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Morphological and molecular evidence for a new endemic freshwater crab, *Sesarma ayatum* sp. n., (Grapsidae, Sesarminae) from eastern Jamaica

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Recent morphological studies have shown that the Jamaican mountain stream crab, *Sesarma bidentatum* Benedict, 1892 (Sensu Abele 1992), consists of several allopatric forms. Three new species have been described from western Jamaica (summarized in Reimer *et al.* 1998) restricting the range of *S. bidentatum* to central and eastern Jamaica. In this study we used morphology and genetic markers to further distinguish crab populations from different East Jamaican river systems. Morphological characters such as the shape of male gonopods, front, chelar carpus, and walking legs, as well as mtDNA sequence (16S rRNA gene) comparisons were consistent in the separation of populations of *S. bidentatum* into two distinct groups. A new species, *Sesarma ayatum* sp. n., is described with a distribution from easternmost Jamaica to the eastern Blue Mountains. © 1998 The Norwegian Academy of Science and Letters

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Introduction

Until 1994, five known endemic species of Sesarminae from Jamaica were well defined in terms of morphology and habitat preferences: *Sesarma bidentatum* Benedict, 1892 thriving along the banks of mountain streams, *Metopaulias depressus* Rathbun, 1896 from water tanks within bromeliad leaf axils, *S. verleyi* Rathbun, 1914 exclusively found in limestone caves, *S. jarvisi* Rathbun, 1914 from terrestrial rock rubble and snail shells of western and central Jamaica, and *S. cookei* Hartnoll, 1971 from terrestrial rock rubble and burrow systems of eastern Jamaica (see Hartnoll 1964, 1971; Diesel & Horst 1995). With the description of *Sesarma windsor* by Türkay and Diesel (1994), a new species was introduced that was morphologically very similar to *S. bidentatum* and also similar in preferred habitat type (freshwater mountain streams and occasionally limestone caves, Peck 1992). The separation of these two species was based on differences in the shape of gonopods and gonopores and the tuberculation of the chelae. Otherwise, the western *S. windsor* and the eastern *S. bidentatum* were noted to be very similar and can be considered as representatives of the same morphotype (here termed *bidentatum*-morphotype) with an island-wide distribution.

In subsequent years, morphological and molecular studies on crabs of the *bidentatum*-morphotype revealed the existence of additional distinct populations and species (Schubart 1997; Schubart *et al.* 1997; Reimer *et al.* 1998). Two additional species were described from western Jamaica on the basis of morphology: *S. fossarum* Schubart, Reimer, Diesel & Türkay, 1997 and *S. dolphinum* Reimer, Schubart & Diesel, 1998. Crabs assigned to *S. fossarum* included type material from *S. windsor* and a redescription of the latter became necessary (Schubart *et al.* 1997).

In this study we analyzed all available material of the *bidentatum*-morphotype from central and eastern Jamaica (east of 77°25′ W). In addition to detailed morphological studies, we used partial DNA sequences of the 16S rRNA mitochondrial gene to establish regional differences among crabs of this morphotype. Two clearly distinct groups could thereby be recognized. Here we describe crabs of the *bidentatum*-morphotype from easternmost Jamaica as a new species, restricting the distribution of *S. bidentatum* to northern Middlesex and the western slopes and central valleys of the Blue Mountains.

Material and methods

Freshwater crabs of the *bidentatum*-morphotype were collected from 27 localities in central and eastern Jamaica encompassing 15 different river systems between 1993 and 1997. In addition, archived material from the collection of Rudolf Diesel (R), the Senckenberg Naturhistorisches Museum (SMF), the National Museum of Natural History, Washington,

DC (USNM) and the Museum of Comparative Zoology, Cambridge (MCZ) was made available. Overall, 267 specimens of *S. bidentatum* were examined.

Crab tissue for DNA sequencing was preserved in 75% ethanol. Genomic DNA was isolated from the muscle tissue of walking legs from 16 crabs using a phenol-chloroform extraction. Only in one case (John Crow Mountains) was more than one specimen used from the same population. Selective amplification of a 520–526 basepair fragment from the mitochondrial large subunit (16S) rRNA gene was carried out by polymerase-chain-reaction (PCR) (33–40 cycles; 1min 94°/1min 50–55°/2.5min 72° denaturing/annealing/extension temperatures) using primers 16sar (5′-CGCCTGTTTATCAAAAACAT -3′) and 16sbr (5′- CCGGTCTGAACTCAGATCACGT -3′) (Palumbi *et al.* 1991) in addition to internal self-designed primers (5′- TGACCGTGCAAAGGTAGCATAA -3′) and (5′-TTATCRCCCCAATAAAATA -3′) (Schubart, Diesel & Hedges 1998). Double-stranded PCR products were purified and used for asymmetric PCR (40–45 cycles; 1min 94°/1min 55-60°/2.5min 72°) (see Gyllenstein and Erlich 1988). The resultant single-stranded DNA was filtered and both strands were sequenced with the primers mentioned above by dideoxy chain termination with S35 radioactive labeling (see Sanger *et al.* 1977). Alignments were done by hand using the multisequence editing program ESEE (Cabot and Beckenbach 1989) and are shown in Appendix 2. Sequences of the seven different haplotypes have been deposited in the EMBL database (AJ005951-AJ005957, see also AJ225890-AJ225891 from Schubart *et al.* 1998).

Results

Morphological comparisons showed consistent differences of crab populations from central Jamaica and the western slopes and central valleys of the Blue Mountains from those of the eastern slopes of the Blue Mountains and the John Crow Mountains (Table I). These differences include the shape of the male gonopods, thus suggesting reproductive isolation.

The comparison of 526 basepairs of mtDNA sequence (16S rRNA gene) from 16 crabs revealed the existence of seven different haplotypes (ht) (Fig. 1) that, according to genetic distances, can be separated into two distinct groups. Populations from the eastern slopes of the Blue Mountains and the John Crow Mountains (ht 1 to 4) differed from each other by a maximum of three mutations, but were clearly distinct (11–14 mutations) from haplotypes representing populations from central Jamaica and the western slopes and central valleys of the Blue Mountains (ht 5 to 7) (Table II). Between haplotypes from the latter populations there was again a low genetic variation of no more than two mutations (Table II). Percentage genetic deviation and types of mutations established between all haplotypes are shown in Table II. Two haplotypes (ht 1 and 5) were clearly most abundant, both being found in five crab specimens. Between the two major groups of similar haplotypes, 11 diagnostic sites (2 transversions, 4 transitions and 5 indels) consistently support separation.

Morphological and molecular methods thus render similar results, clearly suggesting the existence of two distinct species among the studied material, all of which would have been previously assigned to *Sesarma bidentatum*. The original description of this species (Benedict 1892) is very brief and applies to all crabs of the *bidentatum*-morphotype. The holotype, an immature female, was found in the vicinity of Port Henderson (Kingston Harbour) and most probably was washed down from the western Blue Mountains. We therefore attribute the name *S. bidentatum* to the western Blue Mountain population and the new species is described from material of the eastern Jamaican populations.

Systematic Account

Family GRAPSIDAE
Subfamily SESARMINAE

Sesarma ayatum sp. n. (Figs 2–4)

Sesarma bidentatum Benedict, 1892.—Abele 1992: 17, Figs 4e, 5e, 10, 11s–x [in part, including figured material].— Reimer *et al.* 1998 [data on egg size].
Sesarma sp.— Schubart *et al.* 1998.
Material examined. Holotype: SMF 23715, male, 25 April 1994, coll. R. Diesel and R. Schieke. Small river near John Crow Forest Road, east of Ecclesdown, tributary to Drivers River, John Crow Mountains, Portland, Jamaica. Paratypes: USNM 260855, 1 male and 1 female from SW Ecclesdown, Drivers River drainage (~2000′ elev.), John Crow Mountains, Portland, Jamaica, June 1976; coll. L.G. Abele & D.B. Means; SMF 23716, 2 males and 2 females, ZMB 27248, 1 male and MCZ 13339, 1 female all same data as holotype; USNM 260854, 2 males and 2 females from same locality as holotype, 5 March 1995; coll. R. Diesel and C.D. Schubart. Other material: R-341, 1 female from tributary to Swift River, near Ythanside, Portland, Jamaica (3 March 1995; coll. C.D. Schubart), DNA sequence; R-370, 1 female from Somerset Falls, Portland, Jamaica (17 May 1993; coll. R. Diesel and M. Schuh), DNA sequence; R-367, 3 males and 1 female from tributary to Rio Grande, Millbank, Portland, Jamaica (20 March 1997; coll. R. Diesel, J. Reimer and C.D. Schubart); SMF 19531-19534, 2 males and 2 females (dry) and R-40, 1 female from tributary to Rio Grande, Bowden, St. Thomas, Jamaica (20 March 1987; coll. R. Diesel); R-127, 1 male and 1 female from Three Finger Spring, 1 km southeast of Four Feet, tributary to Rio Grande, St. Thomas, Jamaica (2 September 1992; coll. G. Bäurle), DNA sequence; R-368, 6 males and 3 females from Non Such pumphouse, John Crow Mountains, Portland, Jamaica (21 March 1997; coll. R. Diesel, J. Reimer and C.D. Schubart); USNM 260856, 5 males and 1 female same data as USNM 260855; SMF 19535, 2 males (dry) from tributary to Drivers River, Ecclesdown water pump, John Crow Mountains, Portland, Jamaica (22 March 1987; coll.

Table I. *Morphological differences between crabs from central Jamaica and the western and central Blue Mountains* (Sesarma bidentatum *Benedict*, 1892) *and those of the eastern Blue Mountains and the John Crow Mountains* (S. ayatum sp. n.).

	Sesarma ayatum sp. n.	*Sesarma bidentatum*
Front (Fig. 3A–B)	anterior lateral lobes narrow, with anterior border subparallel to front	anterior lateral lobes broader, with an oblique anterior border
Carpus of chelae (Fig. 4A–B)	row of granules at dorsal interior border unevenly long and pointed at sharp bend to distal end	row of granules at dorsal interior border evenly sized and blunt at smooth and rounded bend to distal end
Male gonopod (Fig. 4C–F)	slender and distally elongate long horny apex	broader, distally shorter and more deflexed shorter horny apex
Meri of walking legs	2.5–2.7 times as long as broad	2.65–2.85 times as long as broad[1]

[1] with exception of westernmost population from Roaring River, St. Ann.

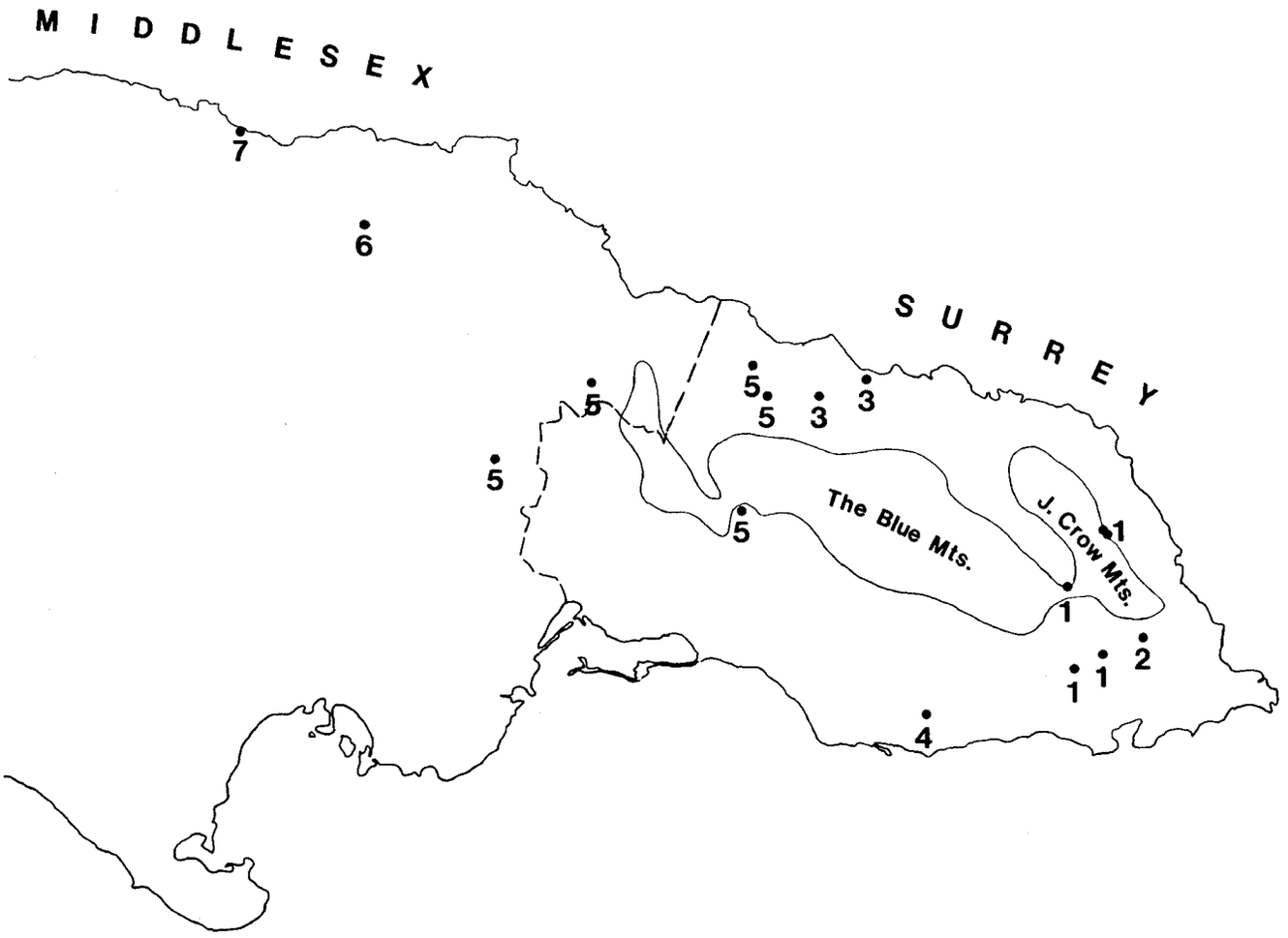

Fig. 1. Map of central and eastern Jamaica showing the distribution of seven different haplotypes of the *bidentatum*-morphotype as established by DNA sequencing of fractions of the 16S rRNA gene.

R. Diesel); R-126, 1 male from Drivers River cave, 250 m above Reach Falls, John Crow Mountains, Portland, Jamaica (18 August 1992; coll. G. and W. Bäurle); R-273, 1 female from dry river bed under stones, John Crow Forest Road, Portland, Jamaica (12 February 1993; coll. R. Diesel and C.D. Schubart) DNA sequence; R-256, 12 females same data as holotype; R-280, 7 females and 3 males same data as USNM 260854, DNA sequence; R-284, 1 male from same locality as holotype (23 March 1995; coll. R. Diesel and C.D. Schubart); R-248, 1 male and 2 females from north of Hordley, St. Thomas, Jamaica (8 March 1994; coll. R. Diesel and R. Schieke); R-297, 15 males and 14 females from Johnson Mountain, St. Thomas, Jamaica (April 1994; coll. R. Diesel and R. Schieke), DNA sequence; USNM 260857, 11 males and 10 females from Bath, in stream at Spa (~380′ elev.), St. Thomas, Jamaica, (3 May 1976; coll. L.G. Abele & D.B. Means); R-79, 1 male from Sulphur River, St. Thomas, Jamaica (26 June 1992; coll. Z. Sary), DNA sequence; R-80, 9 males and 1 female from Dry River, St. Thomas, Jamaica (26 June 1992; coll. G. Bäurle), DNA sequence; R-54, 4 males and 3 females from Simo Spring, Yallahs Hill, St. Thomas, Jamaica (30 December 1992; coll. R. Diesel), DNA sequence; R-78, 4 males and 2 females from same locality (9 March 1992; coll. R. Diesel and G. Bäurle).

Etymology. The species derives from the Rastafarian name Ayata, as a tribute to the Jamaican people and to our hosts and friends in Christmas River (Portland). The ending "-um" was selected to fit the neuter gender of the genus *Sesarma*.

Table II. *Percent deviation (upper values) and number of differences (lower numbers) among 7 haplotypes (ht) of 16S mtDNA found in 16 crab specimens (n) of the* bidentatum*-morphotype. Haplotypes* 1–4 *correspond to* Sesarma ayatum *sp. n. and haplotypes* 5–7 *to* S. bidentatum *Benedict*, 1892. *Type of differences abbreviated as follows: v for transversions, s for transitions, i for indels.*

ht	n	percent genetic deviation/number of differences						
		1	2	3	4	5	6	7
1	5	—	0.2	0.2	0.4	2.5	2.3	2.5
2	1	1i	—	0.4	0.6	2.7	2.5	2.7
3	2	1s	1s,1i	—	0.2	2.3	2.1	2.3
4	1	2s	2s,1i	1s	—	2.5	2.3	2.5
5	5	2v,6s,5i	2v,6s,6i	2v,5s,5i	2v,6s,5i	—	0.2	0.4
6	1	2v,5s,5i	2v,5s,6i	2v,4s,5i	2v,5s,5i	1s	—	0.2
7	1	2v,5s,6i	2v,5s,7i	2v,4s,6i	2v,5s,6i	1s,1i	1i	—

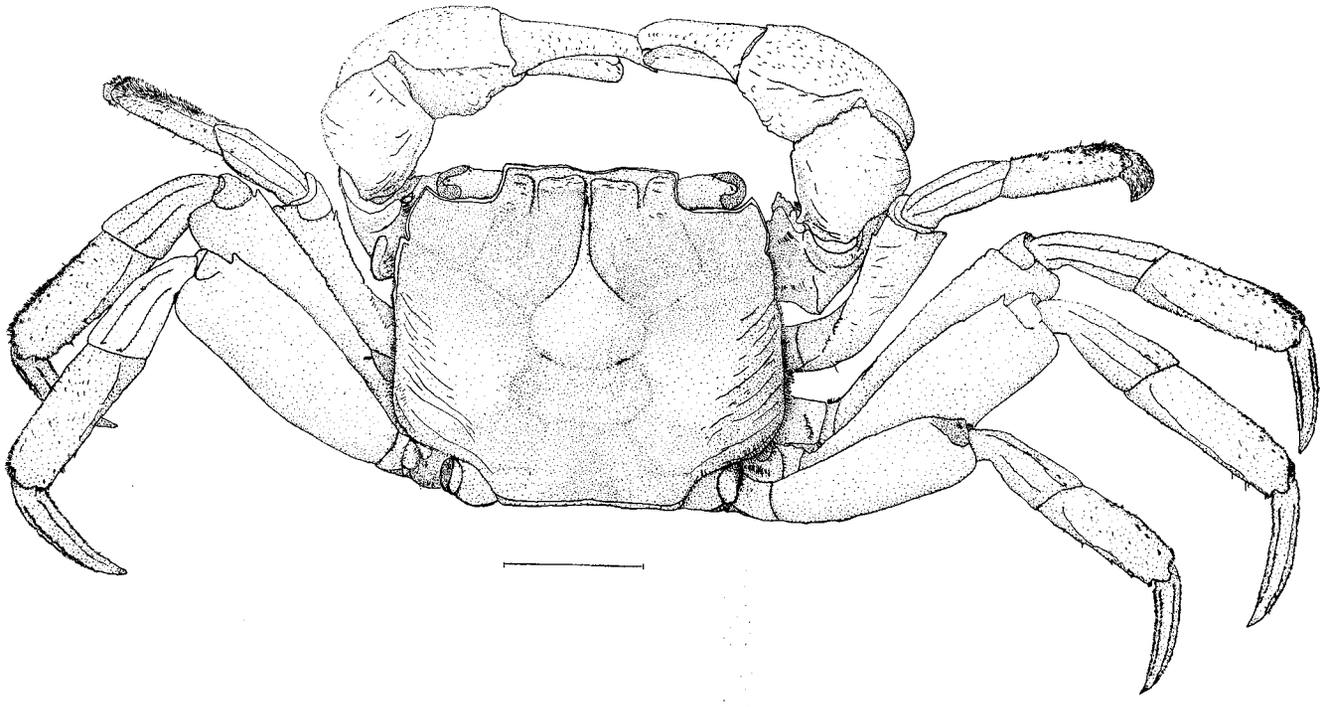

Fig. 2. Sesarma ayatum sp. n., male holotype (SMF 23715); Drivers River tributary, John Crow Mountains, Portland, Jamaica. Scale bar 1 cm.

Diagnosis. General body form flat, height less than 0.5 times carapace width (= cw). This abbreviation will be explained in Description. Carapace broader than long, without dorsal pubescence except few setae on branchial regions. Mesogastric region clearly delimited, other regions less distinct. Posterior frontal lobes lacking, anterior lateral frontal lobes very narrow, anterior border subparallel to front. Anterolateral carapace borders with one epibranchial strong tooth behind long and curved exorbital tooth. Posterior border of orbit subparallel to front. Merus of third maxilliped broad. Upper margin of cheliped palm with a mostly broken line of granules. Row of tubercles on dactyl reaching close to distal end. Chelipeds of adult animals large and strong, weakly sexually dimorphic in adult specimens. Walking legs elongate, fourth pereiopod more than twice as long as carapace length, meri of walking legs 2.5–2.7 times as long as broad. Male first pleopod slender and distally deflexed; horny apex short.

Description. Largest male (R-284) / female (R-78) from the studied material measure: 29.3/26.7 mm carapace width (cw); 25.3/23.9 mm carapace length (cl); 14.3/12.3 mm body height (bh); 13.2/12 mm interorbital width (iw) respectively. *Sesarma ayatum* sp. n. is characterized by a homogeneous dull red coloration of the dorsal carapace. The chelae are bright red to orange distally, ventrally fading to lighter color. Sternum and ventral face of legs cream to light orange.

Flat body form (bh/cw $=0.48\pm0.01$; $n=61$; positive allometric growth). Carapace broader than long (cl/cw $=0.86\pm0.01$; $n=63$), greatest width at posterior angles. Distinct epibranchial tooth at lateral carapace border behind long and curved exorbital tooth (tooth/cl $=0.16\pm0.01$; $n=59$; positive allometric growth); weak second epibranchial tooth sometimes present in larger specimens, otherwise rudimentary denticle.

Carapace dorsally without any pubescence, except few short setae fringing oblique striae of branchial regions (Fig. 2). Interorbital region narrow (iw/cw $=0.45\pm0.01$; $n=63$; negative allometric growth), subdivided into four frontal lobes; lateral lobes very narrow and subparallel to front; posterior lobes reduced to a row of granules (Fig. 3A). Mesogastric region clearly delimited, other regions less distinct. Lateral border of carapace ending shortly before ventral border, at height of third walking leg. Posterior border of orbit subparallel to front; border with row of granules running into orbit. Suborbital border setose; setae in orbit behind eyestalk. Eyes pigmented, cornea wider than eyestalk; eyestalk with 3 sets of setae; small rows of granules proximally.

Epistome and proepistome setose, with two distinct swellings. Interior row of setae fringing Verwey's groove reduced, few setae remaining on proepistome; exterior row of setae fringing Verwey's groove running from tip of lower epistomial edge to ventral border of epistome. Ventral epistome border with endostomial cristae; dorsal border with two sets of 3 to 4 large tubercles; transverse suture between epistome and proepistome medially slightly invaginated. Merus of third maxilliped broad (merus width/merus length $=0.74\pm0.04$; $n=26$).

Chelipeds homochelous, weak sexual dimorphism in adult specimens. Inner face of merus of cheliped oval with two longitudinal rows of setae; lower one continuous over full length; upper one interrupted resulting in small groups of setae, not reaching distal end of merus; few scattered setae below lower row of setae; ventral face triangular, glabrous, and smooth; transverse row of less than 8 granules at distal border. Carpus almost quadrangular; dorsal interior border with row of granules, becoming long and

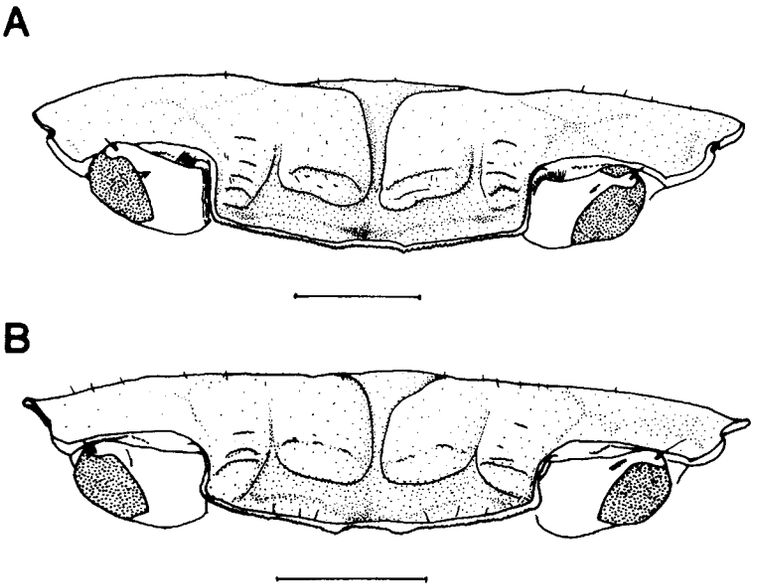

Fig. 3.—A. Sesarma ayatum sp. n. male holotype (SMF 23715), frontal view.—*B. Sesarma bidentatum* Benedict, 1892 (SMF 23878), frontal view. Scale bars 0.5 cm.

pointed at the sharp bent to distal end (Fig. 4A). Interior face of carpus proximally with tuft of grooming setae. Dorsal margin of palm with mostly broken row of granules; inner face with large tubercles, outer face with smaller ones; pollex tapers distally. Dactylus slender, in larger specimens deflexed resulting in an oval gap between closed fingers of chela; dorsal margin with row of tubercles extending close to distal end.

Walking legs elongate (fourth pereiopod/cl $=2.18\pm0.1$; $n=54$; negative allometric growth). Meri broad (2nd–5th pereiopod merus length/merus width $=2.51\pm0.12$; 2.62 ± 0.14; 2.68 ± 0.13; 2.53 ± 0.11; $n=60$ or 61; positive allometric growth). Pubescence mostly confined to distal border of dactylus and propodus. Longitudinal row of granules on anterior face of carpus fringed by scattered setae. Dorsal and ventral margin of propodus setose, increasing distally. Faces of propodus of anterior pereiopods often with scattered tufts of pubescence, glabrous on posterior pereiopods. Dactylus of all walking legs with six longitudinal rows of setae.

Thoracic sternites smooth, and glabrous. Suture between male sternite VII and episternite VII does not reach margin of pleon. Third pleon segment in males broadest, lateral borders convex; fourth segment broader posteriorly; lateral borders concave; fifth and sixth segments narrowing posteriorly; lateral borders convex; telson much narrower at base than base of last pleon segment. Male gonopod slender and distally deflexed; horny apex elongate (Fig. 4C–D).

Distribution. So far known only from eastern Jamaica (Fig. 1). Three morphometrically distinct populations (Schubart 1997) belong to the following geographic regions: (1) northeastern slopes of Blue Mountains and John Crow Mountains (Swift River, Rio Grande, Drivers River), (2) Plantain Garden River and tributaries (Sulphur River, Dry River, Johnson Mountain), (3) Yallahs Hill (Simo Spring).

Occurrence and habitat. The new species inhabits the upper reaches of mountain rivers. It is found mostly under large boulders along the banks of streams, where it retreats during the day. When disturbed, the crabs hide under adjacent stones or move rapidly towards the river. *Sesarma ayatum* sp. n. is also found in moist beds of ephemeral streams or in burrows constructed in vertical slopes of muddy banks.

Discussion

Molecular methods are becoming well established in crustacean systematics. Next to their use in phylogenetic and biogeographic studies, these methods represent an invaluable tool for distinction of morphologically similar species or populations (e.g. Knowlton *et al.* 1993; Felder & Staton 1994; Cuesta & Schubart in press). In population genetics, after several years of a predominance of allozyme studies, the use of DNA-based studies are gaining in popularity (e.g. Geller *et al.* 1997). Universal primers are available for several mitochondrial and nuclear DNA regions and allow the selection of genes according to the degree of variability desired (Palumbi 1996). While the 16S rRNA gene is mostly conserved, it also includes variable regions, most likely due to the secondary structure of the RNA (see Schneider-Broussard & Neigel 1997). In this study, 16S rRNA allowed distinction at three levels of genetic distances. (1) Identical haplotypes were common in contiguous areas, but also between different river systems, and suggest ongoing gene flow or very recent separation. (2) Minor variations (up to 0.6% genetic deviation, Table II) occurred within both of the recognized species and seem to follow geographic distribution, with genetic distances in excess of 0.2% genetic deviation only found between distant localities (Fig. 1). (3) Marked genetic deviation of 2.1–2.7% (including two transversions), clearly suggest a historic speciation event among the studied crab populations (see also Schubart *et al.* 1998). In some cases, haplotypes of these distinct species occur in direct geographic vicinity from each other (e.g. ht 3 and 5 in the northern Blue Mountains, Fig. 1). Differently shaped reproductive

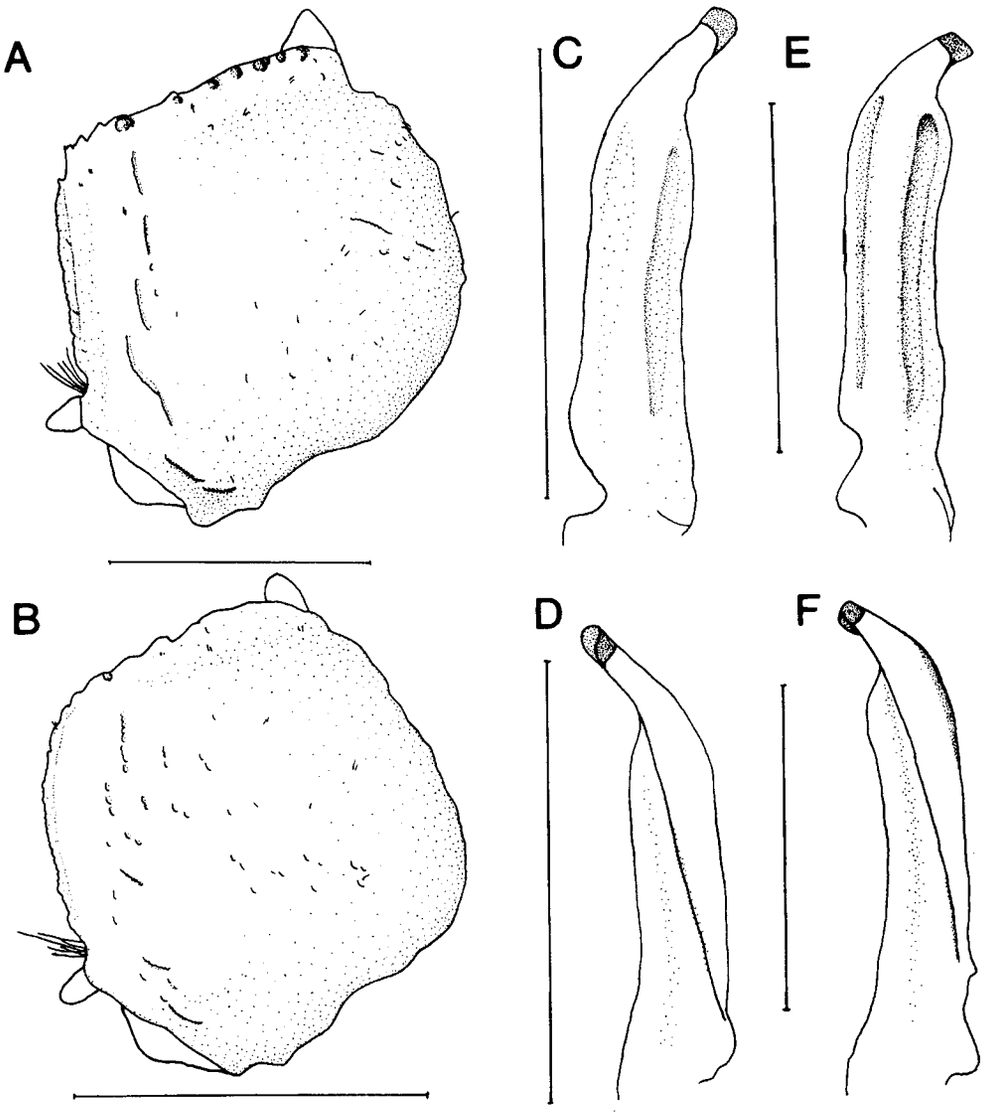

Fig. 4.—A, C, E. Sesarma ayatum sp. n. male holotype (SMF 23715).—*B, D, F. Sesarma bidentatum* Benedict, 1892 (SMF 23878). —*A–B.* Dorsal view of carpus of chelipeds. —*C–D.* Mesio-ventral view of left male gonopod. —*E–F.* Dorsal view of left male gonopod. All scale bars 0.5 cm.

organs (Fig. 4C–F), or ecological mechanisms must be responsible for maintaining a genetic separation between these otherwise very similar species. We did not find evidence of genetic introgression, but sequencing of additional specimens, will be necessary to exclude that possibility.

The description of *S. ayatum* sp. n. from eastern Jamaica completes the series of new species that have been successively discovered after recognizing morphological variation within the *bidentatum*-morphotype (Türkay & Diesel 1994; Schubart *et al.* 1997; Reimer *et al.* 1998). Results are now being corroborated by molecular studies that not only confirm the present separation of *S. ayatum* sp. n. from *S. bidentatum*, but also distinguish between previously described species (Schubart *et al.* 1998). A revision of the distribution, population genetics, and biology of the five species presently comprising the *bidentatum*-morphotype is currently being prepared for submission (Schubart *et al.* unpubl. data).

In this study we refrain from a detailed redescription of *S. bidentatum*. This will, however, be necessary in future to better characterize the morphology of those crab populations that remain in the restricted geographic range of this species. As in *S. dolphinum* (see Reimer *et al.* 1998), *S. windsor*, *S. fossarum*, and *S. ayatum* sp. n., morphometric differences were also found among populations of *S. bidentatum* (see Schubart 1997). They require further study, especially because they may represent short term responses to modified habitats (e.g. cave populations) (see Schubart *et al.* 1997).

The phylogenetic tree of all the American *Sesarma* as presented in Schubart *et al.* (1998: fig. 1) suggests a close genetic relationship of *S. bidentatum* and *S. ayatum* sp. n. (as *Sesarma* sp.) in comparison to the other Jamaican species. Apparently it was the most recent speciation event among the endemic Sesarminae of this island, possibly a consequence of the continuing uplift of the Blue Mountains. This close genetic relationship is reflected in morphology, since *S. ayatum* sp. n. and *S. bidentatum* despite the differences outlined in Table I, share several characteristics which distinguish them from the western three species of the *bidentatum*-morphotype:

1) *Sesarma ayatum* sp. n. and *S. bidentatum* lack a strong sexual dimorphism of the chelae. In contrast, in the western *S. windsor*, *S. fossarum*, and *S. dolphinum* male chelae are

significantly stronger than female chelae (Schubart *et al.* 1997: fig. 2, table I; Reimer *et al.* 1998: table I).

2) Coloration is similar in *S. ayatum* sp. n. and *S. bidentatum* with a mostly red to purple ground color, which tends to be brighter in *S. ayatum* sp. n., but differs from the three western Jamaican species, which have a tan and more marbled ground color.

3) Zoea larvae of *S. ayatum* sp. n. have a bifurcated telson as already described by Hartnoll (1964) for *S. bidentatum*. This character cannot be found in any of the western species of the *bidentatum*-morphotype or American Sesarminae in general (Cuesta, Diesel & Schubart unpubl. data).

With the description of *S. ayatum* sp. n. the Caribbean island of Jamaica clearly is unique within continental America with respect to the diversity of grapsid crabs. Among the 22 presently recognized grapsid species from Jamaica (not including findings of the pelagic *Planes* and subtidal *Euchirograpsus*), nine are endemic and freshwater-related. Such a locally confined diversity of grapsid crabs is unreported and probably occurs similarly only in the Indo–Malayan region (Jones 1984). This reinforces the interest in Jamaica for terrestrial biodiversity studies and shows the need to protect the endangered rain forests of this island. Each deforested river system means the loss of a population of freshwater crabs with its possibly unique local adaptations.

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Appendix I

List of eastern Jamaican freshwater crabs (genus *Sesarma*) used for DNA sequencing of a 16S rRNA gene fraction with locality and date of collection and collection number (see Material examined).

Species	Locality	Collecting date	Coll. #
Sesarma bidentatum	St. Ann: Roaring River	26 May 1992	R-75
Sesarma bidentatum	St. Mary: Lucky Hill Cave	26 March 1996	R-338
Sesarma bidentatum	St. Mary: Wag Water	8 February 1993	R-337
Sesarma bidentatum	St. Catherine: Rio Pedro	1 April 1996	R-336
Sesarma bidentatum	St. Andrew: Yallahs River	30 March 1996	R-334
Sesarma bidentatum	Portland: Mabess River	20 March 1997	R-366
Sesarma bidentatum	Portland: Buff Bay River	22 March 1997	R-364
Sesarma ayatum	Portland: Swift River	3 March 1995	R-341
Sesarma ayatum	Portland: Somerset Falls	17 May 1993	R-370
Sesarma ayatum	St. Thomas: 3-Finger Spring	2 September 1992	R-127
Sesarma ayatum	Portland: Drivers River	12 February 1993	R-273
Sesarma ayatum	Portland: Drivers River	5 March 1995	R-280
Sesarma ayatum	St. Thomas: Johnson Mt.	April 1994	R-297
Sesarma ayatum	St. Thomas: Sulphur River	26 June 1992	R-79
Sesarma ayatum	St. Thomas: Dry River	26 June 1992	R-80
Sesarma ayatum	St. Thomas: Yallahs Hill	30 December 1992	R-54

Appendix 2.

Alignment of 526 basepairs of the 16S rRNA gene in seven haplotypes of *Sesarma ayatum* sp. n. and *S. bidentatum* Benedict.

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S. ayatum ht1      GTCTGTTTGT AGATATAAAA AGTCTAACCT GCCCACTGAT AAA-TAAATT TTAATGGCCG CGGTATTCTG ACTGTGCAAA GGTAGCATAA TAGTTAGTTT
S. ayatum ht2      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S. ayatum ht3      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S. ayatum ht4      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S. bidentatum ht5  .......... ..G....... .......... .......... .......-.. .......... .......... .......... .......... ..........
S. bidentatum ht6  .......... ..G....... .......... .......... .......-.. .......... .......... .......... .......... ..........
S. bidentatum ht7  .......... ..G....... .......... .......... ...A...-.. .......... .......... .......... .......... ..........

S. ayatum ht1      CTTAATTGGA ATCTTGTATG AATGGTTTGA CAAGAAAAAA ACTGTCTCAC AAATTAATTA TTGAATTTAA CTTTTAAGTG AAAAGGCTTA AATAAATTAA
S. ayatum ht2      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S. ayatum ht3      .......... .......... .......... .......... G......... .......... .......... .......... .......... ..........
S. ayatum ht4      .......... .......... .......... .......... G......... .......... .......... .......... .......... ..........
S. bidentatum ht5  .......... .......... .......... .......... G........G G.G....... .......... .......... .......... ..........
S. bidentatum ht6  .......... .......... .......... .......... G........G G.G....... .......... .......... .......... ..........
S. bidentatum ht7  .......... .......... .......... .......... G........G G.G....... .......... .......... .......... ..........

S. ayatum ht1      AAAGACGATA AGACCCTATA AAGCTTAATA TTATTTTTAT TATTTAATAA AATTTTTT-A AATATAAATA TTTAGTAATA ATTTATATTT TATTGGGGTG
S. ayatum ht2      .......... .......... .......... .......... .......... ........T. .......... .......... .......... ..........
S. ayatum ht3      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S. ayatum ht4      .......... .......... .......... .......... .......... .........G .......... .......... .......... ..........
S. bidentatum ht5  .......... .......... .......... .......-.. .......... .......--. .......... ...G...... ....G..... ..........
S. bidentatum ht6  .......... .......... .......... .......-.. .......... .......--. .......... .......... ....G..... ..........
S. bidentatum ht7  .......... .......... .......... .......-.. .......... .......--. .......... .......... ....G..... ..........

S. ayatum ht1      ATAATGATAA AATGATTATT AACTGTTAAT TGTTAAAACA AAAATAAATG AATATAAAGA TTTTGATAAA TGATCCTGTA TTAGAGATTA AAAGTTTAAG
S. ayatum ht2      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S. ayatum ht3      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S. ayatum ht4      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S. bidentatum ht5  .......... .......... .......... .......... .......... .........T .......... G......... .......... ..........
S. bidentatum ht6  .......... .......... .......... .......... .......... .........T .......... G......... .......... ..........
S. bidentatum ht7  .......... .......... .......... .......... .......... .........T .......... G......... .......... ..........

S. ayatum ht1      TTACTTTAGG GATAACAGCG TTATTTTTTT TGAGAGTTCT TATCGAAAAA AGAGTTTGCG ACCTCGATGT TGAATTAAAA TATCTATATA ATTGTAGTAG
S. ayatum ht2      .......... .......... .......... .......... .......... .......... .?........ .......... .........?? ?.........
S. ayatum ht3      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S. ayatum ht4      .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S. bidentatum ht5  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S. bidentatum ht6  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
S. bidentatum ht7  .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

S. ayatum ht1      TTATATAAGA AGGTCTGTTC GACC??
S. ayatum ht2      .......... .......... ......
S. ayatum ht3      .......... .......... ....TT
S. ayatum ht4      .......... .......... ......
S. bidentatum ht5  .......... .......... ....TT
S. bidentatum ht6  .......... .......... ....TT
S. bidentatum ht7  .......... .......... ....TT
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