

# Molecular phylogeny of the western Atlantic species of the genus *Portunus* (Crustacea, Brachyura, Portunidae)

FERNANDO L. MANTELATTO<sup>1\*</sup>, RAFAEL ROBLES<sup>2</sup> and DARRYL L. FELDER<sup>2</sup>

<sup>1</sup>Laboratory of Bioecology and Crustacean Systematics, Department of Biology, FFCLRP, University of São Paulo (USP), Ave. Bandeirantes, 3900, CEP 14040-901, Ribeirão Preto, SP (Brazil)

<sup>2</sup>Department of Biology, Laboratory for Crustacean Research, University of Louisiana at Lafayette, Lafayette, LA 70504-2451, USA

Received March 2004; accepted for publication November 2006

The genus *Portunus* encompasses a comparatively large number of species distributed worldwide in temperate to tropical waters. Although much has been reported about the biology of selected species, taxonomic identification of several species is problematic on the basis of strictly adult morphology. Relationships among species of the genus are also poorly understood, and systematic review of the group is long overdue. Prior to the present study, there had been no comprehensive attempt to resolve taxonomic questions or determine evolutionary relationships within this genus on the basis of molecular genetics. Phylogenetic relationships among 14 putative species of *Portunus* from the Gulf of Mexico and other waters of the western Atlantic were examined using 16S sequences of the rRNA gene. The resultant molecularly based phylogeny disagrees in several respects with current morphologically based classification of *Portunus* from this geographical region. Of the 14 species generally recognized, only 12 appear to be valid. We recommend that *P. vossi* be hereafter regarded as a junior synonym of *P. spinimanus* and that *P. bahamensis* be regarded as a junior synonym of *P. depressifrons*. Our analysis suggests that western Atlantic members of the genus can be subdivided into at least three well-defined clades. Pending further molecular analyses with a large subset of species, it appears that the genus is not monophyletic and that it warrants further taxonomic revision. © 2007 The Linnean Society of London, *Zoological Journal of the Linnean Society*, 2007, 150, 211–220.

ADDITIONAL KEYWORDS: Decapoda – molecular systematics – swimming crab – 16S rRNA gene.

## INTRODUCTION

Swimming crabs of the genus *Portunus* are considered ubiquitous representatives of the portunid fauna in tropical and subtropical waters, and over 80 species (compiled from Rathbun, 1930; Stephenson & Campbell, 1959; Stephenson, 1962, 1972; Stephenson & Rees, 1967) have been assigned to this genus worldwide. Under present systematic treatments, 14 species of *Portunus* have been currently recognized from the western Atlantic: *P. anceps* (de Saussure, 1858); *P. bahamensis* Rathbun, 1930; *P. binoculus* Holthuis, 1969; *P. depressifrons* (Stimpson, 1859); *P. floridanus* Rathbun, 1930; *P. gibbesii* (Stimpson, 1859); *P. ordwayi* (Stimpson, 1860); *P. sayi* (Gibbes, 1850);

*P. spinicarpus* (Stimpson, 1871); *P. spinimanus* Latreille, 1819; *P. rufiremus* Holthuis, 1959; *P. sebae* (H. Milne Edwards, 1834); *P. ventralis* (A. Milne-Edwards, 1879); and *P. vossi* Lemaitre, 1991. Varied diagnostic summaries and key characters based on adult morphology, along with compilations of distribution records, are available in widely cited literature (Rathbun, 1930; Holthuis, 1959, 1969; Williams, 1984; Manning & Chace, 1990; Lemaitre, 1991; Melo, 1996).

Despite the economic and ecological importance of portunid species included in this commonly encountered genus, definitive identification of many species remains difficult. Diagnoses are often based on subtle and inconsistent differences in adult morphology for this supposedly well-known group of swimming crabs. Thus, questions remain as to the validity of several species reported in the literature, while there is also a clear possibility that yet-to-be-named species continue

\*Corresponding author. E-mail: flmantel@usp.br

to go unrecognized. 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) within this group.

We herein analyse phylogenetic relationships among all species of *Portunus* from the western Atlantic Ocean on the basis of partial sequences of the large-subunit 16S rRNA gene. Our analyses also allow us to investigate the taxonomic status of several species erected on the basis of morphological characters that have proven difficult to apply with confidence. We include the genus *Laeonectes* to test molecular support for its separation from *Portunus*, as well as the proximity of its relationship to selected members of *Portunus* among which it was formerly placed.

## MATERIAL AND METHODS

We based our phylogenetic analysis exclusively on a partial fragment of the 16S rRNA gene. Mitochondrial DNA (mtDNA) is a common choice for both population and phylogenetic studies based on several characteristics such as being homoplasmic, maternally inherited (with some exceptions), subject to a high mutation rate, easy to isolate and abundant (Avise *et al.*, 1987; Hartl & Clark, 1997; Rokas, Ladukakis & Zourus, 2003). Absence of recombination might represent a limitation because mtDNA linear evolutionary history can differ from that of the nuclear DNA, which presents a reticulate evolutionary history (Neigel & Avise, 1986). The 16S rRNA gene has nonetheless shown its utility in both phylogenetic and population studies for over a decade (Bucklin, Frost & Kocher, 1995; Schubart, Neigel & Felder, 2000a; Stillman & Reeb, 2001; Schubart, Cuesta & Felder, 2002; Tudge & Cunningham, 2002; Harrison, 2004; Machordom & Macpherson, 2004; Morrison, Rios & Duffy, 2004; Mantelatto *et al.*, 2006; Robles *et al.*, 2007), and it is a common choice for use in phylogenetic studies on decapods (Schubart, Neigel & Felder, 2000a; Mathews *et al.*, 2002; Harrison, 2004; Machordom & Macpherson, 2004; Morrison, Rios & Duffy, 2004).

Crabs used in our analyses were collected from new localities between 2000 and 2001 or were obtained from museum collections (Table 1). Newly collected specimens to be used for DNA analysis were preserved directly in 75–90% ethanol. Species identifications were confirmed on the basis of morphological characters from available references (Rathbun, 1930; Holthuis, 1959, 1969; Williams, 1984; Manning & Chace, 1990; Lemaitre, 1991). Genetic vouchers from which tissue subsamples were obtained are deposited at the University of Louisiana-Lafayette Zoological Collection (ULLZ) or at the United States National Museum of Natural History, Washington (USNM) (Table 1).

Specimens from both collections and also from the Zoology Museum of the University of São Paulo (MZUSP) were loaned and used for morphological comparisons. Tissues from type materials, excised by minimally destructive methods, were sequenced when possible (see Table 1).

Besides the 16 species of *Portunus*, and the recently segregated *Laeonectes vocans*, we included several species representing other genera of the family Portunidae for comparison, to root the analysis more broadly. These consisted of four species of *Callinectes*, one species of *Arenaeus* and two species of *Scylla* as additional representatives of the subfamily Portuninae, along with two species of *Ovalipes* and a species of *Polybius* to represent the subfamily Polybiinae. Some of the comparative sequences included in the analysis were retrieved from GenBank (Table 1).

DNA extraction, amplification and sequencing protocols follow Schubart *et al.* (2000a) with modifications as in Mantelatto *et al.* (2006) and Robles *et al.* (2007). Total genomic DNA was extracted from muscle tissue of walking legs or the chelipeds. Muscle was ground and incubated for 1–12 h in 600 µL lysis buffer at 65 °C; protein was separated by addition of 200 µL 7.5 M ammonium acetate prior to centrifugation. DNA precipitation was made by addition of 600 µL cold isopropanol followed by centrifugation; the resultant pellet was washed with 70% ethanol, dried and resuspended in 10–20 µL TE buffer.

An approximately 560-bp region of the 16S rRNA gene was amplified from diluted DNA by means of polymerase chain reaction (PCR) (thermal cycles: initial denaturation 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 following primers: 16Sar (5'-CGCCTGTTTATCAA AACAT-'), 16Sbr (5'-CCGGTCTGAACTCAGATCAC GT-3'), 16SH4 (5'-GTYGCCCCAACCAAATAAA-3'), 16SL2 (5'-TGCCTGTTTATCAAAAACAT-3'), 16SL15 (5'-GACGATAAGACCCTATAAAGCTT-3') (for references on the primers see Schubart, Neigel & Felder, 2000a and Schubart, Cuesta & Rodriguez, 2001b). We used internal primers 16SH4 and 16SL15 (in combination with 16SL2, 16Sar and 16Sbr) for partial amplification of the possibly formalin-fixed specimens among museum materials. 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 Analyser (Applied Biosystems automated sequencer). All sequences were confirmed by sequencing both strands.

A consensus sequence for the two strands was obtained using the computational program Sequencher 3.0. Sequences were aligned using the Clustal W option as implemented in the sequence alignment editor Bioedit ver. 7 (Hall, 1999). Phylogenetic and

**Table 1.** Portunid crab species used for the phylogeny reconstructions with respective date and site of collection, museum catalogue number, and genetic database accession numbers (GenBank).

Species	Collection site, date	Catalogue no.	GenBank accession no.
<i>Arenaeus cribrarius</i> (Lamarck, 1818)	Venezuela: Falcón, 1999	ULLZ 5173	DQ407667 <sup>d</sup>
<i>Callinectes bocourti</i> A. Milne-Edwards, 1879	Venezuela: Zulia, 1999	–	AJ298177 <sup>a</sup>
<i>Callinectes danae</i> Smith, 1869	Venezuela: Falcón, 1998	–	AJ298184 <sup>a</sup>
<i>Callinectes ornatus</i> Ordway, 1863	Brazil: São Paulo, 1999	–	AJ298186 <sup>a</sup>
<i>Callinectes sapidus</i> Rathbun, 1896	Venezuela: Zulia, 1999	–	AJ298190 <sup>a</sup>
<i>Laleonectes vocans</i> (A. Milne-Edwards, 1878)	USA, Louisiana, 2000	ULLZ 4640	DQ388051
<i>Ovalipes stephensoni</i> Williams, 1976	USA, Florida, 2003	ULLZ 5678	DQ388050
<i>Ovalipes trimaculatus</i> (De Haan, 1833)	Argentina: Mar del Plata, 2001	ULLZ 4773	DQ388049
<i>Polybius henslowii</i> Leach, 1820	Portugal: Cascais, 2001	ULLZ 4755	DQ388059
<i>Portunus anceps</i> (Saussure, 1858)	Belize: Carrie Bow Cay, 1983	ULLZ 4327	DQ388054
<i>Portunus bahamensis</i> Rathbun, 1930*	Bahamas: Eleuthera Island, 1903	USNM 204659	DQ388065
<i>Portunus binoculus</i> Holthuis, 1969†	USA, NW Atlantic, 1965	USNM 113560	DQ388062
<i>Portunus depressifrons</i> (Stimpson, 1859)	USA, Florida, 1996	ULLZ 4442	DQ388064
<i>Portunus floridanus</i> Rathbun, 1930	USA, Gulf of México, 2000	ULLZ 4695	DQ388058
<i>Portunus gibbesii</i> (Stimpson, 1859)	USA, Alabama, 2001	ULLZ 4565	DQ388057
<i>Portunus ordwayi</i> (Stimpson, 1860)†	USA, Florida, 1915	USNM 61174	DQ388066
<i>Portunus pelagicus</i> (Linnaeus, 1758)	India: Gulf of Mainnar, 2003	ULLZ 5682	DQ388052
<i>Portunus rufiremus</i> Holthuis, 1959†	French Guiana: Sinnamary, 1974	USNM 151568	DQ388063
<i>Portunus sayi</i> (Gibbes, 1850)	USA, Louisiana, 2001	ULLZ 4753	DQ388053
<i>Portunus sebae</i> (H. Milne Edwards, 1834)	USA, Florida, 2001	ULLZ 4527	DQ388067
<i>Portunus spinicarpus</i> (Stimpson, 1871)	USA, Florida, 1996	ULLZ 4618	DQ388061
<i>Portunus spinimanus</i> Latreille, 1819	Brazil: Ubatuba Bay, 2000	ULLZ 4754	DQ388056
<i>Portunus trituberculatus</i> (Miers, 1876)	China: Yunnan, 2003	–	AY264913 <sup>b</sup>
<i>Portunus ventralis</i> (A. Milne-Edwards, 1879)	Belize: Carrie Bow Cay, 1983	ULLZ 4440	DQ388060
<i>Portunus vossi</i> Lemaitre, 1991*	USA, Florida, 1980	USNM 239283	DQ388055
<i>Scylla olivacea</i> (Herbst, 1796)	Taiwan, 2003	–	AF109321 <sup>c</sup>
<i>Scylla serrata</i> (Forsk. 1775)	Taiwan, 2003	–	AF109318 <sup>c</sup>

Specimens used for DNA analysis: \*paratype; †holotype.

<sup>a</sup>Schubart *et al.* (2001a), <sup>b</sup>Quan *et al.* (2004), <sup>c</sup>Hideyuki *et al.* (2004), <sup>d</sup>Robles *et al.* (2007).

molecular evolutionary analyses were conducted using MRBAYES software for Bayesian analysis (BAY) and PAUP 4.0 b10 (Swofford, 1998) for the maximum parsimony (MP) and neighbour joining (NJ) analyses. Sequences were first analysed with the software MODELTEST (Posada & Crandall, 1998) in order to find the model of evolution that best fit the data. The BAY analysis was performed by sampling one tree every 100 generations for 1000 000 generations starting with a random tree using the model of evolution obtained with MODELTEST. Preliminary analysis showed that stasis was reached at approximately 10 000 generations, so we used 20 000 generations as a burn-in and discarded all previous trees. A 50% majority rule consensus tree was obtained from the remaining saved trees. NJ analysis was carried out with a maximum-likelihood distance correction set with the parameters obtained by MODELTEST. MP analysis was performed as a heuristic search with random sequence addition of 5000 random trees, includ-

ing tree bisection and reconnection as branch swapping option; ten trees were saved after every repetition; indels were treated as a fifth character. On molecular trees, bootstrap confidence values > 50% were reported for both NJ (1000 bootstraps) and MP (1000 bootstraps). For the BAY analysis, values were shown for posterior probabilities of the nodes among the 19 800 saved trees. Sequences, as well as the complete alignment, have been deposited in GenBank (Table 1).

## RESULTS

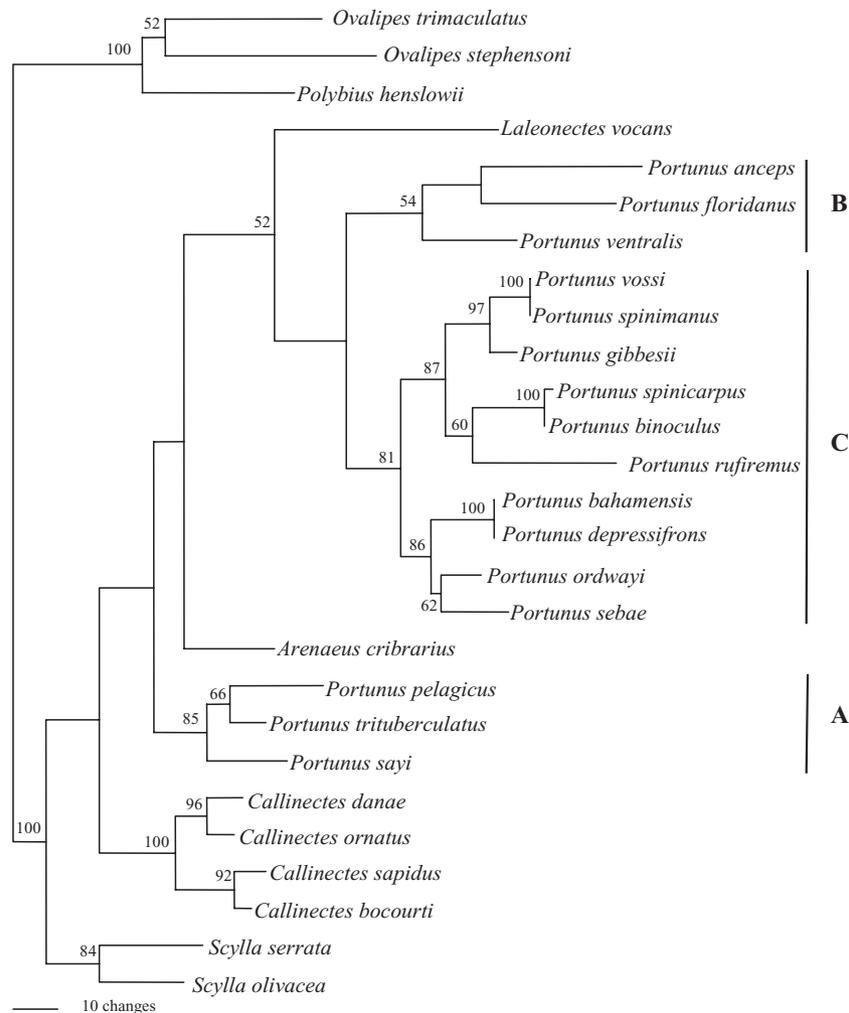
A total of 535 positions of the 16S rRNA gene (not including the primer regions) were aligned for 16 described species of *Portunus*, four species of *Callinectes*, two of *Ovalipes*, two of *Scylla*, *Laleonectes vocans* (A. Milne-Edwards, 1878), *Arenaeus cribrarius* (Lamarck, 1818) and *Polybius henslowii* Leach, 1820. From these, 38 bp could not be aligned and were

removed from the alignment (329–366). Thus, 497 homologous basepairs were used for the phylogenetic analysis, 159 of which were found to be parsimony-informative positions. The optimal model for the data set, selected under the Akaike information criterion (AIC), as implemented in Modeltest (Posada & Crandall, 1998), was the TrN+I+G (Tamura & Nei, 1993 + Invariable sites + Gamma distribution) with the following parameters: assumed nucleotide frequencies A = 0.3802, C = 0.0887, G = 0.1643, T = 0.3668; substitution model A-C = 1.00, A-G = 5.65, A-T = 1.00, C-G = 1.00, C-T = 8.41, G-T = 1.00; proportion of invariable sites I = 0.1920; variable sites follow a gamma distribution with shape parameter = 0.3713. Thus, posterior analyses are based on this evolutionary model.

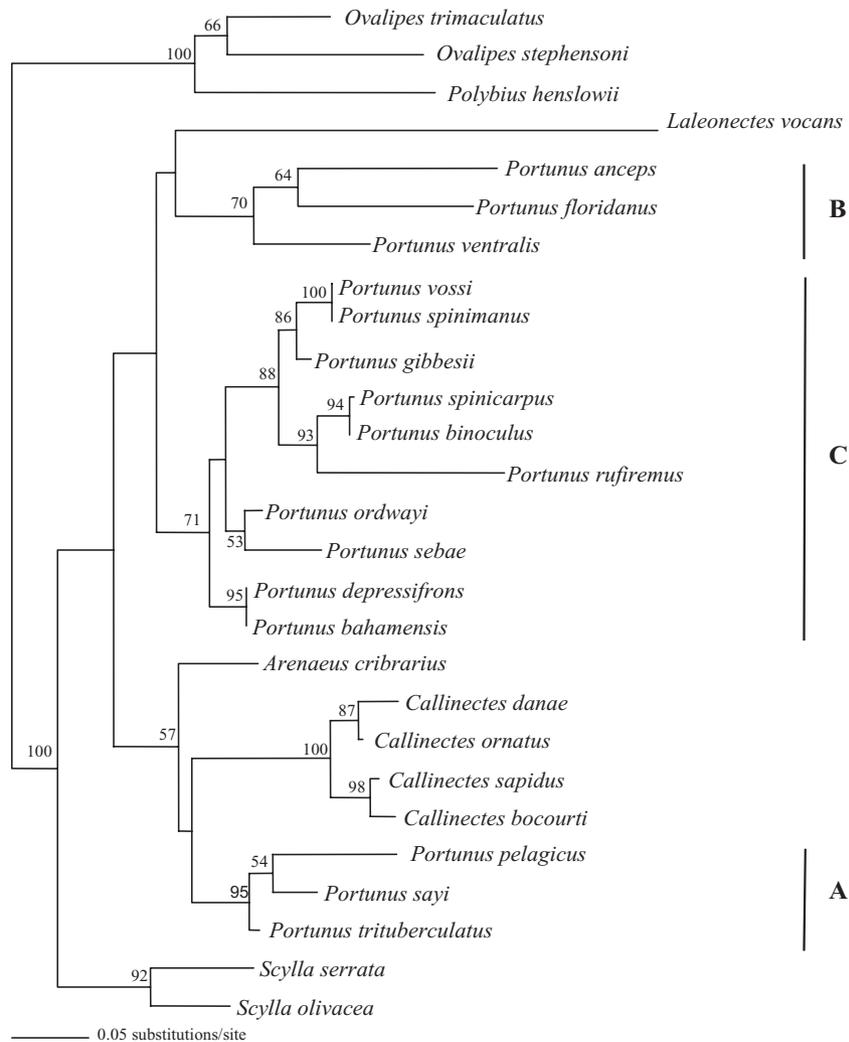
MP analysis yielded three equally parsimonious trees of length 728, with consistency index

(CI) = 0.523 and retention index (RI) = 0.611 (Fig. 1). Except on the position of *Laleonectes*, all trees showed the same topology. Overall, distance, Bayesian and parsimony methods resulted in similar tree topologies, although differences were observed (Figs 1–3).

In our analyses, the genus *Portunus* was separated into at least three lineages (clades A–C, Figs 1–3). *P. trituberculatus*, *Portunus sayi*, and the type species of the genus, *Portunus pelagicus*, clustered together, well separated from the other species presently assigned to *Portunus* (clade A, Figs 1–3). This clade (A) shared a common basal lineage with *Arenaeus* and *Callinectes* in the analysis while the other two major clades (B and C) derived from an independent common lineage. One of these (B) was formed by *P. ventralis*, *P. anceps* and *P. floridanus*, but only under weak support by MP and NJ analyses (54/64 bootstraps, respectively; Figs 1, 2). The other (C) grouped remaining



**Figure 1.** One of the three phylogenetic trees obtained from MP analysis of 16S rRNA gene sequences for the western Atlantic species of *Portunus*, and other selected portunids. Numbers are significance values for 1000 bootstraps; values  $\leq 50\%$  are not shown. Letters to right centre on three major clades (A–C), as discussed in the text.



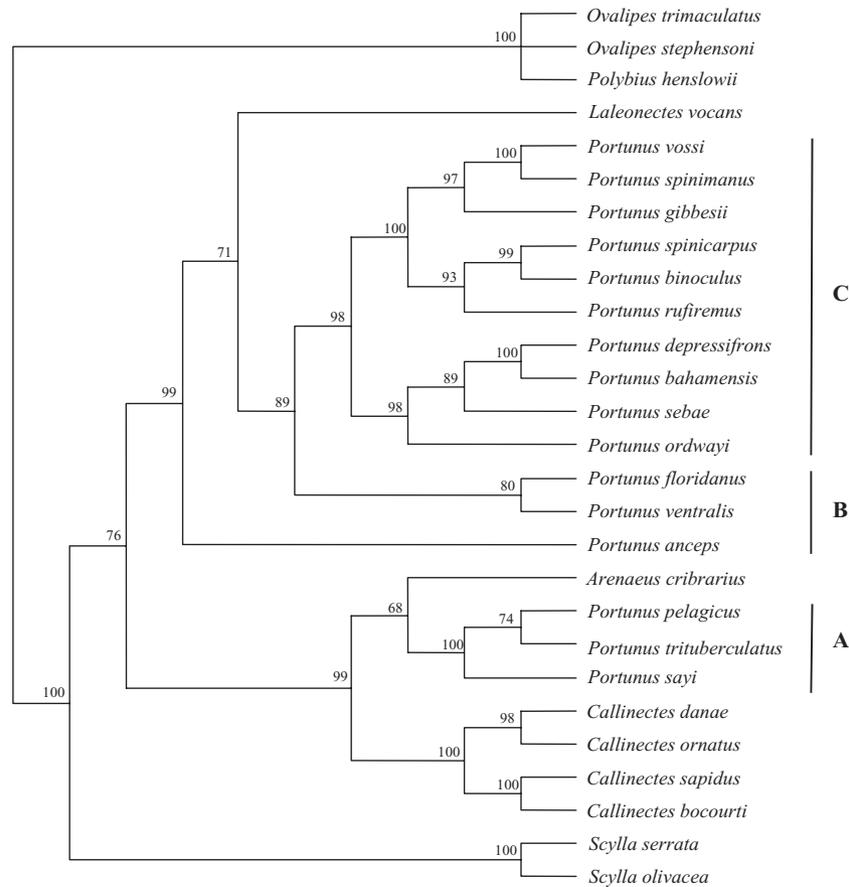
**Figure 2.** Single phylogenetic tree obtained from NJ analysis of 16S rRNA gene sequences for the western Atlantic species of *Portunus*, and other selected portunids. Numbers are significance values for 1000 bootstraps; values  $\leq 50\%$  are not shown. Letters to right centre on three major clades (A–C), as discussed in the text.

species of *Portunus* in our analysis and was supported by all three analyses, although some internal nodes were poorly resolved. Both MP and NJ analyses placed *L. vocans* in a separate lineage from clades A, B and C. However, in BAY analysis *P. anceps* was placed at the base of clades B and C; thus, *L. vocans* was included in a large clade that also contained clades B and C of *Portunus*.

Comparison of a paratype of *P. vossi* from Florida with Brazilian materials identifiable as *P. spinimanus* did not reveal a single diagnostic molecular character in our sequenced fragment of 16S mtDNA (comparisons made prior to the phylogenetic analysis); the same was true in our sequence-based comparison of materials identifiable as *P. depressifrons* from Florida with a paratype of *P. bahamensis* from Eleuthera Island, Bahamas.

## DISCUSSION

Here we report the first molecularly based phylogenetic analysis of western Atlantic swimming crabs in the genus *Portunus*. Although we do not include representation of all species assigned worldwide to this genus, monophyly of *Portunus* was not supported for the western Atlantic species by any of the three analyses that we based on part of the 16S rRNA gene. It would thus appear that the genus has been maintained in its presently broad composition on the basis of morphological characters that do not accurately reflect evolutionary history of the group, and that further generic-level revisions or elevations of subgenera in the group may be required. A complete analysis of the genus and its relationships to other members of the family is clearly warranted.



**Figure 3.** Consensus phylogenetic tree obtained from BAY analysis (50% majority consensus of 9800 trees) of 16S rRNA gene sequences for the western Atlantic species of *Portunus*, and other selected portunids. Numbers are posterior probabilities; values  $\leq 50\%$  are not shown. Letters to right centre on three major clades (A–C), as discussed in the text.

We included the western Atlantic representative of the genus *Laleonectes*, *L. vocans*, in an attempt to confirm its taxonomic status. Members of this genus have long been assigned to *Portunus* because of general morphological similarity to most members of that genus (Stephenson, Williams & Lance, 1968; Manning & Holthuis, 1981; Abele & Kim, 1986). However, the presence of a stridulating apparatus in both species of *Laleonectes*, as reflected in the configuration of the pterygostomial region of the carapace and merus of the cheliped, makes this species and its congener, *L. nipponensis*, unique (Manning & Chace, 1990). Our molecular analysis supports the morphologically based conclusion that *L. vocans* should not be considered a species of *Portunus*. This is made apparent by the distance at which *Laleonectes* is positioned from clade A, which includes *Portunus pelagicus*, the type species of the genus. However, this also implies that, if generic distinction is warranted for *Laleonectes*, it is no less appropriate for other clades presently treated under *Portunus*, but that are well diverged from clade A. Eventual inclusion of *L. nipponensis* in the analy-

sis, along with broader representation of world portunid genera, will be required to clarify further the relationship between *Laleonectes* and lineages of the Portunidae overall.

Of the 14 species of *Portunus* usually recognized from the western Atlantic Ocean, only 12 can be confirmed as valid species on the basis of our current sequence analyses. *Portunus vossi* and *P. spinimanus* did not exhibit a single difference in the sequences that we examined, and we thus cannot support their separation. As described by Lemaitre (1991), *Portunus vossi* was noted to be morphologically more similar to *P. ordwayi* than to *P. spinimanus*. One of us (D.L.F.) has examined and photographed series of juvenile *P. spinimanus* that also exhibit this superficial resemblance to *P. ordwayi*. Ostensibly, *P. vossi* should be distinguishable from both *P. ordwayi* and *P. spinimanus* by, among other features, the rounded shape of its frontal teeth (Lemaitre, 1991); however, we find that these teeth are also commonly rounded in juvenile specimens of *P. spinimanus*. Although morphological differences in armature of the swimming leg merus and differences

in iridescence of the chelae also served to distinguish *P. vossi* from *P. ordwayi*, those reported for *P. vossi* rather closely conform to character states in juvenile specimens of *P. spinimanus* that we have examined from throughout the Gulf of Mexico, Venezuela and Brazil (ULLZ and MZUSP). In fact, we sequenced three specimens of *P. spinimanus* from the Gulf of Mexico and Venezuela (ULLZ 4774, 4757) and their sequences were 100% equal to those from the four specimens from Brazil (ULLZ 4754, 4776, 4777; MZUSP 12786) and to that of *P. vossi* included in our analysis.

The lack of differences in 16S mtDNA sequences between *P. vossi* and *P. spinimanus* leads us to conclude that the former is a junior synonym of the later. The morphological variations used to differentiate *P. vossi* appear to be attributable to the relatively small size of the types and other specimens (carapace widths, with lateral spines, 11.0–34.0 mm) examined in the course of the description (Lemaitre, 1991). These specimens of *P. vossi* were also all found on mud, sand with sea grasses, and algal rubble (Lemaitre, 1991), habitats in which we commonly encounter juveniles of *P. spinimanus*. *Portunus spinimanus* in general can reach sizes of 65 × 110 mm (carapace length × width), with adults sometimes occurring alongside juveniles in the aforementioned habitats. However, the adults are also found in more open habitats on sand banks with some cover, on coral reefs, along beaches under *Sargassum*, in deeper channels of lagoons and embayments, and sometimes in the water column (Williams, 1984; D.L.F., pers. observ.).

*Portunus depressifrons* and *P. bahamensis* constitute another pair of species that have long been separated on the basis of minor morphological differences, but that were indistinguishable on the basis of our 16S mtDNA sequences. *Portunus bahamensis* was originally proposed to be an endemic species from the Bahamas (Rathbun, 1930). However, ranges of the two species were thereafter reported to overlap (Garth, 1978), and the range of *P. bahamensis* was subsequently reported to extend outside the Bahamas (Lemaitre, 1984). A close relationship between these two species has long been suggested (Stephenson *et al.*, 1968; Garth, 1978). In the course of her very brief description of *P. bahamensis*, Rathbun (1930: 90) noted its very close resemblance to *P. depressifrons*, and then based the separation almost entirely on minor differences between the species in relative sizes of anterolateral teeth, relative carapace dimensions, sinuosity of selected granular lines and a few other qualitative features of the pereopods. The morphological characters usually cited for the separation of these species are in our opinion vague and difficult to apply with rigour, which is probably why few definitive listings of the species have appeared since the original description. From our examination of a

series of *P. depressifrons* and meagre holdings assigned to *P. bahamensis* (USNM; ULLZ), we conclude that characters used to separate *P. bahamensis* probably represent ontological and other interspecific variations of *P. depressifrons*. Taken together, molecular and morphological evidence lead us to conclude that *P. bahamensis* should be regarded as a junior synonym of *P. depressifrons*.

*Portunus binoculus* and *P. spinicarpus* comprise one additional western Atlantic species pair separated by such comparatively minor morphological differences that we were led to question the validity of the separation. The major character used to support the original separation was colour pattern (Holthuis, 1969), on both the chelipeds and the carapace. The presence of two submedian red spots in the middle of the carapace of *P. binoculus* makes this species easily separable from *P. spinicarpus* even after long periods of preservation in ethanol. At the molecular level, we were able to separate these two species on the basis of 16S rRNA gene sequences. However, differences between the two species were very limited (three transitions, four transversions, four indels) compared with those observed among other species of *Portunus*. In some brachyuran groups, even such limited differences in 16S mtDNA sequences can be diagnostic for separate species (see Schubart, Neigel & Felder, 2000b; Schubart *et al.*, 2001a). Thus, pending future studies that might involve larger samples for population genetic analyses, we continue to recognize the separation between these two species.

Our present analysis indicates that the genus *Portunus* comprises at least three lineages, each of which may warrant independent generic rank. One of these clades appears to be variously allied to species of the genus *Callinectes* in our analyses (Figs 1–3, clade A) and in the western Atlantic is represented exclusively by *P. sayi*. From our presently limited sampling, this clade includes at least the Indo-Pacific type species of the genus, *Portunus pelagicus*, along with *P. trituberculatus*. This close molecular relationship to *Callinectes* corroborates the proposition made previously on the basis of numerical and morphological data by Stephenson *et al.* (1968), who suggest *P. sayi* to be the sixth species belonging to the so-called ‘*P. pelagicus* group’, which includes *P. sanguinolentus*, *P. pubescens*, *P. convexus*, *P. trituberculatus* and *P. pelagicus*. The aforementioned authors also postulated that, among all members of the ‘*P. pelagicus* group’, *P. sayi* was possibly the closest relative to both *Callinectes* and *Arenaeus*. However, there is no evidence in our analyses that *P. sayi* is more closely related to *Callinectes* and *Arenaeus* than are the other two members of this clade that we examined.

We cannot argue on the basis of our presently limited molecular analyses whether the entire

'*P. pelagicus* group' comprises a monophyletic group. However, the three species of that grouping in this analysis clearly constitute a clade of apparent generic rank, comparable with the sister groups *Callinectes*, *Arenaeus* and *Scylla*, with which they share a common node. As membership of this clade includes *P. pelagicus*, type species of the genus, we must conclude that it represents *Portunus* s.s. Further molecular analyses of additional candidate species should readily confirm its full membership.

Another of the apparent clades in our molecularly based phylogeny consists of *P. ventralis*, *P. floridanus* and *P. anceps* (Figs 1, 2, clade B). However, this grouping is weakly supported (70 and 54% bootstrap support in NJ and MP analyses, respectively; no BAY support), which suggests that it could either become subdivided into two clades or a compound single group once a more thorough coverage of putative worldwide congeners can be included in an analyses. Stephenson *et al.* (1968) considered *P. floridanus* and *P. anceps* a part of a '*P. bahamensis* group', which unlike our groupings also included *P. bahamensis* and *P. depressifrons*. Their '*P. bahamensis* group' was something of a default grouping, suggested by these authors not because of the close relationship among the mentioned species but because they all lacked close similarities to both the '*P. pelagicus* group' and the '*P. xantusii* group'. The questionably delimited group included 15 species (Stephenson *et al.*, 1968), nine of them distributed in the eastern Pacific (western American), and it clearly deserves additional study.

In our analyses, most species of the '*P. bahamensis* group' (subject to the synonymies we propose above and including *P. binoculus* which was described after the analyses by Stephenson *et al.*, 1968) resolve into a large and complex group (Figs 1–3, clade C) that includes *P. spinimanus*, *P. gibbesii*, *P. spinicarpus*, *P. binoculus*, *P. rufiremus*, *P. depressifrons*, *P. ordwayi* and *P. sebae*. This finding in many respects agrees with the groupings that Stephenson *et al.* (1968) based upon numerical analysis of morphology. Their morphological groupings differ from our molecular phylogeny primarily in that the former included *P. ventralis* but excluded both *P. rufiremus* and *P. depressifrons* (plus its junior synonym *P. bahamensis*) from the '*P. xantusii* group'. However, *P. ventralis* was found along the borderline of the morphologically based '*P. xantusii* group' and could as easily have been treated as a separate clade, much as suggested by our molecularly based phylogenetic tree.

The internal relationships within clade C will perhaps be much better resolved when re-analysed with the inclusion of additional species of *Portunus*, as well as additional genes. However, the present molecularly based analysis does reveal two well-supported

branches, one including *P. rufiremus*, *P. spinicarpus*, *P. binoculus*, *P. spinimanus* and *P. gibbesii*, the other including *P. depressifrons*, *P. ordwayi* and *P. sebae*. It was somewhat surprising to find *P. gibbesii* as the closest relative to *P. spinimanus* and *P. rufiremus* closely related to both *P. spinicarpus* and *P. binoculus*. Because they share a broader carapace than the other species mentioned, a feature commonly used as a character of subgenera and in identification keys, *Portunus rufiremus* and *P. gibbesii* were expected to be close relatives to one another. This suggests that carapace width should be regarded as a potentially convergent character in this group, rather than a diagnostic feature for any major clade.

By relegation of *P. vossi* and *P. bahamensis* to the status of junior synonyms, our account of the western Atlantic *Portunus* becomes limited to 12 species. The phylogenetic relationship among them cannot be entirely understood on the basis of our presently limited study of molecular genetics encompassing only '14' of the almost 80 worldwide recognized species. However, even this initial analysis clearly demonstrates that the genus is not monophyletic and that the group should be subjected to further taxonomic revision.

#### ACKNOWLEDGEMENTS

F.L.M. is grateful to FAPESP (Grant no. 00/11220-1) for financial support during his UL-Lafayette senior postdoctoral affiliation, to CNPq for Research Scholarships (PQ 300279/95-7; 301261/04-0) and Research Grant (472746/04-9). We thank Augusto Flores, Jack O'Brien and Marcelo A. Scelzo for making available some essential fresh specimens, and Rafael Lemaitre and Gustavo Melo for comments on an earlier draft of this manuscript and loans of materials from USNM and MZUSP collections, respectively. We wish to express our gratitude to anonymous reviewers for critical reading and valuable suggestions on the manuscript. This study was supported under funding from the US National Science Foundation (grant Nos. DEB-0315995 and EF 0531603) and the US Department of Energy (grant No. DE-FG02-97ER1220) to D.L.F. This is contribution number 112 from the UL-Lafayette Laboratory for Crustacean Research.

#### REFERENCES

- Abele LG, Kim W. 1986. An illustrated guide to the marine decapod crustaceans of Florida. *State of Florida Department of Environmental Regulation Technical Series* 8 (1): 1–760.
- Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA, Saunders NC. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics* 18: 484–522.

- Bucklin A, Frost BW, Kocher TD. 1995.** Molecular systematics of six *Calanus* and three *Metridia* species (Copepoda: Calanoida). *Marine Biology* **121**: 655–664.
- Garth JS. 1978.** Marine biological investigations in the Bahamas. 19. Decapoda Brachyura. *Sarsia* **63**: 317–333.
- Hall TA. 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 JS. 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 DL, Clark AG. 1997.** *Principles of population genetics*, 2nd edn. Sunderland, MA: Sinauer Associates.
- Hideyuki I, Cheng JH, Hamasaki K, Numachi KI. 2004.** Identification of four mud crab species (genus *Scylla*) using ITS-1 and 16S rDNA markers. *Aquatic Living Resources* **17**: 31–34.
- Holthuis LB. 1959.** The Crustacea Decapoda of Suriname (Dutch Guiana). *Zoologische Verhandlungen* **44**: 1–296.
- Holthuis LB. 1969.** *Portunus binoculus* n. sp., a new deep-water swimming crab from the Caribbean region (Crustacea, Decapoda, Brachyura). *Bulletin of Marine Science* **19**: 409–427.
- Lemaitre R. 1984.** Decapod crustaceans from Cay Sal Bank, Bahamas, with notes on their zoogeographic affinities. *Journal of Crustacean Biology* **4** (3): 425–447.
- Lemaitre R. 1991.** To the memory of Gilbert L. Voss: *Portunus vossi*, a rare new species of swimming crab (Decapoda: Brachyura: Portunidae) from the west Coast of Florida. *Bulletin of Marine Science* **49** (1/2): 546–551.
- 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.
- Manning R, Chace FA Jr. 1990.** Decapod and stomatopod Crustacea from Ascension Island, South Atlantic Ocean. *Smithsonian Contributions to Zoology* **503**: 1–91.
- Manning R, Holthuis LB. 1981.** West African brachyuran crabs (Crustacea: Decapoda). *Smithsonian Contributions to Zoology* **306**: 1–379.
- Mantelatto FLM, Robles R, Biagi R, Felder DL. 2006.** Molecular analysis of the taxonomic and distributional status for the hermit crab genera *Loxopagurus* Forest, 1964, and *Isocheles* Stimpson, 1858 (Decapoda, Anomura, Diogenidae). *Zoosystema* **28**: 495–506.
- Mathews LM, Schubart CD, Neigel JE, Felder DL. 2002.** Genetic, ecological, and behavioural divergence between two sibling snapping shrimp species (Crustacea: Decapoda: *Alpheus*). *Molecular Ecology* **11**: 1427–1437.
- Melo GAS. 1996.** *Manual de identificação dos Brachyura (Caranguejos e Siris) do litoral brasileiro*. São Paulo: Fundação de Amparo à Pesquisa do Estado de São Paulo.
- Morrison CL, Harvey AW, Lavery S, Tieu K, Huang Y, Cunningham CW. 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 CL, Rios R, Duffy JE. 2004.** Phylogenetic evidence for an ancient rapid radiation of Caribbean sponge-dwelling snapping shrimps (*Synalpheus*). *Molecular Phylogenetics and Evolution* **30**: 563–581.
- Neigel JE, Avise JC. 1986.** Phylogenetic relationships of mitochondrial DNA under various demographic models of speciation. In: Karlin S, Nevo E, eds. *Evolutionary processes and theory*. Academic Press, New York, 515–534.
- Posada D, Crandall KA. 1998.** MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14** (9): 817–818.
- Quan J, Zhuang Z, Deng J, Dai J, Zhang YP. 2004.** Phylogenetic relationships of 12 Penaeoidea shrimp species deduced from mitochondrial DNA sequences. *Biochemical Genetics* **42**: 331–345.
- Rathbun MJ. 1930.** The Cancroid crabs of America of the families Euryalidae, Portunidae, Atelecyclidae, Cancridae, and Xanthidae. *United States Natural Museum Bulletin* **152**: 1–609.
- Robles R, Schubart CD, Conde JE, Carmona-suárez C, Alvarez F, Villalobos JL, Felder DL. 2007.** Molecular phylogeny of the American *Callinectes* Stimpson, 1860 (Brachyura: Portunidae), based on two partial mitochondrial genes. *Marine Biology* **150**: 1265–1274.
- Rokas A, Ladukakis A, Zouros E. 2003.** Animal mitochondrial DNA recombination revisited. *Trends in Ecology and Evolution* **18** (8): 411–417.
- Schubart CD, Conde JE, Carmona-Suárez C, Robles R, Felder DL. 2001a.** Lack of divergence between 16S mtDNA sequences of the swimming crabs *Callinectes bocourti* and *C. maracaiboensis* (Brachyura: Portunidae). *Fishery Bulletin* **99**: 475–481.
- Schubart CD, Cuesta JA, Felder DL. 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.
- Schubart CD, Cuesta JA, Rodriguez A. 2001a.** Molecular phylogeny of the crab genus *Brachynotus* (Brachyura: Varunidae) based on the 16S rRNA. *Hydrobiologia* **449**: 41–46.
- Schubart CD, Neigel JE, Felder DL. 2000a.** Use of the mitochondrial 16S rRNA gene for phylogenetic and population studies of Crustacea. *Crustacean Issues* **12**: 817–830.
- Schubart CD, Neigel JE, Felder DL. 2000b.** Molecular phylogeny of mud crabs (Brachyura: Panopeidae) from the northwestern Atlantic and the role of morphological stasis and convergence. *Marine Biology* **137**: 11–18.
- Stephenson W. 1962.** Evolution and ecology of portunid crabs, with special reference to Australian species. In: Leeper GW, ed. *The evolution of living organisms. Symposium of the Royal Society of Victoria*. Melbourne: Melbourne University Press, 311–327.
- Stephenson W. 1972.** An annotated check list and key to the Indo-West-Pacific swimming crabs (Crustacea: Decapoda: Portunidae). *Royal Society of New Zealand* **1**: 3–63.
- Stephenson W, Campbell B. 1959.** The Australian portunids (Crustacea: Portunidae). III. The genus *Portunus*.

- Australian Journal of Marine and Freshwater Research* **10** (1): 84–129.
- Stephenson W, Rees M. 1967.** Some Portunidae crabs from the Pacific and Indian oceans in the collections of the Smithsonian Institution. *Proceedings of the United States of Natural Museum* **120** (3556): 1–114.
- Stephenson W, Williams WT, Lance GN. 1968.** Numerical approaches to the relationships of certain American swimming crabs (Crustacea: Portunidae). *Proceedings of the United States of Natural Museum* **124** (3645): 1–25.
- Stillman JH, Reeb CA. 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 DL. 1998.** *PAUP\**. *Phylogenetic analysis using parsimony (\*and other methods)*, Version 4. Sunderland, MA: Sinauer Associates.
- Tamura K, Nei M. 1993.** Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in human and chimpanzees. *Molecular Biology and Evolution* **10**: 512–526.
- Tudge CC, 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.
- Williams AB. 1984.** *Shrimps, lobsters, and crabs of the Atlantic coast of the eastern United States, Maine to Florida*. Washington, DC: Smithsonian Institution Press.