Isocheles*), as also mentioned by Forest & de Saint Laurent (1968). We observed such differences in chela morphology in *L. loxochelis* and *I. sawayai*. Furthermore, from previous larval descriptions by Negreiros-Fransozo & Hebling (1983) and Bernardi (1986) for both species, we recognized additional important differences that were not reported previously, especially in maxilla and maxilliped setation and structure of the antenna in zoea I, II and III. In addition, there are clear ecological differences, at least as observable in southern Atlantic shallow waters: *L. loxochelis* inhabits cold waters at depths as great as 35m and salinities exceeding 30‰ (Mantelatto *et al.* 2004), while *I. sawayai* is found in warmer shallow water generally less than 10 m deep and of salinity less than 20‰, often close to some freshwater influence (Martinelli *et al.* 2002). However, there are likewise shared attributes that suggest these genera are of close relationship; Scelzo *et al.* (2004) and Mantelatto *et al.* (unpublished data) found that members of *L. loxochelis* and *I. sawayai* are very closely allied species on the basis of spermatophore morphology.

Among the species and populations of *Isocheles* included in our analyses, the present molecular phylogeny showed a 100% similarity between a sample of *I. sawayai* from Brazil and a sample from Venezuela, the latter of which may represent populations that were assigned to *I. wurdemanni* by Rodríguez (1980). At the molecular level, we are not able to separate these two populations on the basis of 16S rDNA sequences, since we did not find a single basepair difference between the specimens analyzed. Ranges of the two western Atlantic species (*I. wurdemanni* and *I. sawayai*) were recently reported by Nucci & Melo (2000) to overlap in Venezuela, under the assumption that *I. sawayai*, previously considered endemic to Brazil, had extended its distribution to Venezuela. Without question, we can corroborate that *I. sawayai* occurs in Venezuela where Nucci & Melo (2000) reported it too, as our specimens came from the same site (Isla Margarita). They are clearly not identifiable with *I. wurdemanni*, as we sequenced specimens of the latter species from the type locality in the Gulf of Mexico (Texas) for comparison to the Venezuelan materials and found basepair divergence to exceed 4.6%. However, we also now question the supposition that ranges of these two species overlap in Venezuela. Since the materials furnished to us from there were those typically identified as *I. wurdemanni*, but were found in molecular analyses to be *I. sawayai*, we feel it is possible that only the latter species occurs there and that records to date may be in error.

Our present analysis infers that the closest relative to *I. sawayai* is *I. pilosus* from the eastern Pacific, rather than any other Atlantic species of *Isocheles* or *Loxopagurus*. Differences between these two species were limited (3.1% of difference: 17 transitions, 5 transversions, 5 indels) compared to those observed among most other species of Diogenidae that we have sequenced. Provenzano (1959) previously commented on "*Holopagurus pilosus* Holmes, 1900" bearing close resemblance to an overgrown specimen of his therein described *I. wurdemanni* (from Texas), noting the tendency of larger specimens in the latter species to have slightly unequal chelipeds and questioning the interpretation of this variable character. The present analysis shows that *Holopagurus* is correctly synonymized with *Isocheles* and that eastern Pacific and western Atlantic forms in this genus are closely related, rather than being separated into geographically defined branches.

Our findings also argue that, in addition to re-examination of adult and larval morphological characters, phylogenetic studies can benefit from less commonly applied characters such as spermatophore morphology. The phylogenetic proximity between *Loxopagurus* and *Clibanarius* or *Calcinus*, demonstrated in our analysis as well as in previous works (McLaughlin 1983; Morrison *et al.* 2002), is corroborated by the recent observations of spermatophore (Scelzo *et al.* 2004; unpublished data) and sperm (Scelzo *et al*. in press) morphology obtained for species of the Diogenidae from the South Atlantic.

Previous phylogenetic hypotheses based on limited data have suggested that *Paguristes* has close affinities to *Pagurus* (Morrison *et al.* 2002). However, our work suggests instead that *Paguristes* is more

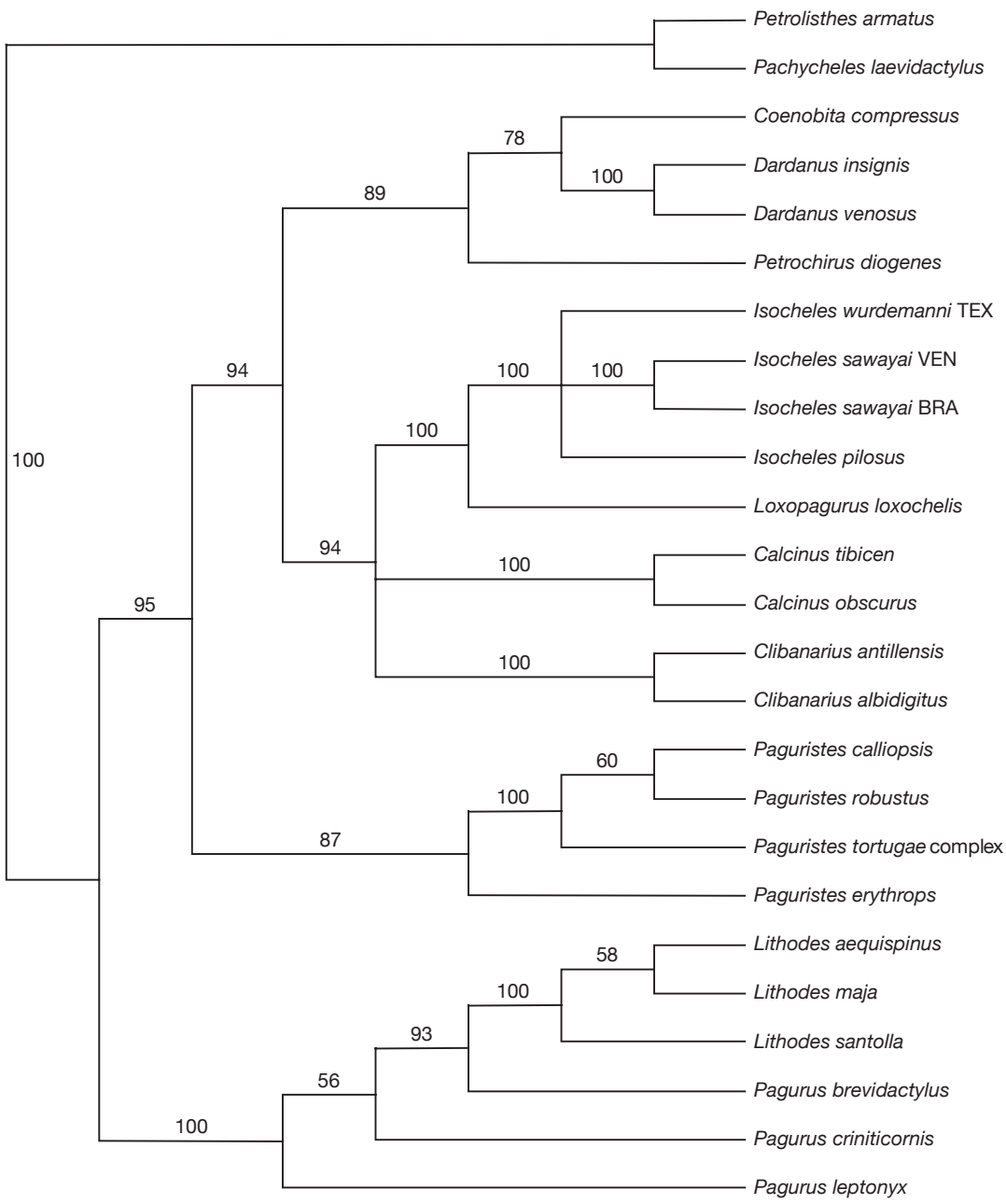

FIG. 3. — Consensus phylogenetic tree obtained from BAY analysis (50% majority consensus of 19800 trees) of 16S sequences for *Loxopagurus*, *Isocheles*, and other selected anomurans. Numbers are significance values of posterior probabilities; values ≤ 50% are not shown. Abbreviations: **BRA**, Brazil; **TEX**, Texas; **VEN**, Venezuela.

closely allied to the remaining diogenid species used in the analysis. Our findings are preliminary and underscore the need for comprehensive studies of Paguridae and Diogenidae throughout American waters.

Dedication

The authors are honored to recognize the many achievements of our friend and colleague, Patsy A. McLaughlin, by this contribution to this commemorative issue.

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REFERENCES

AKAIKE H. 1974. — A new look at the statistical model identification. *IEEE Transactions on Automatic Controls* 19: 719-723.

BERNARDI J. V. E. 1986. — *Desenvolvimento larval de* Loxopagurus loxochelis *(Moreira, 1901) (Crustacea, Decapoda, Diogenidae) em laboratório*. Bachelor unpublished Thesis, UNESP, Rio Claro, Brazil, 33 p.

BUCKLIN A., FROST B. W. & KOCHER T. D. 1995. — Molecular systematics of six *Calanus* and three *Metridia* species (Copepoda: Calanoida). *Marine Biology* 121: 655-664.

COSTA H. R. 1962. — Ocorrência do gênero *Isocheles* Stimpson na Costa Brasileira. *Centro de Estudos Zoológicos* 17/18: 1-3.

FOREST J. 1964. — Sur un nouveau genre de Diogenidae (Crustacea Paguridea) de l'Atlantique sud-américain, *Loxopagurus* gen. nov., établi pour *Pagurus loxochelis* Moreira. *Zoologische Mededelingen* 39: 279-296.

FOREST J. & SAINT LAURENT M. DE 1968. — Campagne de la *Calypso* au large des côtes atlantiques de l'Amérique du Sud (1961-1962). 6. Crustacés décapodes : pagurides. *Annales de l'Institut océanographique*, Monaco 45: 47-169.

HALL T. A. 1999. — BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95-98.

HARRISON J. S. 2004. — Evolution, biogeography, and the utility of mitochondrial 16s and COI genes in phylogenetic analysis of the crab genus *Austinixa*. *Molecular Phylogenetic and Evolution* 30: 743-754.

HARTL D. L. & CLARK A. G. 1997. — *Principles of Population Genetics*. 2nd ed. Sinauer Associates, Sunderland, 682 p.

HARVEY A. W. & SANTO E. M. DE 1996. — On the status of *Pachycheles laevidactylus* Ortmann, 1892 (Crustacea: Decapoda: Porcellanidae). *Proceedings of Biological Society of Washington* 109 (4): 707-714.

HEBLING N. J., NEGREIROS-FRANSOZO M. L. & BERNARDI J. V. E. 2000. — Larval development of *Loxopagurus loxochelis* (Moreira, 1901) (Crustacea, Decapoda, Diogenidae), obtained in laboratory, *in I Congresso Brasileiro sobre Crustáceos* 18: 51.

HEDGES S. B. 2002. — The origin and evolution of model organisms. *Nature Reviews Genetics* 3: 838-849.

MACHORDOM A. & MACPHERSON E. 2004. — Rapid radiation and cryptic speciation in squat lobsters of the genus *Munida* (Crustacea, Decapoda) and related genera in the South West Pacific: molecular and morphological evidence. *Molecular Phylogenetics and Evolution* 33: 259-279.

MANTELATTO F. L. M., MARTINELLI J. M. & FRANSOZO A. 2004. — Temporal-spatial distribution of the hermit crab *Loxopagurus loxochelis* (Decapoda: Diogenidae) from Ubatuba Bay, São Paulo State, Brazil. *Revista de Biologia Tropical* 52 (1): 47-55.

MANTELATTO F. L. M., ROBLES R. & FELDER D. L. in press. — Molecular phylogeny and taxonomic ap-

proach of the crab genus *Portunus* (Crustacea, Portunidae) from Western Atlantic. *Zoological Journal of the Linnean Society*.

MARTINELLI J. M., MANTELATTO F. L. M. & FRANSOZO A. 2002. — Population structure and breeding season of the South Atlantic hermit crab, *Loxopagurus loxochelis* (Anomura, Diogenidae) from Ubatuba region, Brazil. *Crustaceana* 75 (6): 791-802.

MATHEWS L. M., SCHUBART C. D., NEIGEL J. E. & FELDER D. L. 2002. — Genetic, ecological, and behavioural divergence between two sibling snapping shrimp species (Crustacea: Decapoda: *Alpheus*). *Molecular Ecology* 11: 1427-1437.

MELO G. A. S. 1999. — *Manual de identificação dos Crustacea Decapoda do litoral brasileiro: Anomura, Thalassinidea, Palinuridea, Astacidea*. Plêiade, São Paulo, 551 p.

MCLAUGHLIN P. A. 1983. — Hermit crabs – are they really polyphyletic? *Journal of Crustacean Biology* 3: 608-621.

MCLAUGHLIN P. A. 2003. — Illustrated keys to families and genera of the superfamily Paguroidea (Crustacea: Decapoda: Anomura), with diagnoses of genera of Paguridae, *in* LEMAITRE R. & TUDGE C. C. (eds), Biology of the Anomura. Proceedings of a symposium at the Fifth International Crustacean Congress, Melbourne, Australia, 9-13 July 2001. *Memoirs of Museum Victoria* 60 (1): 111-144.

MOREIRA C. 1901. — Contribuições para o conhecimento da fauna brasileira. Crustáceos do Brasil. *Archivos do Museo Nacional do Rio de Janeiro* 11: 1-151, pls 1-5.

MORRISON C. L., HARVEY A. W., LAVERY S., TIEU K., HUANG Y. & CUNNINGHAM C. W. 2002. — Mitochondrial gene rearrangements confirm the parallel evolution of the crab-like form. *Proceedings of the Royal Society of London*, Part B, Biological Sciences 269 (1489): 345-350.

MORRISON C. L., RIOS R. & DUFFY J. E. 2004. — Phylogenetic evidence for an ancient rapid radiation of Caribbean sponge-dwelling snapping shrimps (*Synalpheus*). *Molecular Phylogenetics and Evolution* 30: 563-581.

NEGREIROS-FRANSOZO M. L. & HEBLING N. J. 1983. — Desenvolvimento pós-embrionário de *Isocheles sawayai* Forest & Saint Laurent, 1968 (Decapoda, Diogenidae) em laboratório. *Papéis Avulsos de Zoologia* 35 (4): 41-53.

NUCCI P. R. 2002. — *Taxonomia e biogeografia da Superfamília Paguroidea Latreille (Crustacea, Decapoda, Anomura) no litoral brasileiro.* Ph.D. unpublished Thesis, UNESP, Rio Claro, Brazil, 218 p.

NUCCI P. R. & MELO G. A. S. 2000. — Range extensions for eight species of western atlantic hermit crabs (Crustacea, Paguroidea). *Nauplius* 8 (1): 141-147.

PÉREZ-LOSADA M., JARA C. G., BOND-BUCKUP G. & CRANDALL K. A. 2002. — Phylogenetic relationships among the species of *Aegla* (Anomura: Aeglidae) freshwater crabs from Chile. *Journal of Crustacean Biology* 22 (2): 304-313.

POSADA D. & CRANDALL K. A. 1998. — MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14 (9): 817-818.

PROVENZANO A. J. JR. 1959. — The shallow-water hermit crabs of Florida. *Bulletin of Marine Science of the Gulf of Caribbean* 9 (4): 349-420.

ROBLES R., SCHUBART C. D., CONDE J. E., CARMONA-SUÁREZ C., ALVAREZ F., VILLALOBOS J. L. & FELDER D. L. in press. — Molecular phylogeny of the American *Callinectes* Stimpson, 1860 (Brachyura: Portunidae), based on two partial mitochondrial genes. *Marine Biology.*

RODRÍGUEZ G. 1980. — *Los Crustaceos Decápodos de Venezuela*. IVIC, Caracas, 494 p.

RODRÍGUEZ R., OLIVER J. L., MARTIN A. & MEDINA J. R. 1990. — The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology* 142: 485-501.

SCELZO M. A., MANTELATTO F. L. M. & TUDGE C. C. 2004. — Spermatophore morphology of the endemic hermit crab *Loxopagurus loxochelis* (Anomura, Diogenidae) from Southwestern Atlantic-Brazil and Argentina. *Invertebrate Reproduction and Development* 46 (1): 1-9.

SCELZO M. A., MEDINA A. & TUDGE C. C. in press. — Spermatozoal ultrastructure of the hermit crab *Loxopagurus loxochelis* (Moreira, 1901) (Anomura, Diogenidae) from the Southwestern Atlantic. *Crustacean Research*.

SCHUBART C. D., NEIGEL J. E. & FELDER D. L. 2000a. — Use of the mitochondrial 16S rRNA gene for phylogenetic and population studies of Crustacea. *Crustacean Issues* 12: 817-830.

SCHUBART C. D., CUESTA J. A., DIESEL R. & FELDER D. L. 2000b. — Molecular phylogeny, taxonomy, and evolution of nonmarine lineages within the American grapsoid crabs (Crustacea: Brachyura). *Molecular Phylogenetics and Evolution* 15 (2): 179-190.

SCHUBART C. D., CUESTA J. A. & FELDER D. L. 2002. — Glyptograpsidae, a new brachyuran family from Central America: larval and adult morphology, and a molecular phylogeny of the Grapsoidea. *Journal of Crustacean Biology* 22: 28-44.

STILLMAN J. H. & REEB C. A. 2001. — Molecular phylogeny of eastern Pacific porcelain crabs, genera *Petrolisthes* and *Pachycheles*, based on the mtDNA 16S rDNA sequence: phylogeographic and systematic implications. *Molecular Phylogenetics and Evolution* 19: 236-245.

SWOFFORD D. L. 2003. — *PAUP. Phylogenetic Analysis Using Parsimony (and Other Methods).* Version 4. Sinauer Associates, Sunderland, Masschusetts.

TUDGE C. C. & CUNNINGHAM C. 2002. — Molecular phylogeny of the mud lobsters and mud shrimps (Crustacea: Decapoda: Thalassinidea) using nuclear 18S rDNA and mitochondrial 16S rDNA. *Invertebrate Systematics* 16: 839-847.

YOUNG A. M., TORRES C., MACK J. E. & CUNNINGHAM C. 2002. — Morphological and genetic evidence for vicariance and refugium in Atlantic and Gulf of Mexico populations of the hermit crab *Pagurus longicarpus. Marine Biology* 140: 1059-1066.

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