Journal of
Molluscan Studies

The Malacological Society of London

Journal of Molluscan Studies (2015) **81**: 66–84. doi:10.1093/mollus/eyu062
Advance Access publication date: 28 August 2014

Systematics and phylogeography of *Cerion sensu stricto* (Pulmonata: Cerionidae) from Aruba, Curaçao and Bonaire

M. G. Harasewych

Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, PO Box 37012, MRC 163, Washington, DC 20013-7012, USA

Correspondence: M.G. Harasewych; e-mail: harasewych@si.edu

(Received 6 June 2013; accepted 12 June 2014)

ABSTRACT

The systematic relationships of the *Cerion uva* complex and its constituent taxa are reviewed based on partial sequences of the cytochrome *c* oxidase I and 16S rDNA genes from 19 populations spanning the geographic range of the species complex and including the type localities of 8 of the 9 subspecies and forms. Molecular data support the conclusion of prior morphometric studies that all living *Cerion* inhabiting Aruba, Curaçao and Bonaire are members of a single species, *C. uva*. Sequence variability among and within populations is not sufficiently discontinuous to segregate populations into discrete, species-level taxa. Three of four subspecies, proposed on the basis of geographic isolation during the Quaternary, *C. uva uva* (Linnaeus, 1758), the nominotypical subspecies from eastern Curaçao, *C. uva knipensis* Baker, 1924, from western Curaçao, and *C. uva bonairensis* Baker, 1924, from Bonaire, are all supported by distinctive haplotypes. *Cerion uva arubanum* Baker, 1924, a taxon based on living specimens from Aruba, is shown to be a synonym of *C. uva uva*, with which it shares a preponderance of haplotypes. It is conjectured that *C. uva* was widespread on Aruba during the Quaternary, but had become extinct on that island, and was reintroduced from a population near Willemstad in eastern Curaçao by humans (either by Caquetío Indians or by European settlers) within the past 800 years. Further investigation is needed to determine if Quaternary Aruban *Cerion* warrant subspecific recognition. On the island of Curaçao, molecular data lend support to the partition of the *Cerion* fauna into *C. uva knipensis*, which is confined to an isolated western region, as defined by Baker, and *C. uva uva*, which inhabits a broad, eastern region that is composed of Baker's central and eastern regions. A population at Ronde Klip in eastern Curaçao has remained genetically isolated, and retains subspecific status as *C. uva diablensis* Baker, 1924. A neotype is designated for *Turbo uva* Linnaeus, 1758, as is necessary to provide an objective standard of reference for this species-group taxon, and for the genus- and family-level taxa based upon it.

INTRODUCTION

The nominotypical subgenus of *Cerion* Röding, 1798, which is endemic to the islands of Aruba, Curaçao and Bonaire, has been separated from all other living members of the family Cerionidae by the Caribbean Tectonic Plate, a portion of Pacific Ocean sea floor that has been moving eastward since the Late Cretaceous (Harasewych, 2012). Molecular data have shown this lineage to be the sister group to the Cerionidae inhabiting the Greater Antilles, western Virgin Islands, Bahamas, Turks and Caicos Islands and Florida (Harasewych, Sikaroodi & Gillevet, 2011: figs 16, 17). This subgenus has a long and complex taxonomic history and has been extensively studied during the 20th century. Multiple subspecies and forms have been described and synonymized, based on morphometric analyses of shell size and shape, and their distributions used to define faunal areas (Baker, 1924; Wagenaar Hummelinck, 1940b; Gould, 1969, 1971, 1984; de Vries, 1974).

The relationships of the subgenus *Cerion* and its constituent taxa are reviewed based on partial sequences of their cytochrome *c* oxidase I (COI) and 16S rDNA genes. Samples from the type localities of all but one of the named taxa of *Cerion sensu stricto* are included in these analyses in order to infer patterns of interrelatedness among populations on Aruba, Curaçao and Bonaire. The present study includes a review of the taxonomic history of *Cerion s. s.* and is the first to analyse the systematics and population structure of the *Cerion* of these islands from a molecular perspective.

TAXONOMIC HISTORY

Following the settlement of Curaçao by the Dutch West India Company in 1634, shells of *Cerion* became well known to pre-Linnean authors (e.g. Buonanni, 1681: fig. 140; 1684: fig. 140; 1709: fig. 140; Lister, 1685–1962: pl. 588, fig. 47; Petiver,

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1709: pl. 27, fig. 2; Gualtieri, 1742: pl. 58, fig. D; Klein, 1753: 33), many of whom published stylized illustrations (Fig. 1A–D). In his brief description of *Turbo uva*, Linnaeus (1758: 765) cited the illustrations of Petiver (1709) and Gualtieri (1742). Later, he (Linnaeus, 1767: 1239) slightly expanded the description and added references to illustrations in Buonanni (1684) and Seba (1758). The habitat of *T. uva* was unknown at the time the species was described. Müller (1775: 554) was the first to associate this taxon with the island of Curaçao. Baker (1924: 14, 98) restricted the type locality to Schaarlo, back of Willemstad, Curaçao, noting "As Willemstad is the principle port of the islands, it is probable that most of the species described from Curaçao by the earlier writers came from its near vicinity; and I am regarding the Schaarlo as the type locality for all of them that occur there."

Dodge (1959: 236) discussed at length the discrepancies between Linnaeus' description and figure citations, particularly his use of the terms "*cancellata*" and "*imbricatis*" for a shell that is neither cancellate nor imbricate according to the modern concept of the species. Dodge concluded that "the description is not only too defective to be used as a factor in identification but is strangely inharmonious with the majority of the figures in the synonymy and with the undocumented specimen of the *uva* of authors in the Linnaean collection." Citing Hanley's (1855: 343) confirmation that the specimen in the Linnaean collection is identical to the *uva* of authors, and noting that the serial number of *uva* in Linnaeus' copy of the *Systema Naturae* is underlined, Dodge inferred that a specimen was present in Linnaeus' collection and concluded that "we are therefore justified in treating it as his type specimen, although only on a 'probable' basis." However, the Linnean Collection contains 14 specimens, of which only 13 are referable to *T. uva* of authors. The 14th specimen is of a species from Great Inagua (southern Bahamas) that was subsequently named *C. rubicunda* Menke, 1829, and assigned to the subgenus *Diacerion* Dall, 1894 (Pilsbry, 1902: 271). A note accompanying the specimens states that they were in a J. E. Smith tray, and that "there is no proof of the authenticity of these specimens". Hanley (1855: 2–3) reported that when the collection was in the custody of Sir James Smith, "numerous other specimens were mingled with the ancient ones. This ill-advised admixture has not merely augmented to an almost inconceivable degree the difficulties of investigation, but has too frequently proved fatal to any accurate decision." Dance (1967) documented further instances of additions to, and sales from, Linnaeus' collection following his death, and questioned the significance of the underlining, noting (p. 11) that there is "altogether too little real evidence to prove that the underlinings indicate Linnaeus' possession of certain species."

As there remains a question as to which, if any, of the specimens currently in the Linnaean collection were present in the collection at the time Linnaeus formulated his description of *T. uva*, their status as syntypes is also in doubt (ICZN, 1999: Articles 72.1.1, 72.4.1 and 72.4.1.1).

Dodge (1959) clearly documented the vagaries and contradictions in the original description of *T. uva* and demonstrated the need for a name-bearing type to define this taxon objectively. This need is compounded by the fact that this taxon has come to serve as the type species of the genus *Cerion* Röding, 1798, which, in turn, is the type genus of the family Cerionidae Pilsbry, 1901. The lot labelled *Turbo uva* in the Linnean collection (LSL 530) contains multiple specimens representing two clearly distinct species. As their status as syntypes cannot be established, a single specimen (LSL 530/1, Fig. 2A) from among the 13 specimens that correspond to *uva* of authors is here designated as the neotype of *T. uva* in order to provide an objective standard of

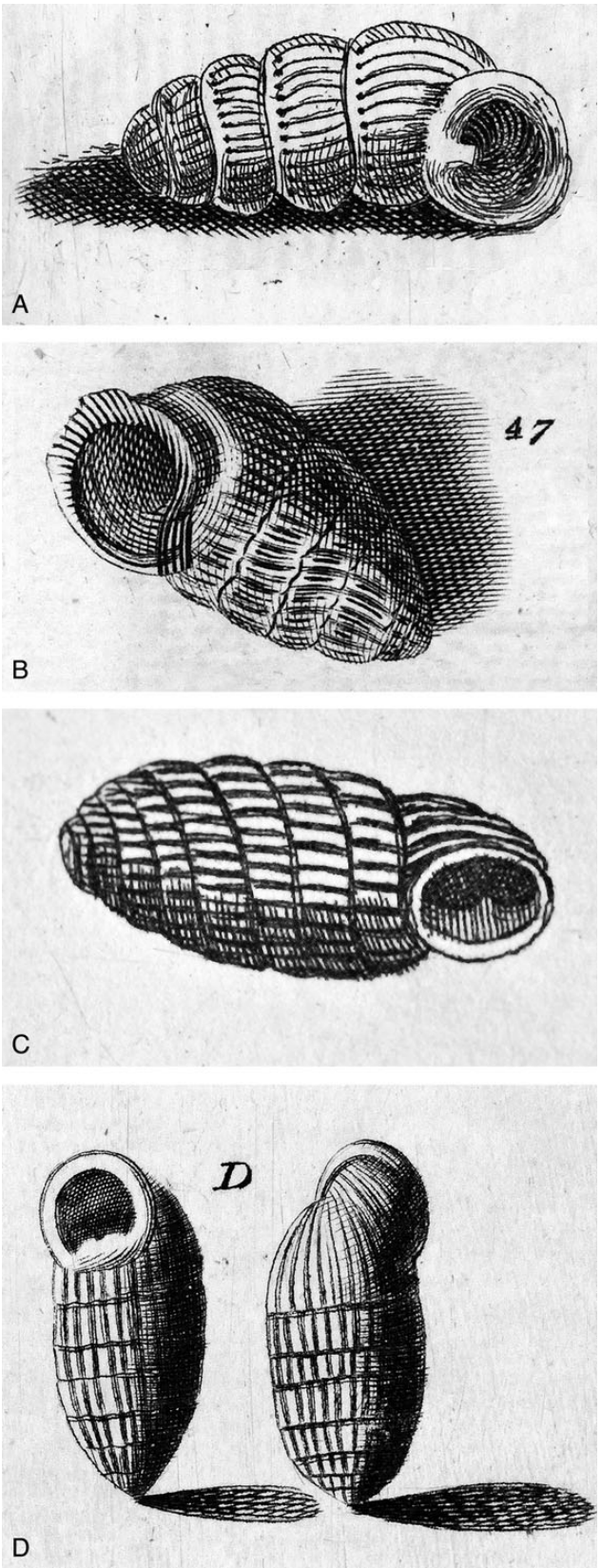

Figure 1. Pre-Linnean illustrations of *Cerion uva*. **A.** Buonanni, 1681: 140. **B.** Lister, 1685–1962: tab. 588, fig. 47. **C.** Petiver, 1709: tab. 27, fig. 2. **D.** Gualtieri, 1742: tab. 58, fig. D.

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Figure 2. Primary type specimens of the named subspecies and forms of *Cerion uva* (Linnaeus, 1758). Apertural, lateral and dorsal views of: **A**. Neotype of *Turbo uva* Linnaeus, 1758 (LSL 530/1). **B.** Lectotype of *C. uva desculptum* Pilsbry & Vanatta, 1896 (ANSP 257540). **C.** Holotype of *C. uva uva* form *diablensis* Baker, 1924 (UMMZ 31760). **D.** Holotype of *C. uva uva* form *hatoensis* Baker, 1924 (UMMZ 31768). **E.** Holotype of *C. uva knipensis* Baker, 1924 (UMMZ 31768). **F.** Holotype of *C. uva knipensis* form *djerimensis* Baker, 1924 (UMMZ 31768). **G.** Holotype of *C. uva arubanum* Baker, 1924 (UMMZ 31752). **H.** Holotype of *C. uva bonairensis* Baker, 1924 (UMMZ 31756). **I.** Holotype of *C. uva bonairensis* form *kralendijki* Baker, 1924 (UMMZ 31863). Scale bar = 1 cm for all images.

reference for this species-group taxon, as well as the genus-group and family-group taxa based upon it, that is concordant with accepted usage and is not dependent on the uncertain provenance of the specimens. This neotype conforms to the detailed subsequent descriptions for this taxon (e.g. Pilsbry, 1901: 180–181; Wagenaar Hummelinck, 1940b: 47). Although locality data are lacking for the neotype, its morphology and proportions (21.5 × 9.0 mm, $11\frac{1}{2}$ whorls) fall within the range reported by Baker (1924: 100, table 11) for Schaarlo, the subsequently identified type locality for this species.

Dodge (1959: 237–241) also enumerated in detail the early misapplications of the name and subsequent generic allocations of *T. uva*, now *C. uva*, while Wagenaar Hummelink (1940b: 44–47) provided a comprehensive synonymy of this species spanning the years 1688–1940 that was updated by de Vries (1974: 85).

Cerion uva was considered to be the only species of *Cerion* to inhabit the Dutch Leeward Islands during the 18th and most of the 19th centuries. Pilsbry & Vanatta (1896: 328) differentiated the variety *C. uva desculptum* as a morphotype that lacks the strong, regular ribs characteristic of *C. uva uva*. The three specimens in the type series [lectotype (Fig. 2B) ANSP 257540, subsequently designated by Baker (1963: 206)] are from Curaçao, but lack more detailed data. Baker (1924: 100) considered this to be a synonym of *C. uva uva*, commenting that the exact type

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locality of this very conspicuous form is unknown, but is probably in the vicinity of Sint Anna Baai, on Campo Blenheim, or some similar hill in this vicinity, as it forms here a small proportion of the *Cerion* population. Subsequent authors (e.g. Wagenaar Hummelinck, 1940b: 49; de Vries, 1974: 94) universally regarded *C. uva desculptum* as a synonym of *C. uva uva*, noting that this morphotype had also been collected at additional localities on western, central and eastern Curaçao, and from fossil localities in southeastern Aruba.

Following an extensive survey of the terrestrial molluscs of the Dutch Leeward Islands, Baker (1924) partitioned *C. uva* into four subspecies and further subdivided three of the subspecies into infrasubspecific forms (Table 1, Fig. 2C–I). On Curaçao, he recognized three faunal areas based on molluscan distribution "coincident with degrees of present or former isolation": a small, eastern area surrounding Tafelberg Santa Barbara; a small, western area along the southern shore north of Bullen Baai, including the adjacent hills and the large central area spanning the intervening southern coast as well as the entire northern coastal plain (Baker, 1924: 12). Based primarily on measurements of shell length, maximum diameter and number of whorls, Baker (1924: 99) concluded that within each area, variation in shell size was a consequence of differences in habitat among populations, and distinguished several extremes in size with form names. Noting that the molluscan fauna of Aruba is, in general, more closely related to that of western Curaçao, Baker (1924: 114) nevertheless recognized that *C. uva arubanum* is most similar to *C. uva uva* from eastern Curaçao. He also commented on the 'peculiar' restricted distribution of *Cerion* in the Recent fauna to a small region along the central western coast of Aruba, despite its nearly ubiquitous presence on the island as fossils and subfossils. Baker reported that the fauna of Bonaire and Klein Bonaire is the most distinctive within the Dutch Leeward Islands, and noted the similarities in whorl height between *C. uva bonairensis* and *C. uva knipensis* from western Curaçao.

After obtaining similar measurements from a much larger number of samples from throughout these islands, Wagenaar Hummelinck (1940a, b) concluded that the morphological variation among populations was not sufficient to justify subdivision of *C. uva* into subspecies. Gould (1969) reanalysed Baker's and Wagenaar Hummelinck's data using multivariate techniques and reported that separate names (of subspecific rank) for the four geographic regions (i.e. Aruba, western Curaçao, eastern Curaçao and Bonaire) were warranted, but that the "cumbersome nomenclature for intraregional diversity should be dropped." After greatly enlarging the number of population samples and expanding the character matrix to include information on rib count and spacing, de Vries (1976) concluded that "no obvious geographic variation in *Cerion uva* exists." Gould (1984) produced a new data matrix based on 135 populations of *C. uva*, each containing 20 specimens, and scored each shell for 19 measurements. He demonstrated that each of the four regions is distinguished by consistent differences displayed in covariance sets. He also showed that differences in shell size were present in all regions, and were likely ecophenotypic and nonadaptive.

Working with the distribution of a different land snail genus (*Tudora*), Hovestadt (1987) hypothesized the existence of four island refugia within Curaçao during interglacial high sea-level stands, corresponding to Christoffelberg, Ceru Grande, Ronde Klip and Tafelberg Santa Barbara (Fig. 4C). In a review of the molluscan biogeography of Curaçao, Wagenaar Hummelinck (1990: 186) supported the premise that Curaçao was repopulated from the nuclei of the eastern and western highlands following the interglacial transgression that resulted in the formation of the limestones of the High Terrace. He concurred with Gould's (1969) partition of Curaçao into western and eastern regions and rejected Baker's (1924) hypothesis that the island could be divided into three faunal areas (western, central and eastern).

Table 1. Taxa included in *Cerion sensu stricto* by Baker (1924).

Curaçao	*C. uva uva* (Linnaeus, 1758)
	Type locality: Schaarlo, Willemstad [12°5.11′N, 68°54.21′W]
	Type material: neotype, LSL 530/1
	C. uva desculptum Pilsbry and Vanatta, 1896
	Type locality: Curaçao
	Type material: Lectotype, ANSP 257540
	C. uva uva form *diablensis* Baker, 1924*
	Type locality: the top of Ronde Klip [12°8.98′N, 68°52.02′W]
	Type material: holotype, UMMZ 31760
	C. uva uva form *hatoensis* Baker, 1924*
	Type locality: eastern escarpment of Seroe Spelonk, near Landhuis Hato [12°10.71′N, 68°57.92′W]
	Type material: holotype, UMMZ 31768
	C. uva knipensis Baker, 1924
	Type locality: valley between Seroes Palomba and Baha Hoendoe [12°20.34′N, 69°9.10′W]
	Type material: holotype, UMMZ 31768
	C. uva knipensis form *djerimensis* Baker, 1924†
	Type locality: top of the shore cliffs near Plaja Djermimi [12°21.24′N, 69°9.83′W]
	Type material: holotype, UMMZ 31768
Aruba	*C. uva arubanum* Baker, 1924
	Type locality: Baranca Alto [12°28.50′N, 69°57.77′W]
	Type material: holotype, UMMZ 31752
Bonaire	*C. uva bonairensis* Baker, 1924
	Type locality: Porta Spaño [12°14.06′N, 68°16.68′W]
	Type material: holotype, UMMZ 31756
	C. uva bonairensis form *kralendijki* Baker, 1924†
	Type locality: south of Kralendijk [12°8.08′N, 68°16.68′W], along the highway near the western shore
	Type material: holotype, UMMZ 31863

Type localities and repositories of primary type material are given. Abbreviations: ANSP, Academy of Natural Sciences of Philadelphia; LSL, Linnaean Society, London; UMMZ, University of Michigan, Museum of Zoology.

*Baker (1925: 43) used the taxa *C. uva diablensis* and *C. uva hatoensis* as trinomena, indicating subspecies status (ICZN, 1999: 5; Article 5.2), thus satisfying the provisions of Article 45.6.4.1 (ICZN, 1999: 50) and making these two taxa available as subspecific names from their original publication (Baker, 1924).

†These form names were clearly proposed as infrasubspecific names intended to distinguish populations within subspecies by Baker (1924). Therefore, they are not available names (ICZN, 1999: 49; Articles 45.5; 45.6.4).

MATERIAL AND METHODS

Taxon sampling

Nineteen populations of *Cerion uva* were sampled from localities that spanned the islands and regions within the range of this species (Table 2, Figs 3, 4). These samples included the type localities of all the taxa treated by Baker (1924), except *C. uva desculptum*, as neither a population nor individuals conforming to this phenotype were found despite searches at localities listed by Baker (1924), Wagenaar Hummelinck (1940b) and de Vries (1974). Spelling of local place names follows usage by original authors. Over the years, the local language Papiamentu has

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Table 2. Populations of *Cerion* sampled for DNA sequences of COI and 16S genes.

Taxon	No. ind.	USNM no.	Station no.	Locality	COI		16S		Concatenated
					No. hap.	GenBank nos	No. hap.	GenBank nos	No. hap.
Aruba									
C. uva arubanum	5	1183676	A1	Spanish Lagoon, Aruba	2	*	2	*	2
C. uva arubanum	**12**	**1183677**	**A2**	**Baranca Alto, Aruba**	**2**	*	**3**	*	**3**
C. uva arubanum	8	1183678	A3	Parkietenbos, Aruba	2	*	2	*	2
Bonaire									
C. uva bonairensis	**15**	**1156515**	**B1**	**NW of Rincon, Bonaire**	**1**	*	**1**	*	**1**
C. uva bonairensis kralendijki	**11**	**1156516**	**B2**	**S of Kralendijk, Bonaire**	**4**	*	**4**	*	**6**
Curaçao									
C. uva uva	**10**	**1153961**	**C1**	**Schaarlo, Curaçao**	**3**	*	**2**	*	**4**
C.n uva uva	10	1155587	C4	Tafelberg Santa Barbara, Curaçao	6	*	6	*	7
C. uva uva	8	1162081	C13	St. Joris Baai, Curaçao	3	*	6	*	6
C. uva uva	10	1155591	C8	Boca Wandomi, Curaçao	4	*	7	*	9
C. uva uva	10	1155594	C11	Boca Patrick, Curaçao	7	*	7	*	9
C. uva uva	7	1183666	C14	Kueba di Brua, Curaçao	3	*	5	*	6
C. uva uva	10	1155592	C9	Playa Canoa, Curaçao	7	*	5	*	8
C. uva uva	8	1155595	C12	Sint Willibrordus, Curaçao	4	*	4	*	4
C. uva uva	6	1183668	C15	Boca Sint Michiel, Curaçao	4	*	6	*	6
C. uva diablensis	**9**	**1155588**	**C5**	**N slope, Ronde Klip, Curaçao**	**3**	*	**4**	*	**6**
C. uva hatoensis	**5**	**1153964**	**C2**	**E of Hato Airport, Curaçao**	**4**	*	**3**	*	**4**
C. uva knipensis	**10**	**1155593**	**C10**	**Landhuis Knip, Curaçao**	**4**	*	**5**	*	**6**
C. uva knipensis djerimensis	**10**	**1155590**	**C7**	**Plaja Jeremi, Curaçao**	**3**	*	**4**	*	**5**
C. uva knipensis	10	1153966	C3	Plaja Abao, Curaçao	3	*	3	*	4
Total	174				69		79		98
Outgroups									
Albinaria caerulea	1	Complete mitochondrial genome *ex* GenBank			1	NC 001761	1	NC 001761	1
C. striatellum	2	1158944	PR1	Tamarindo Beach, Puerto Rico	2	KJ934716-7	2	KJ636083-4	2
C. malonei	2	1002335	LI9	E of Bains, Long Is., Bahamas	2	KJ934718-9	2	KJ636085-6	2
C. stevensoni	2	1002176	LI23	Wemyss, Long Is., Bahamas	2	KJ934720-1	1	KJ636087-8	2
C. caerulescens	2	1002449	LI7	Whitehouse, Long Is., Bahamas	2	KJ934722-3	2	KJ636089-90	2

The numbers of individuals sequenced from each population for both genes, the numbers of different haplotypes within each population for each of the partial gene sequences, as well as for the concatenated sequences are given. Samples from the type localities of named taxa are in bold.

*GenBank numbers for the various haplotypes of *C. uva* are provided in Appendix 1.

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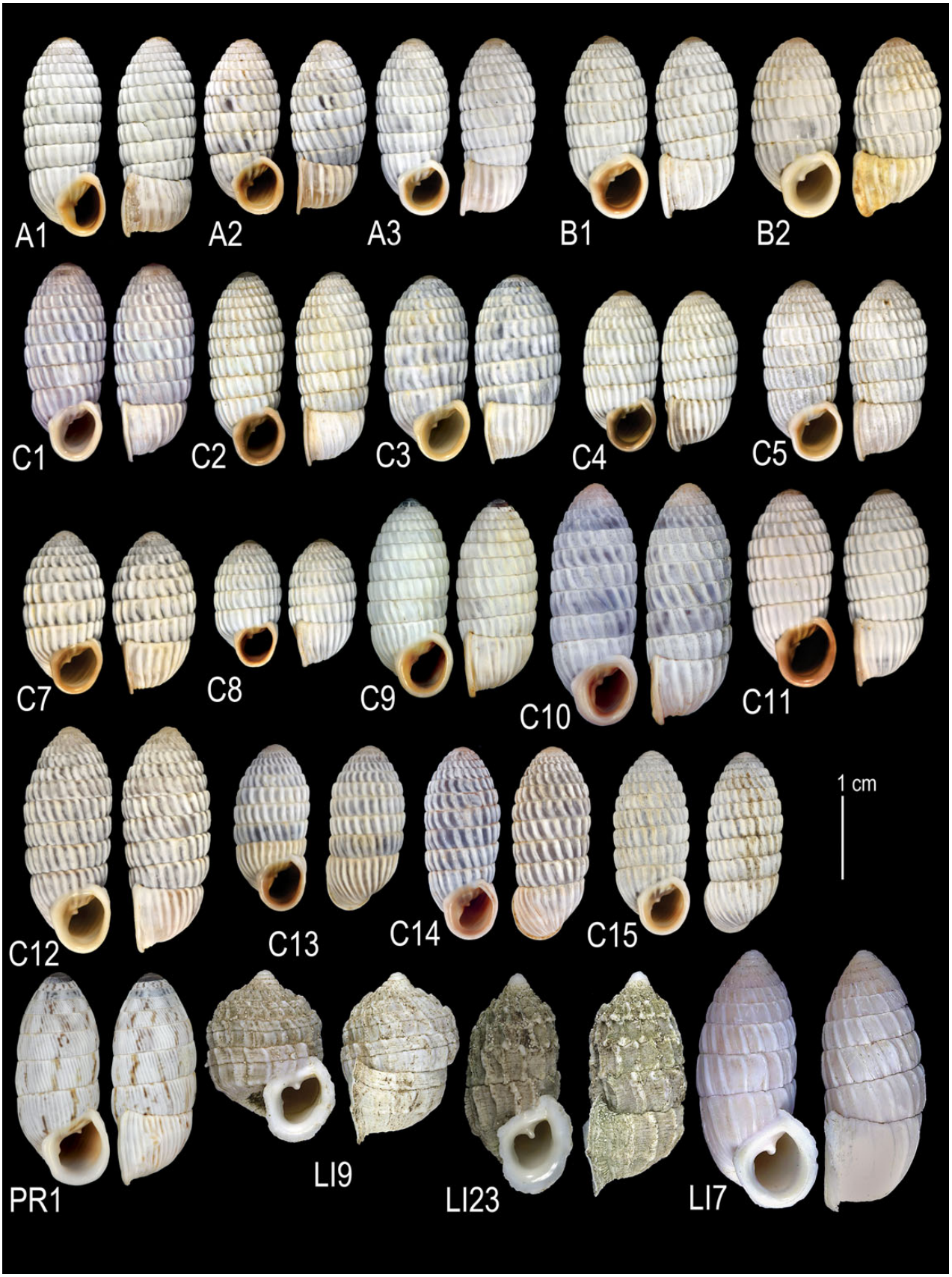

Figure 3. Representative specimens of *Cerion* populations used in this study. Labels correspond to station numbers in Table 2.

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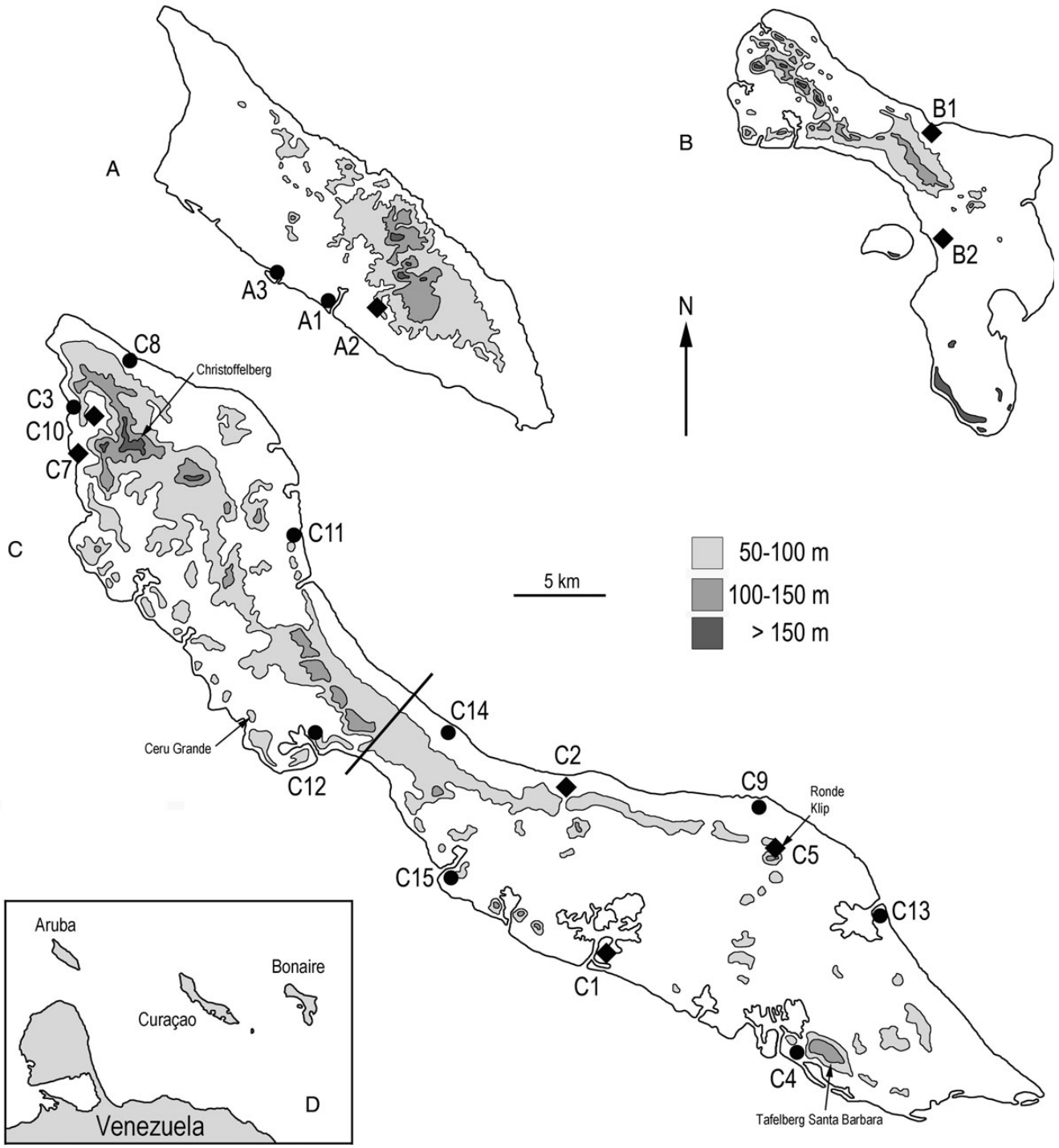

Figure 4. Maps. **A**. Aruba. **B**. Bonaire. **C**. Curaçao. **D**. The relative positions of these islands and the coast of Venezuela. Locations of sampled populations (solid circles) are identified by alphanumeric station numbers (see Table 2). Diamonds correspond to type localities for named subspecies and forms of *Cerion uva*. The line between stations C12 and C14 separates the regions previously identified as eastern and western Curaçao (Gould, 1984: fig. 8; Hovestadt, 1987: map). Locations of the four island refugia during interglacial high stands (Christoffelberg, Ceru Grande, Ronde Klip and Tafelberg Santa Barbara) (Hovestadt, 1987) are indicated.

had various different systems of spelling (e.g. Seroe, Ceru, Seru, for hill). Baker (1924) used Schaarlo, more recently spelled Scharloo.

DNA extraction, PCR amplification and sequencing

DNA was obtained by removing the dorsum of the shell with a Wizard Model 100 Saw (Diamond Pacific Tool Corp.) and dissecting a *c*. 20 mg portion of buccal muscle from the living animal. Genomic DNA was extracted using Autogeneprep965 (Autogen) employing the mouse-tail tissue protocol with a final elution volume of 50 μl. The shells were retained as voucher specimens and individually identified with a sequential letter suffix to the USNM catalogue number assigned to each population. [Images of sequenced specimens are appended to their catalogue records: http://collections.nmnh.si.edu/search/iz/ (search

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by catalogue number in Table 2) and also appear on the *Cerion* website: http://invertebrates.si.edu/cerion/ under the taxon names and station numbers shown in Table 2].

Portions of two mitochondrial genes were amplified: the barcoding region of the COI gene using the primers HCO1490 and LCO2198 (Folmer *et al*., 1994), and a section of the 16S rDNA gene using 16Sar and 16Sbr primers (Palumbi, 1996). PCR reactions were performed in 20-μl volumes. COI reactions contained: 10 μl of Promega Go-Taq® Hotstart Master Mix (1 unit Promega Go-Taq®, 400 μM dNTPs, 4 mM $MgCl_2$), 0.3 μM of each primer, 0.25 μl of BSA, 1.25% DMSO and 1 μl of template DNA. Amplification consisted of an initial denaturation step at 95 °C for 7 min, followed by 45 cycles of denaturation at 95 °C for 45 s, annealing at 42 °C for 45 s and extension at 72 °C for 1 min. The final extension was at 72 °C for 5 min. Reactions for the 16S PCR, also in 20-μl volumes, contained: 1 unit Biolase DNA Polymerase (Bioline), 2 μl 10× reaction buffer, 500 μM dNTPs, 2 mM $MgCl_2$, 0.25 μl of BSA, 0.3 μM of each primer and 1 μl of template DNA. Amplification consisted of an initial denaturation step at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 48 °C for 30 s and extension at 72 °C for 45 s. The final extension was at 72 °C for 3 min. Resulting PCR products were visualized by agarose gel electrophoresis (1.5% agarose) and purified, with ExoSAP-IT (Affymetrix) according to manufacturer's protocols, prior to sequencing.

Sequencing reactions were performed using 1 μl of purified PCR product in a 10-μl reaction containing 0.5 μl primer, 1.75 μl Big Dye buffer and 0.5 μl Big Dye (ABI, Foster City, CA, USA) and run in the thermal cycler for 30 cycles of 30 s at 95 °C, 30 s at 50 °C, 4 min at 60 °C and then held at 10 °C. These sequencing reactions were purified using Millipore Sephadex plates (MAHVN-4550; Millipore, Billerica, MA, USA) according to the manufacturer's instructions and sequenced on an ABI 3730XL automated DNA sequencer. Sequencher v. 4.7 (GeneCodes, Ann Arbor, MI, USA) was used to visualize, trim, edit and assemble contigs from forward and reverse sequences. All sequences have been deposited in GenBank (NCBI). Accession numbers are listed in Table 2 and in Appendix 1.

Data analysis

Gene sequences for two specimens each of *C. malonei* Clench, 1937, *C. stevensoni* Dall, 1900 and *C. caerulescens* Maynard & Clapp, 1920, from Long Island, Bahamas, and *C. striatellum* (Guerin, 1829), from Puerto Rico were added to each of the datasets (COI and 16S) to serve as outgroups. Sequences for the COI and 16S genes were aligned against those of *Albinaria caerulea* derived from its complete mitochondrial genome (1,529 bp) (Hatzoglou, Rodakis & Lecanidou, 1995; GenBank NC 001761) using Clustal X v. 2.1 (Larkin *et al*., 2007). Mega v. 5.05 (Tamura *et al*., 2011) was used to calculate pairwise differences for each of the genes within and between *Cerion* species and among populations of *C. uva*.

Pairwise distance matrices were produced using MEGA v. 5.05 (Jukes-Cantor model, with pairwise deletion of missing data) for each of the three datasets (COI, 16S and COI + 16S) and analysed using the automatic barcode gap discovery method (ABGD; Puillandre *et al*., 2012, http://wwwabi.snv.jussieu.fr/public/abgd/) under default parameters, to determine patterns and gaps in genetic distances and evaluate hypotheses of species delimitation.

In order to expedite Bayesian analyses, Mega v. 5.05 and MacClade v. 4.08 (Maddison & Maddison, 2005) were used to identify and collapse identical sequences at each locality for the individual genes as well as for the concatenated sequences. In these analyses, such sequences are identified by the station number, representing geographic origin, followed by the letter designations of all specimens from that locality with identical sequences (e.g. C1ACD refers to a sequence occurring at station C1 that is shared by individuals A, C and D from that population).

The COI, 16S and concatenated datasets (with identical sequences collapsed) were analysed using Bayesian inference with MrBayes v. 3.2.1 (Ronquist *et al*., 2012). Default settings in jModelTest v. 2.1.3 (Darriba *et al*., 2012) were used to evaluate nucleotide substitution models. The Bayesian information criterion selected the HKY + G model for the COI data and the TVM + G model for the 16S data. The concatenated dataset was analysed keeping the two partitions unlinked, using the corresponding models for each partition. Metropolis-coupled Markov chain Monte Carlo simulations were performed with four chains for 4,000,000 generations for each of the individual genes, and 6,000,000 generations for the concatenated data. Trees were sampled every 100 generations, starting after a burn-in of 2,500 generations. The standard deviation of split frequencies was below 0.01, and the potential scale reduction factor was 1 for all analyses.

Haplotype networks were estimated for both the COI and 16S rDNA datasets (with outgroups removed) using the program TCS v. 1.21 (Clement, Posada & Crandall, 2000) under default settings (gaps treated as a fifth base, 95% confidence interval for statistical parsimony). Correlations between haplotypes and specimens at the various localities are shown in Appendix 1.

RESULTS

Sequences for portions of the COI and 16S rDNA genes were obtained from 174 individuals of *Cerion uva* spanning the geographic range of this species (Table 2).

Data analysis

For the COI gene, an alignment of 655 bp, corresponding to positions 39–693 of the COI gene in *Albinaria caerulea*, was produced after primers were trimmed. The COI alignment contained 260 (39.69%) variable and 220 (33.59%) parsimony-informative sites. The Clustal X alignment of the *C. uva* 16S rDNA sequences produced an alignment of 507 bp, corresponding to positions 491–933 of this gene in *A. caerulea*. Addition of eight outgroup sequences and subsequent realignment using Clustal X extended the 16S alignment to 518 positions. Of these 518 positions, 274 (52.90%) were variable and 206 (39.77%) were parsimony informative. The concatenated dataset consisted of 1,173 position, of which 535 (45.61%) were variable and 428 (36.49%) were parsimony informative.

Only a single population (*C. uva bonairensis* B1, $n = 15$) was monomorphic for either the COI or 16S gene. Maximum sequence diversity among both Bonaire populations reached 0.31% for COI and 0.79% for 16S genes. All populations from the three Aruban localities were represented by two to three haplotypes for each gene, with sequence diversity reaching 0.15% for COI and 0.39% for 16S. The majority of populations from Curaçao had four or more haplotypes for each of the two genes. On Curaçao, the greatest haplotype diversity occurred at the western (C8 and C11) and eastern (C9) margins of the northern coastal plain, and at the base of Tafelberg Santa Barbara (C4). Despite the large number of haplotypes, the number of nucleotide differences within populations of *C. uva* was low for both genes (Table 3), with the highest values (1.83% for COI at C8; 1.97% for 16S at C10), in populations from west of Christoffelberg. Greatest differences were between populations from Bonaire (B1, B2), the region west of Christoffelberg (C3, C7, C8, C10) in western Curaçao and Ronde Klip (C5) in eastern Curaçao (2.44% for COI and 2.76% for 16S),

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Table 3. Nucleotide differences within and between and populations of *Cerion uva*.

	A1	A2	A3	B1	B2	C1	C2	C3	C4	C5	C7	C8	C9	C10	C11	C12	C13	C14	C15
A1 ($n = 5$)	1 **1**	0–1	0–1	10–11	10–13	0–3	3–7	8–12	1–5	7–9	10–13	3–13	2–6	5–13	3–8	3–7	3–5	3–6	4–7
A2 ($n = 12$)	**0–2**	1 **1–2**	0–1	10–11	10–13	0–3	3–7	8–12	1–5	7–9	10–13	3–13	2–6	5–13	3–8	3–7	3–5	3–6	4–7
A3 ($n = 8$)	**0–1**	**0–2**	1 **1**	10–11	10–13	0–3	3–7	8–12	1–5	7–9	10–13	3–13	2–6	5–13	3–8	3–7	3–5	3–6	4–7
B1 ($n = 15$)	**7–8**	**7–8**	**7–8**	0 **0**	0–2	10–12	10–13	8–11	8–10	11–12	10–14	7–12	8–11	9–12	7–11	7–10	8–10	7–9	8–10
B2 ($n = 11$)	**7–11**	**7–11**	**7–11**	**1–4**	1–3 **1–6**	10–14	10–15	8–12	8–12	11–14	10–16	7–13	8–13	9–13	7–13	7–12	8–12	7–11	8–12
C1 ($n = 10$)	**0–1**	**0–1**	**0–1**	**7**	**7–11**	1–3 **1**	2–8	8–12	1–5	7–10	10–14	4–13	2–7	6–13	4–9	3–8	3–6	3–7	4–8
C2 ($n = 5$)	**0–2**	**0–2**	**0–2**	**5–8**	**5–12**	**0–2**	2–7 **1–3**	8–12	3–7	5–11	10–14	3–14	4–9	5–14	3–10	3–9	3–7	3–8	4–9
C3 ($n = 10$)	**5–10**	**5–10**	**5–10**	**6–11**	**6–13**	**5–10**	**3–11**	1–4 **3–6**	6–11	9–13	0–9	4–11	6–12	0–10	5–12	5–11	6–11	6–10	6–11
C4 ($n = 10$)	**0–5**	**0–5**	**0–5**	**5–9**	**5–13**	**0–5**	**0–6**	**3–12**	1–4 **1–6**	5–8	8–12	1–12	1–7	3–11	1–7	1–6	1–4	1–5	2–6
C5 ($n = 10$)	**4–7**	**4–7**	**4–7**	**5–8**	**5–12**	**4–7**	**2–8**	**3–11**	**2–9**	1 **1–4**	11–16	4–14	5–9	6–14	4–9	4–8	6–8	4–7	5–8
C7 ($n = 10$)	**5–10**	**5–10**	**5–10**	**8–11**	**8–14**	**5–10**	**3–11**	**2–10**	**3–12**	**5–11**	4–9 **2–5**	4–14	8–15	0–13	7–15	7–14	8–12	8–13	8–14
C8 ($n = 10$)	**2–7**	**2–7**	**2–7**	**5–9**	**5–13**	**2–7**	**0–8**	**1–10**	**0–9**	**2–9**	**3–10**	2–12 **1–8**	1–13	2–12	1–13	0–12	2–12	0–11	1–12
C9 ($n = 10$)	**2–3**	**2–3**	**2–3**	**5–6**	**5–10**	**2–3**	**0–4**	**1–9**	**0–5**	**2–6**	**3–9**	**0–7**	1–3 **1–2**	3–13	1–7	1–7	3–7	1–6	2–7
C10 ($n = 10$)	**2–10**	**2–10**	**2–10**	**5–11**	**5–13**	**2–10**	**0–11**	**1–10**	**0–12**	**2–11**	**1–10**	**0–10**	**0–9**	1–11 **1–10**	2–13	2–11	4–11	2–10	3–12
C11 ($n = 10$)	**2–6**	**2–6**	**2–6**	**5–8**	**5–12**	**2–6**	**0–7**	**0–12**	**0–8**	**2–9**	**3–12**	**0–10**	**0–5**	**0–11**	1–6 **1–7**	1–7	3–7	1–6	1–7
C12 ($n = 8$)	**4–5**	**4–5**	**4–5**	**7–8**	**7–11**	**4–5**	**2–6**	**3–10**	**2–7**	**3–8**	**5–10**	**2–9**	**2–4**	**2–10**	**2–7**	1–5 **2–5**	2–6	0–4	0–6
C13 ($n = 8$)	**1–3**	**1–3**	**1–3**	**6–8**	**6–12**	**1–3**	**1–4**	**1–11**	**1–6**	**3–8**	**4–11**	**1–9**	**1–4**	**1–11**	**1–7**	**2–6**	1–2 **1–2**	2–5	3–6
C14 ($n = 7$)	**2–5**	**2–5**	**2–5**	**5–8**	**5–12**	**2–5**	**0–6**	**1–11**	**0–7**	**2–8**	**3–11**	**0–9**	**0–4**	**0–11**	**0–7**	**0–6**	**1–4**	1–3 **1–5**	1–5
C15 ($n = 6$)	**2–5**	**2–5**	**2–5**	**5–8**	**5–12**	**2–5**	**0–6**	**1–11**	**0–7**	**2–8**	**3–11**	**0–9**	**0–4**	**0–11**	**0–7**	**0–6**	**1–5**	**0–6**	1–5 **1–5**

Within-population differences are shown on the diagonal, with total number of nucleotide differences in the cytochrome *c* oxidase I gene (COI) (of 655 bp) above the line and **total number of nucleotide differences** in the 16S rDNA gene (of 507 bp) below the line. Differences in the COI gene between populations are shown above the diagonal, while differences in the 16S rDNA gene between populations are shown below the diagonal.

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Table 4. Nucleotide differences within and between taxa in the portions of the COI and 16S rDNA genes used in this study.

	Albinaria	*Cerion striatellum*	*C. maloneі*	*C. stevensoni*	*C. caerulescens*	*C. uva*
Albinaria ($n = 1$)	0/0.00 **0/0.00**	172–173/26.26–26.41	166–170/25.34–25.95	171/26.11	170–171/25.95–26.11	142–148/21.68–22.59
C. striatellum ($n = 2$)	**150/28.96**	12/1.83 **4/0.77**	140–150/21.37–22.90	150–153/22.90–23.36	146–149/22.29–22.75	146–154/22.29–23.51
C. maloneі ($n = 2$)	**150–153/28.96–29.54**	**118–122/22.78–23.55**	15/2.29 **9/1.74**	51–55/7.79–8.40	54–56/8.24–8.55	135–149/19.85–22.75
C. stevensoni ($n = 2$)	**154/29.73**	**122–124/23.55–23.94**	**46–49/8.88–9.55**	1/0.15 **0/0.00**	14–16/2.14–2.44	138–144/21.07–21.98
C. caerulescens ($n = 2$)	**147–148/28.38–28.57**	**123–125/23.74–24.13**	**44–49/8.49–9.55**	**13–15/2.51–2.90**	1/0.15 **2/0.39**	138–145/21.07–22.14
C. uva ($n = 174$)	**132–136/25.48–26.25**	**120–128/23.66–24.71**	**131–142/25.29–27.41**	**126–132/24.32–25.48**	**127–134/24.52–25.87**	0–16/0.00–2.44 **0–14/0.00–2.76**

Within-species differences are shown on the diagonal, with total number of nucleotide differences/*percent nucleotide differences* in the COI gene above the line and **total number of nucleotide differences/*percent nucleotide differences*** in the 16S rDNA gene below the line. Differences in the COI gene between species are shown above the diagonal in the format total number of nucleotide differences/*percent nucleotide differences*. Differences in the 16S rDNA gene between species are shown below the diagonal in the format **total number of nucleotide differences/*percent nucleotide differences***.

comparable with the differences between *C. caerulescens* and *C. stevensoni* (Table 4), two species inhabiting Long Island (Bahamas) that have been assigned to separate subgenera, *Strophiops* and *Umbonis*, respectively. Sequence differences between species of *Cerion* inhabiting different island groups were 21.07–23.51% for COI and 22.78–27.41% for 16S, while differences among species inhabiting a single island (Long Island, Bahamas), each representing a different subgenus, were 2.14–8.55% for COI and 2.51–9.55% for 16S (Table 4).

Results of ABGD analyses of distance matrices for each of the three datasets (COI, 16S and COI + 16S) are shown in Table 5. When applied to the COI and concatenated datasets, the ABGD method could not identify a gap sufficient to partition the *C. uva* complex into taxonomic subunits and assigned all samples of *C. uva* to a single group in all partitions. Only the first two partitions based on the 16S data failed to include all samples of *C. uva* within a single group. Subsequent partitions based on the 16S data combined all *C. uva* within a single group and converged on the results of COI only and concatenated analyses by the fifth partition.

Phylogenetic analyses using Bayesian inference of the COI, 16S and concatenated datasets each resolved the relationships among the outgroup taxa and the *C. uva* complex consistently and with high support (≥0.96 posterior probability, PP) (Fig. 5), corroborating the prior finding (Harasewych *et al.*, 2011) that the subgenus *Cerion s. s.* is a monophyletic sister group to the cerionid fauna of the Greater Antilles, western Virgin Islands, Bahamas, Turks and Caicos Islands and Florida, a divergence that dates to the Oligocene (Harasewych, 2012).

Within the *C. uva* complex, all samples from Bonaire (Fig. 5: B) and most samples from the region near Christoffelberg (Fig. 5: C) were resolved as distinct and well supported (≥0.97 PP) clades in all analyses. The population from a monadnock at Ronde Klip (Fig. 5: RK) also emerged as a clade that was well supported by the 16S and concatenated data (≥0.96 PP), but not by the COI data (0.54 PP). The populations from Aruba were not resolved as a clade in any of the analyses, but were grouped with populations from southeastern Curaçao, particularly those from Schaarlo (C1), with which they share both COI and 16S haplotypes (Fig. 5: A + E). The remaining samples from Curaçao were poorly resolved.

The program TCS sorted the COI sequences from the 174 specimens of *C. uva* into 60 haplotypes, with more than half (31 of 60) represented by single individuals (singletons), and 7 of the 60 present in more than one population. The corresponding 16S sequences were apportioned among 59 haplotypes, of which 30 were limited to single specimens and 6 were present in multiple populations (Appendix 1). Haplotype networks were estimated for each gene (Fig. 6), with haplotypes from Aruba, Bonaire, western and eastern Curaçao (boundary as shown in Fig. 4) each shaded in different tones. Haplotypes present in more than one of the four areas are proportionally shaded.

Populations from Bonaire were characterized by distinct haplotypes that require a minimum of seven mutational steps to connect to the nearest Curaçao population for the COI gene, and a minimum of five mutational steps for the 16S gene. In contrast, COI haplotypes occurring on Aruba are either identical to those present in eastern Curaçao (C1) or distinguished by a single mutational step. Aruban 16S haplotypes are also either identical to those that occur in three populations in eastern Curaçao (C1, C2, C4) or differ by a single mutational step.

Within both the COI and 16S networks, relationships among the haplotypes occurring on Curaçao are represented by complex patterns containing multiple loops and interior haplotypes (haplotypes with two or more mutational connections). Several haplotype clades are evident that either distinguish individual populations or group together geographically proximal localities. Specimens from Ronde Klip (C5) in eastern Curaçao

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Table 5. Results of automatic barcode gap discovery analyses of distance matrices for each of the three datasets (COI, 16S and COI + 16S).

Partition	No. groups found COI gene (78 haplotypes)	No. groups found 16S rDNA gene (87 haplotypes)	No. groups found COI + 16S (107 haplotypes)	Prior maximum distance
1	5	52	5	$P = 0.001000$
2	5	52	5	$P = 0.101668$
3	5	7	5	$P = 0.002783$
4	5	7	5	$P = 0.004642$
5	5	5	5	$P = 0.007743$
6	5	5	5	$P = 0.012915$
7	5	5	5	$P = 0.021544$
8	4	4	4	$P = 0.035938$
9	4	4	4	$P = 0.059948$
10	4	4	4	$P = 0.100000$

Composition of groups resulting from analysis of only the COI sequences (78 haplotypes).

Five groups = *Albinaria* + all *C. uva* + *C. striatellum* + *C. malonei* + (*C. stevensoni* + *C. caerulescens*).

Four groups = *Albinaria* + all *C. uva* + *C. striatellum* + (*C. malonei* + *C. stevensoni* + *C. caerulescens*).

Composition of groups resulting from analysis of only the 16S rDNA sequences (87 haplotypes).

52 groups = *Albinaria* + (A1AGH + A1DE + A2AF + A2HM + A2CDEIJLNO + A3ADEFG + A3BCH + C1ABCDF + C1HIJKL + C4D + C2IJ) + (B1ABCDEFGHIJKLMNO + B2ABCDEFL) + B2HI + B2J + B2K + (C4ABCGI + C11A) + C4E + (C4F + C8CI + C8E + C8F + C11CJ + C14D + C9AE + C15D + C15F + C15H + C2B + C10J) + C4H + C4J + (C13AH + C13B + C13CD + C13E) + C13F + C13G + C8AG + C8BJ + C8D + C8H + C11BEF + C11D + C11G + C11H + C11I + C14A + C14BE + (C14C + C12GI + C15B) + C14FH + (C9BDFGH + C9I + C9J) + C9C + C12A + C12CDFH + C12J + C15E + C15G + C5A + C5D + C5G + C5BCEFIJ + C2CF + C10AEH + C10BDF + C10C + (C10GI + C3ABCDEGIJ) + C7ABCGI + C7DJ + C7EH + C7F + C3H + C3F + *C. striatellum* + *C. malonei* + *C. stevensoni* + *C. caerulescens.*

Seven groups = *Albinaria* + all *C. uva* + *C. striatellum* + *C. malonei* + *C. malonei* + *C. stevensoni* + *C. caerulescens.*

Five groups, as in COI analysis.

Four groups, as in COI analysis.

Composition of groups resulting from analysis of concatenated COI + 16S rDNA sequences (107 haplotypes).

Five groups, as in COI analysis.

Four groups, as in COI analysis.

Deleting *Albinaria* reduces the number of groups by 1, but does not change the composition of the remaining groups.

have distinct COI haplotypes (20, 21) that are four steps removed from a wide-ranging haplotype spanning northwestern and central Curaçao. 16S haplotypes (97–101) from Ronde Klip form a group that is a minimum of two steps from a wide-ranging haplotype present in western and central Curaçao, and five steps from a haplotype endemic to Bonaire. Populations from an area west of Christoffelberg corresponding to the western region circumscribed by Baker (1924) were also segregated by both their COI and 16S haplotypes as being four or more steps from the next nearest haplotype. Several other, smaller haplotype clades were also present, especially in the COI network, but were generally composed of subsets of haplotypes from single, highly diverse populations and were unsupported in the 16S network.

DISCUSSION

Among land snail taxa, *Cerion uva* is certainly one of the most extensively analysed species from the standpoint of shell morphometrics (Baker, 1924; Wagenaar Hummelinck, 1940a, b; Gould, 1969, 1971, 1984; de Vries, 1974). The conclusion advanced on the basis of shell morphology, that all cerionid populations inhabiting Aruba, Curaçao and Bonaire comprise a single species, *C. uva*, is supported by molecular data. Within the *C. uva* complex, levels of sequence divergence between geographic regions do not occur in patterns that can be parsed into discrete, reciprocally monophyletic groups, as evidenced by the results of ABGD and Bayesian analyses.

Subdivision of *C. uva* by geographical regions is supported by molecular data, although in some cases with different boundaries than have been indicated by previous authors (Table 6). Populations on Bonaire have distinct and well differentiated haplotypes (12–15, 71–75) that are endemic to the island. Bayesian parsimony and network analyses clearly support the distinctiveness of the Bonaire populations, which therefore retain the rank of subspecies, *C. uva bonairensis*. This subspecies had been further partitioned (Baker, 1924) to distinguish between populations of smaller individuals that inhabit the lower, southeastern portion of the island (B2) (as form *kralendijki*) from the typical, larger individuals from the northern portion of the island (B1). These two regions share the most common COI haplotype, which is ubiquitous in the northern population, and the most prevalent of four haplotypes occurring in the southeastern population. Corollaries of coalescent theory predict that the most common haplotype is generally the oldest (Watterson & Guess, 1977), most widely distributed geographically and more likely to have more mutational connections than younger haplotypes (Posada & Crandall, 2001). This would suggest that rarer haplotypes (13–15) present in the population from the southeastern lowlands are derived from an older haplotype (12) present in the highland population in the northern portion of the island. While these populations do not share any of the four 16S haplotypes endemic to Bonaire, the highland population has a single 16S haplotype (75) that differs from the most common lowland haplotype (71) by a single mutation. The increase in haplotype diversity in the southern lowland region, which was repopulated from the northern highlands

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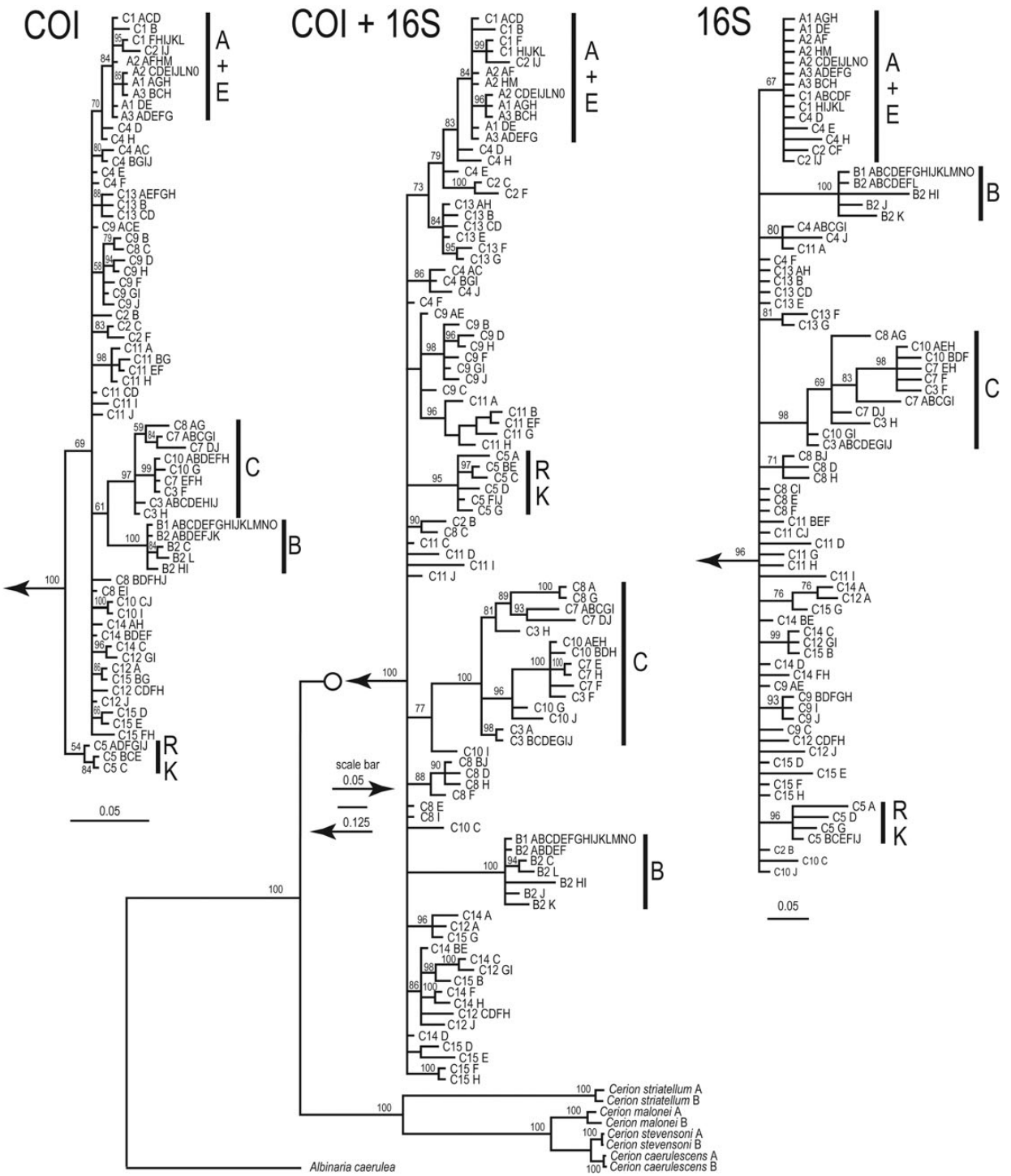

Figure 5. The 50% majority-rule consensus of post burn-in sampled trees from Bayesian inference analyses of DNA sequences of COI and 16S genes analysed separately and as a concatenated dataset (COI + 16S). Resolution of outgroups is common to all trees. Numbers above nodes are Bayesian inference posterior probabilities. Terminal taxa are haplotypes identified by the station number, representing geographic origin, followed by the letter designations of all specimens with identical sequences. Abbreviations: A + E, Aruba and eastern Curaçao; B, Bonaire; C, west of Christoffelberg, Curaçao; RK, Ronde Klip, Curaçao.

following the last interglacial period, is similar to the pattern found in central Curaçao.

On Curaçao, clades of haplotypes (1–11, 61–70) largely confined to a region southwest of Christoffelberg (within Baker's western faunal area) are differentiated from the remaining populations of the island by four or more mutational steps. The haplotypes composing these clades are also highly divergent, with some separated by as many as four mutational steps from their nearest neighbour within the clade. Several of the more prevalent haplotypes are likely of comparable age based on their

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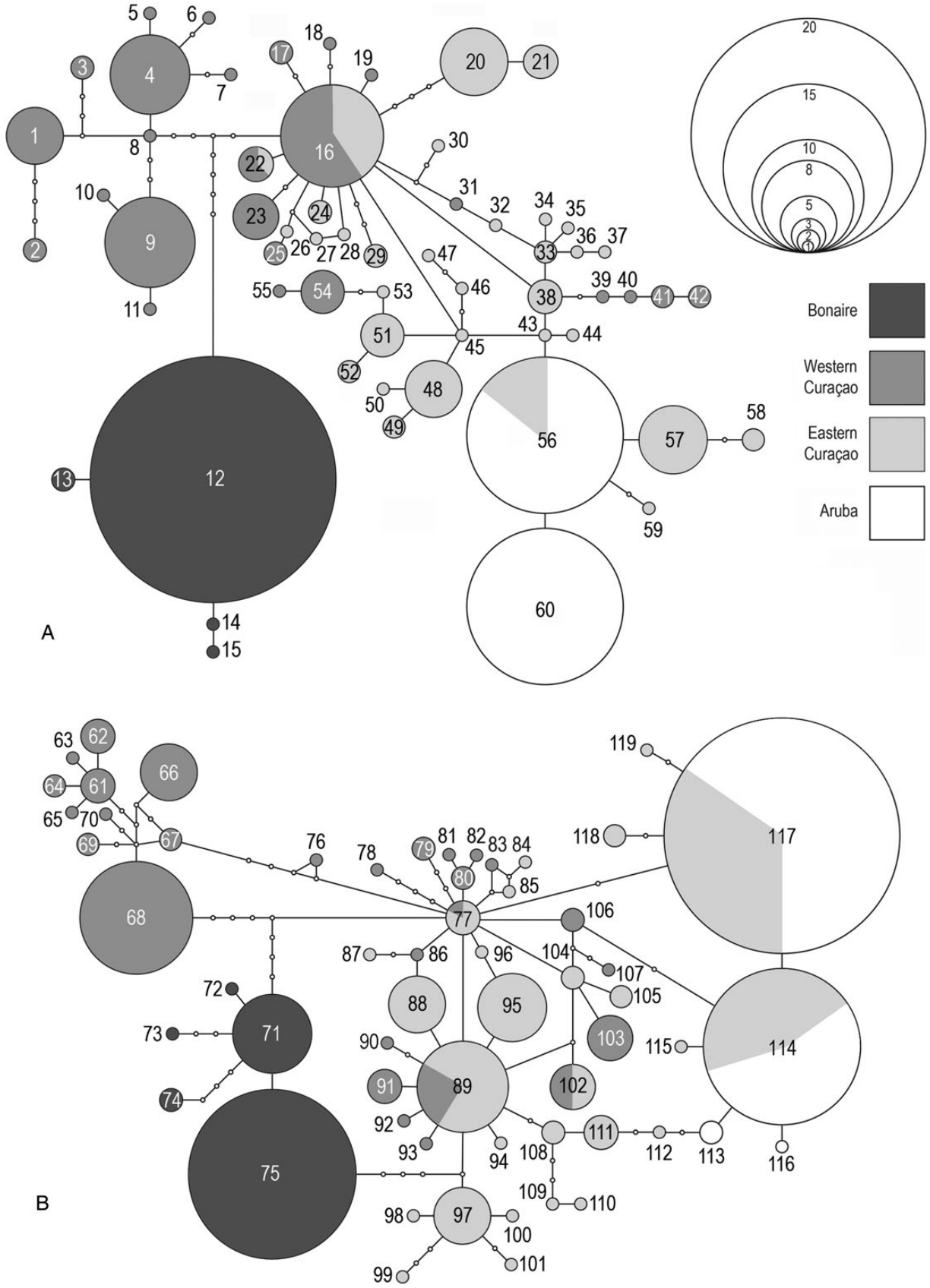

Figure 6. TCS networks based on COI (**A**) and 16S haplotypes (**B**) of the *Cerion uva* complex. Haplotypes are sequentially numbered. In instances where identical haplotypes occur within more than one region, circles are proportionally shaded. (See Appendix 1 for correlation of network haplotype numbers with individuals and populations as they appear in Bayesian analyses.)

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Table 6. Partition of the subgenus *Cerion sensu stricto* based on morphological (†) and molecular (‡) studies.

	Linnaeus, 1758 (†)	Pilsbry & Vanatta, 1896 (†)	Baker, 1924 (†)	Wagenaar Hummelinck, 1940b (†)	Gould, 1969 (†)	de Vries, 1974 (†)	Gould, 1984 (†)	Present study (‡)
Aruba	*Turbo uva*	*C. uva uva*	*C. uva arubanum*	*C. uva*	*C. uva arubanum*	*C. uva*	*C. uva arubanum*	*C. uva uva*
Curaçao		*C. uva uva*	*C. uva uva*		*C. uva uva*		*C. uva uva*	
		C. uva desculptum	*C. uva uva* form *hatoensis*					
			C. uva uva form *diablensis*					*C. uva diablensis*
			C. uva knipensis		*C. uva knipensis*		*C. uva knipensis*	*C. uva knipensis*
			C. uva knipensis form *djerimensis*					
Bonaire		*C. uva uva*	*C. uva bonairensis*		*C. uva bonairensis*		*C. uva bonairensis*	*C. uva bonairensis*
			C. uva bonairensis form *kralendijki*					

frequencies within the populations. It would appear that the *Cerion* inhabiting this valley are descendants of a genetically diverse population isolated on Christoffelberg during an interglacial period, and subsequently confined by the valley walls when they moved down-slope as sea levels receded. The upper portion of this valley contains the type locality of *C. uva knipensis* (C10), while the shore cliffs near its southern limit serve as the type locality of *C. uva knipensis* form *djerimensis* (C7). Subspecific rank (*C. uva knipensis*) is warranted for the *Cerion* inhabiting this valley, as shown by maximum-parsimony and haplotype-network analyses. However, further partition of the smaller forms inhabiting the coastal cliffs is not, as coastal populations inhabiting the northern (C3) and southern (C7) regions of the valley share common haplotypes with the inland population from the grounds of Landhuis Knip (C10). Other progeny of the interglacial population at Christoffelberg likely descended along its northern slope to become part of the fauna of the northern coastal plain. Both COI and 16S haplotypes related to this lineage persist as minor components of the Boca Wadomi (C8) population. The presence of haplotypes more closely related to those from central Curaçao (17, 89, 90) in two individuals (C10CJ) from Landhuis Knip invites more conjectural explanations, ranging from the presence of these highly divergent haplotypes in the interglacial population on Christoffelberg, to more recent anthropogenic transport of individuals (commerce between landhuizen).

Schaarlo (C1), in eastern Curaçao, was designated by Baker (1924: 14) as the type locality of the nominotypical subspecies *C. uva uva*. In the COI network, the Schaarlo population shares a haplotype (56) with all populations in Aruba, including the type locality of *C. uva arubanum* (A2). This haplotype is grouped in a clade that includes the other haplotype present in Aruba (60) and several others present in Schaarlo (57, 59) and is one step from a haplotype (43) occurring along the southwestern slope of Tafelberg Santa Barbara (C4). The 16S network reiterates this grouping, but also includes several individuals from southwest of Tafelberg Santa Barbara (C40). The most common of both the COI and 16S haplotypes are shared by the Schaarlo and all Aruba populations, with all the remaining haplotypes on Aruba differing by a single mutation. Given the close genetic relatedness of all sampled populations from Aruba to the population from the type locality of the nominotypical subspecies, *C. uva arubanum* is considered to be a synonym of *C. uva uva*.

Populations from central Curaçao and the northern coastal plain, a region inundated during the last interglacial period and subsequently repopulated from the eastern and, to a lesser extent, the western highlands, are characterized by their large numbers of haplotypes. Several of the populations along the northern coastal plain, especially along its eastern portion, have haplotypes that are not present at other localities (i.e. populations C2, C9 and C13 have distinctive COI haplotypes, populations C11 and C13 have diagnostic 16S haplotypes). Populations from the western and central portions of the northern coastal plain (C8, C11, C14) as well as those from the southern coast of the central region (C12, C15) have combinations of unique and wide-ranging haplotypes for one or both genes. A small proportion of these haplotypes (16, 77, 89) are exceptionally wide ranging, and several, particularly the 16S haplotypes, may span the length of the island (Fig. 7). Relationships among many of the haplotypes occurring in the central region are depicted as complex networks with multiple loops and a disproportionately high number of interior haplotypes, which are indicative of interbreeding among previously isolated populations as sea levels receded.

Unlike the other samples from eastern Curaçao, the population from Ronde Klip, a monadnock of older limestone that is the type locality of *C. uva diablensis* (C5), has distinctive haplotypes (20, 21, 97, 98, 99, 100, 101) that form a well differentiated clade in Bayesian and network analyses. The Ronde Klip haplotypes are separated from the remaining Curaçao haplotypes by four mutations steps in the COI network and two mutational steps in the 16S network, levels similar to those distinguishing the subspecies *C. uva knipensis* of western Curaçao from the remaining Curaçao populations. This population was distinguished morphologically by Baker (1924: 100) on the basis of extremely dwarfed shells. *Cerion uva diablensis* should be retained as a subspecies on the basis of genetic data. The range of this subspecies appears confined to the immediate slopes of Ronde Klip, and does not extend to the eastern shores of Sint Joris Baai (C13), as suggested by Baker (1924: 16) on the basis of shell morphology.

Baker (1924: 100) also distinguished a large, heavy shelled phenotype from near Landhuis Hato (C2) as *C. uva hatoensis*. Haplotypes from this locality were anomalously heterogeneous, and several were far more closely related to haplotypes from distant populations than to other haplotypes from this location. The presence of a rubbish dump near the collecting site raises the possibility that this sample may include recently introduced individuals from other regions of Curaçao. More rigorous resampling will be required to produce an accurate assessment of the relationships of *Cerion* from this area.

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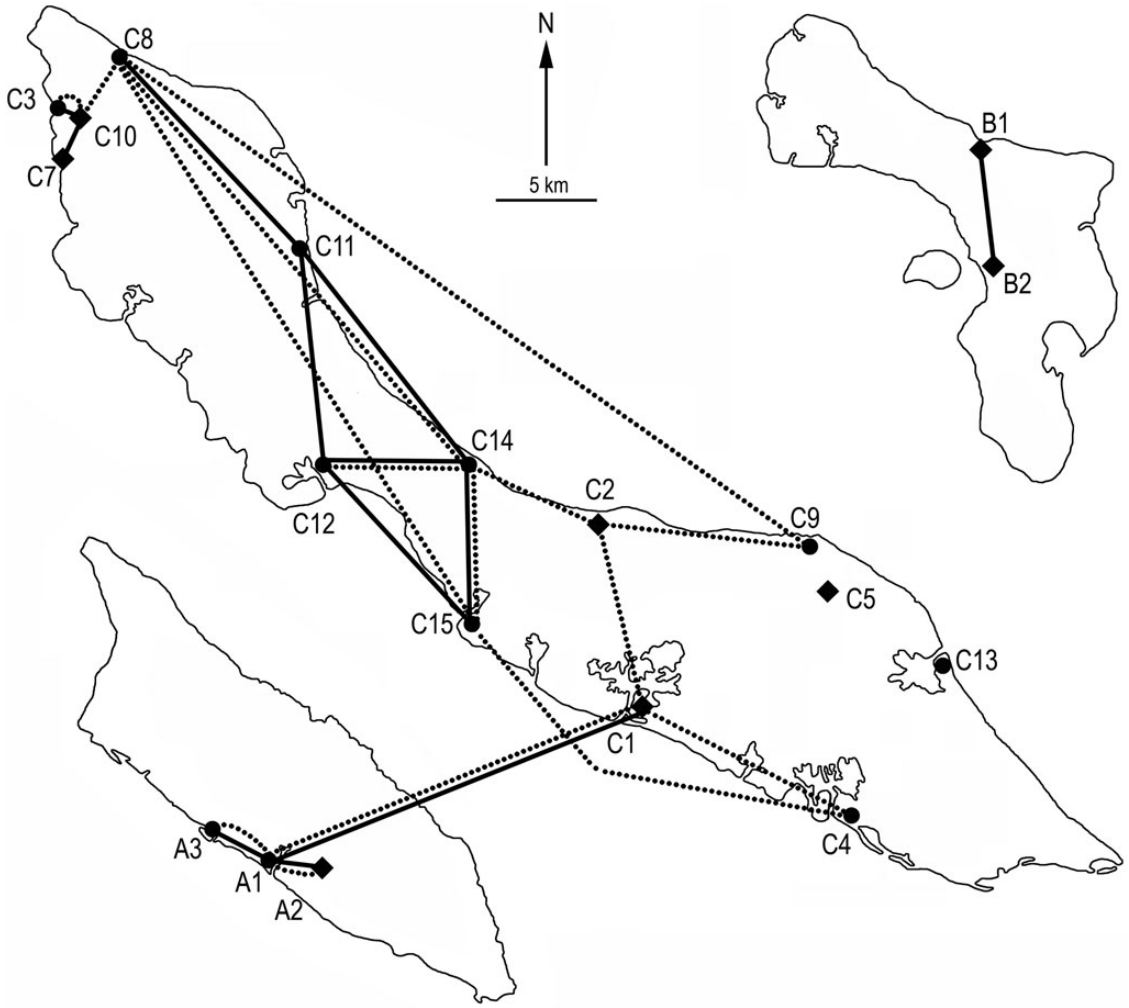

Figure 7. Map showing sampled *Cerion* populations that share one or more COI (solid lines) or 16S (dotted lines) haplotypes. Relative positions of the islands have been altered.

The two sampled populations from Bonaire shared a COI haplotype. Both COI haplotypes present on Aruba occurred in all populations, and one was shared with one population in eastern Curaçao. On Curaçao, COI haplotypes are shared only among populations from the western region, and also among populations from the northern and western portion of the central region of the island (Fig. 7). In contrast, 16S haplotypes were more widely distributed on Curaçao, with several (77, 89) spanning the length of the island. Bonaire populations did not have any 16S haplotypes in common. The two prevalent 16S haplotypes on Aruba (114, 117) were shared among all populations, as well as with populations on eastern Curaçao.

In some cases, discordant patterns of variation in the two mitochondrial genes appear incompatible with the general view that mitochondrial genomes are uniparentally transmitted and nonrecombining. Possible explanations for such patterns range from convergent sequence evolution in wide-ranging haplotypes, to the occurrence of heteroplasmy and recombination in mitochondrial genomes, as has been reported in cases of hybridization (e.g. Ballard & Whitlock, 2004; Filipowicz *et al.*, 2008).

The most unexpected finding of this study has been the presence of identical haplotypes in all populations from Aruba (A1–A3) and the Schaarlo population from eastern Curaçao (C1), indicating that the *Cerion* of Aruba are genetically as close or closer to those from Schaarlo than are other populations on Curaçao. The taxon *C. uva arubanum*, described as being endemic to Aruba, is therefore a synonym of *C. uva uva*. This is especially surprising, as *C. uva* is well represented in fossil deposits of Quaternary age on Aruba, Curaçao and Bonaire (Wagenaar Hummelinck, 1940b: 47; de Vries, 1974: 85). In his description of *C. uva arubanum*, Baker (1924: 104) commented on the peculiar distribution of living *Cerion* on Aruba (Fig. 4A), which is restricted to a narrow region along the central southwestern coast flanking a natural harbour, in contrast to the 'almost universal' distribution of fossils on the island. He (Baker, 1924: 114) went on to observe that while the molluscan fauna of Aruba is most closely related to that of western Curaçao, "*Cerion uva arubanum* appears most closely related to *Cerion uva uva* from central Curaçao." Gould (1969: 195) demonstrated that fossil *Cerion* from Aruba are morphologically distinct and group with samples from western Curaçao and Bonaire, while modern samples from Aruba group with eastern Curaçao. In a subsequent study using multivariate techniques, Gould (1984: 223) again concluded that Late Pleistocene or Holocene fossils from Aruba "do not share the characteristic regional morphology of living samples."

In an intervening study, Gould (1971: table 1) reported that, on Curaçao, middens at several Meso-Indian sites with radiocarbon ages of 3,530–4,705 YBP contained shells or fragments from a variety of marine molluscs (gastropods, bivalves and chitons), but most common were shells of *Cerion* that bore characteristic

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apical breaks indicating that they had been eaten. He noted that these *Cerion* shells were larger than any found on Curaçao today. Berry (1934) reported the presence of *Cerion* shells in Indian middens of unspecified age along Lake Tacarigua [=Lake Valencia], Venezuela. As living *Cerion* are confined to the Dutch West Indies, Gould (1971: 21) concluded that these *Cerion* must have been transported to Venezuela along trade routes.

In contrast, several Neo-Indian middens from sites on Aruba, with radiocarbon ages of 815–2,000 YBP, contained shell remains of an assortment of marine gastropods, bivalves and chitons similar to those found in the middens on Curaçao, but shells of *Cerion* were notably absent. Sites at Malmok (Versteeg, Tacoma & van der Velde, 1997) and Tanki Flip (Versteeg & Rostain, 1997) are situated some distance from localities where living *Cerion* are present. However, Parkietenbos (A3), a region where *Cerion* are common today, is directly south of the Neo-Indian site at Ceru Canashito (Gould, 1971), and along the shortest distance from this site to the ocean, where the marine molluscs would have been collected. The absence of *Cerion* in the Neo-Indian middens at Ceru Canashito may indicate that dietary preferences had changed to the exclusion of *Cerion* over the intervening millennia, or that living *Cerion* were not present on Aruba at that time.

The new molecular findings add to a body of evidence that is concordant with an alternative hypothesis, one that postulates that *Cerion* inhabiting Aruba today are not descendants of the fossil and subfossil *Cerion* found on Aruba, but rather are derived from propagules that have been reintroduced to Aruba from the vicinity of Willemstad (Schaarlo area) by humans, either by the Dutch West India Company in the 17th century, or possibly earlier by Caquetío Indians, who travelled between Venezuela, Curaçao and Aruba by canoe (Fig. 8). A corollary of this hypothesis is that *Cerion* present on Aruba during the Quaternary died off prior to the arrival of humans. Should this prove to be true, it may well be that the fossil specimens from Aruba merit recognition as a subspecies. The name *C. uva arubanum* would not be available, as it is based on a holotype from an introduced modern population that is neither descended from nor closely related to the fossil populations.

It is difficult to envision an event that would lead to the extinction of *Cerion* on Aruba, but not on Curaçao or Bonaire, yet allow for the persistence of other terrestrial molluscs endemic to Aruba. Selivanov (1992) estimated that mean sea level rose at most by 7 m during the early Late Pleistocene interglacial, while Scheffers & Kelletat (2006: fig. 20) document several tsunamis with amplitudes greater than 10 m impacting the Dutch Antilles within the past 3,500 years, noting the difficulties inherent in identifying palaeotsunamis of greater age.

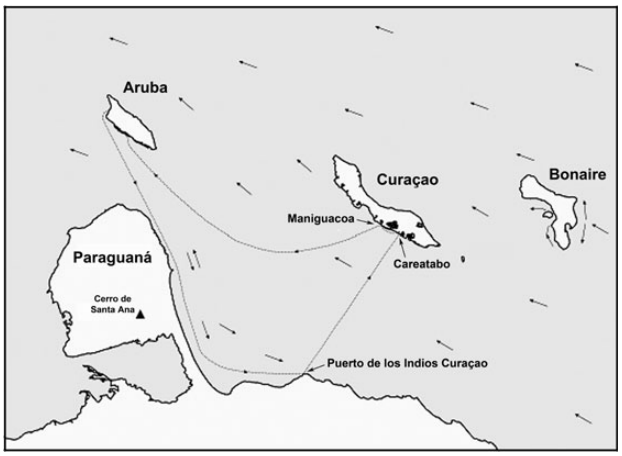

Figure 8. Map showing hypothesized trade routes of the Caquetío Indians between the coast of Venezuela, Curaçao and Aruba (after van Buurt, 2009).

The highest point on Aruba (Seroe Jamanota, 188.3 m) is lower than the highest points on Bonaire (Seroe Brandaris, 240.4 m) or Curaçao (Christoffelberg, 372.4 m) (Baker, 1924). However, these peaks achieved their present elevations as the result of uplifting during the Quaternary, and many show evidence of fossil corral terraces at considerable elevations. De Buisonjé (1964a, b) recognized a series of five emerged Pleistocene shorelines and terraces ranging between present sea level and an elevation of 200 m, with the oldest terraces generally having higher elevations than the younger ones. The Lower Terrace is the youngest of these terraces (dated as 122 k YBP by Schellmann *et al.*, 2004), encircling the islands of Aruba, Bonaire and Curaçao, with a mean altitude of 10 m asl. The two Middle Terraces (I and II) have been uplifted by 25–45 m asl and have similar elevations on Aruba, Bonaire and Curaçao. Middle Terrace I was estimated to have been formed 400–500 k ybp (Schellmann *et al.*, 2004). The Higher Terrace has an elevation ranging from 80 to 55–60 m on the seaward side, indicating that sea levels were decreasing at the time this terrace was formed. The Highest Terrace, with an elevation of 100–150 m (with an eastern dip), is present only on Curaçao, and is also a regressive terrace, formed by slowly receding sea levels. De Buisonjé (1964b: 70) noted that this terrace consists of a discontinuous row of limestone caps that represented a series of small islands emerging in a shallow area between the larger islands that had already emerged (Christoffelberg and Sta Barbara). While this Highest Terrace is the oldest and highest of the depositional terraces, he identified two additional terraces, one on Tafelberg Sta Barbara at an elevation of 150 m and one on Tafelberg St Hieronymus at an elevation of 210 m, that may be either erosional or depositional in character. The highest points on Curaçao and Bonaire are taller than 210 m, the highest point on Aruba is not.

The *Cerion* fauna of Aruba, Curaçao and Bonaire is believed to be related to the genus *Brasilennea*, which inhabited eastern Brazil during the Palaeocene, and a sister group to the Cerionidae that colonized the Greater Antilles via the GAARlandia land bridge during the 1–2 Myr interval at the Eocene-Oligocene boundary (Harasewych, 2012). Although Cretaceous outcrops are present in the Dutch Antilles, the earliest records of *Cerion s. s.* are so far confined to the Quaternary deposits of these islands (Gould, 1969). Molecular data indicate that the most genetically differentiated populations inhabit the island of Bonaire, a valley in western Curaçao and an isolated hill in eastern Curaçao. Morphometric analyses of fossil *Cerion* from Aruba place them close to living populations from Bonaire and western Curaçao. Molecular and morphometric data show that living Aruban *Cerion* are most closely related to a population from Willemstad in eastern Curaçao, and lead to the hypothesis that *Cerion* became extinct on Aruba during the Quaternary or Holocene, and were reintroduced within the past 800 years to a confined region along the central southwestern coast of Aruba, either by Indian canoes or Dutch vessels. On Bonaire, the area around Seroe Brandaris served as a highland refuge during interglacial periods from which the lowlands along the southern portions of the island were subsequently repopulated.

The topology of Curaçao is more complex, with peaks in both the western and eastern portions of the island connected by a narrower lowland area. Several authors (e.g. Baker, 1924; Gould, 1984; Wagenaar Hummelinck, 1990) considered the peaks of Christoffelberg (372.4 m) in western Curaçao and Tafelberg Sta Barbara (193.8 m) in eastern Curaçao to be nuclei for recolonization of the lowland regions following their inundation during interglacial periods. Others (Hovestadt, 1987) included Ceru Grande (73.2 m) in western Curaçao and Ronde

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Klip (129 m) in eastern Curaçao (Fig. 4) as island refugia during interglacial high sea-level stands. Baker (1924) discerned western, central and eastern faunal regions on Curaçao, while most subsequent workers recognized only western and eastern regions.

Molecular data clearly identify a well differentiated group of haplotypes confined to western Curaçao in the vicinity of Christoffelberg. As sea levels receded, the *Cerion* inhabiting Christoffelberg, a separate island during the interglacial period, migrated down-slope. Those migrating to the south and west of the peak appear to have been isolated within a valley that extends to the present coastline. Additional sampling will be required to determine how far along the coastline these haplotypes extend, and thus the degree of concordance with Baker's western region, which runs southward along the western shoreline. Those *Cerion* migrating down-slope along the northern face of Christoffelberg would have reached the western portion of the Hato Plain that stretches the length of the northern coast of Curaçao and come in contact with populations expanding from the east.

In contrast, haplotypes occurring in populations around Tafelberg Sta Barbara in eastern Curaçao are more diverse and less well differentiated, connecting with those from progressively more geographically distant sampling sites through a succession of interior haplotypes that rarely include multiple mutational steps. Several haplotypes are present at multiple localities. Most commonly, such haplotypes are shared by populations in central Curaçao, but one ranges from the base of Tafelberg Sta Barbara to the western reaches of the Hato Plain. A notable distinction occurs at Ronde Klip, an isolated hill along the eastern margin of the Hato Plain. This population has haplotypes that are not shared with any other population, and require multiple mutational steps (2–4) to join the next most similar haplotype. While this hill (129 m) may have served as an island refuge during an interglacial period as suggested by Hovestadt (1987), the *Cerion* from its base have remained comparatively isolated genetically. With the exception of the population at Ronde Klip, there is continuity between populations of eastern and central Curaçao, but a sharp distinction between these regions and western Curaçao. Curaçao may therefore be divided into western and eastern regions. The boundaries of the western region are as described by Baker (1924), while the eastern region is a combination of Baker's central and eastern regions.

ACKNOWLEDGEMENTS

I am most grateful to Gerard van Buurt for sharing his invaluable knowledge of the terrestrial fauna, geology and history of the Dutch Antilles, as well as for his guidance and assistance in collecting *Cerion* on Curaçao. Thanks are due to Daniel Keil and Cheryl Ames for collecting and providing the specimens from Bonaire that were used in this study. This research was conducted while participating in the Smithsonian Institution's Deep Reef Observation Project (DROP) operating at the Curaçao Seaquarium. Support from this programme is gratefully acknowledged. Thanks also to Adriaan Schrier and the staff and crew of Substation Curaçao and the Seaquarium for their gracious hospitality and support. Special thanks to Carole Baldwin, Cristina Castillo and Martha Nizinski for assistance in the field, and to Robin Turner and Yolanda Villacampa for their contributions in the laboratory. I am grateful to Dr Herman Wirshing for instructions on the use of MrBayes. Thanks also to Richard E. Petit for helpful discussions on nomenclature.

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APPENDIX

Appendix 1. Correlation between sequentially numbered haplotypes (bold) in the network analyses in Figure 6 [COI (1–60) and 16S (61–119)] and alphanumeric locality haplotypes (italics) used in Bayesian analyses in Figure 5. GenBank accession number in square brackets following haplotype number.

COI Haplotypes: **1**[KJ624920] ($n = 5$) = *C7ABCGI*; **2** [KJ624921] ($n = 2$) = *C7DJ*; **3** [KJ624922] ($n = 2$) = *C8AG*; **4** [KJ624923] ($n = 8$) = *C3ABDEFGI* + *C10G*; **5** [KJ624924] ($n = 1$) = *C3J*; **6** [KJ624925] ($n = 1$) = *C10I*; **7** [KJ624926] ($n = 1$) = *C3C*; **8** [KJ624927] ($n = 1$) = *C3H*; **9** [KJ624928] ($n = 7$) = *C7EH* + *C10ABDEF*; **10** [KJ624929] ($n = 1$) = *C7F*; **11** [KJ624930] ($n = 1$) = *C10H*; **12** [KJ624931] ($n = 22$) = *B1A* → *O* + *B2ABDEFJK*; **13** [KJ624932] ($n = 2$) = *B2HI*; **14** [KJ624933] ($n = 1$) = *B2C*; **15** [KJ624934] ($n = 1$) = *B2L*; **16** [KJ624935] ($n = 9$) = *C8EI* + *C11CD* + *C12J* + *C14BDEF*; **17** [KJ624936] ($n = 2$) = *C10CJ*; **18** [KJ624937] ($n = 1$) = *C11I*; **19** [KJ624938] ($n = 1$) = *C11J*; **20** [KJ624939] ($n = 6$) =*C5ADFGIJ*; **21** [KJ624940] ($n = 3$) = *C5BCE*; **22** [KJ624941] ($n = 3$) = *C12A* + *C15BG*; **23** [KJ624942] ($n = 4$) = *C12CDFH*; **24** [KJ624943] ($n = 2$) = *C14AH*; **25** [KJ624944] ($n = 2$) = *C12GI*; **26** [KJ624945] ($n = 1$) = *C14C*; **27** [KJ624946] ($n = 1$) = *C15D*; **28** [KJ624947] ($n = 1$) = *C15E*; **29** [KJ624948] ($n = 2$) = *C15FH*; **30** [KJ624949] ($n = 1$) = *C2B*; **31** [KJ624950] ($n = 1$) = *C8C*; **32** [KJ624951] ($n = 1$) = *C9B*; **33** [KJ624952] ($n = 2$) = *C9GI*; **34** [KJ624953] ($n = 1$) = *C9J*; **35** [KJ624954] ($n = 1$) = *C9F*; **36** [KJ624955] ($n = 1$) = *C9H*; **37** [KJ624956] ($n = 1$) = *C9D*; **38** [KJ624957] ($n = 3$) = *C9ACE*; **39** [KJ624958] ($n = 1$) = *C11A*; **40** [KJ624959] ($n = 1$) = *C11H*; **41** [KJ624960] ($n = 2$) = *C11EF*; **42** [KJ624961] ($n = 2$) = *C11BG*; **43** [KJ624962] ($n = 1$) = *C4H*; **44** [KJ624963] ($n = 1$) = *C4D*; **45** [KJ624964] ($n = 1$) = *C4E*; **46** [KJ624965] ($n = 1$) = *C2C*; **47** [KJ624966] ($n = 1$) = *C2F*; **48** [KJ624967] ($n = 5$) = *C13AEFGH*; **49** [KJ624968] ($n = 2$) = *C13CD*; **50** [KJ624969] ($n = 1$) = *C13B*; **51** [KJ624970] ($n = 4$) = *C4BGIJ*; **52** [KJ624971] ($n = 2$) = *C4AC*; **53** [KJ624972] ($n = 1$) = *C4F*; **54** [KJ624973] ($n = 4$) = *C8BDHJ*; **55** [KJ624974] ($n = 1$) = *C8F*; **56** [KJ624975] ($n = 14$) = *C1ACD* + *A1DE* + *A2AFHM* + *A3ADEFG*; **57** [KJ624976] ($n = 6$) = *C1FHIJKL*; **58** [KJ624977] ($n = 2$) = *C2IJ*; **59** [KJ624978] ($n = 1$) = *C1B*; **60** [KJ624979] ($n = 14$) = *A1AGH* + *A2CDEIJLNO* + *A3BCH*.

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Number of COI haplotypes (number of individuals): 31(1), 13(2), 3(3), 3(4), 2(5), 2(6), 1(7), 1(8), 1(9); 2(14), 1(22).

COI haplotypes occurring at multiple localities: 4/9/12/16/22/56/60.

16S haplotypes: **61** [KJ636091] ($n = 3$) = *C10AEH*; **62** [KJ636092] ($n = 3$) = *C10BDF*; **63** [KJ636093] ($n = 1$) = *C7F*; **64** [KJ636094] ($n = 2$) = *C7EH*; **65** [KJ636095] ($n = 1$) = *C3F*; **66** [KJ636096] ($n = 5$) = *C7ABCGI*; **67** [KJ636097] ($n = 2$) = C7DJ; **68** [KJ636098] ($n = 10$) = *C3ABCDEGIJ* + *C10GI*; **69** [KJ636099] ($n = 2$) = *C8AG*; **70** [KJ636100] ($n = 1$) = *C3H*; **71** [KJ636101] ($n = 7$) = *B2ABCDEFL*; **72** [KJ636102] ($n = 1$) = *B2J*; **73** [KJ636103] ($n = 1$) = *B2K*; **74** [KJ636104] ($n = 2$) = *B2HI*; **75** [KJ636105] ($n = 15$) = *B1A→O*; **76** [KJ636106] ($n = 1$) = *C11D*; **77** [KJ636107] ($n = 3$) = *C4F* + *C8E* + *C15H*; **78** [KJ636108] ($n = 1$) = *C11I*; **79** [KJ636109] ($n = 2$) = *C11CJ*; **80** [KJ636110] ($n = 2$) = *C8BJ*; **81** [KJ636111] ($n = 1$) = *C8D*; **82** [KJ636112] ($n = 1$) = *C8H*; **83** [KJ636113] ($n = 1$) = *C12A*; **84** [KJ636114] ($n = 1$) = *C14A*; **85** [KJ636115] ($n = 1$) = *C15G*; **86** [KJ636116] ($n = 1$) = *C11A*; **87** [KJ636117] ($n = 1$) = *C4J*; **88** [KJ636118] ($n = 5$) = *C4ABCGI*; **89** [KJ636119] ($n = 9$) = *C2B* + *C8CI* + *C9AE* + *C10J* + *C14D* + *C15DE*; **90** [KJ636120] ($n = 1$) = *C10C*; **91** [KJ636121] ($n = 3$) = *C11BEF*; **92** [KJ636122] ($n = 1$) = *C11G*; **93** [KJ636123] ($n = 1$) = *C11H*; **94** [KJ636124] ($n = 1$) = *C9C*; **95** [KJ636125] ($n = 6$) = *C9BDFGHI*; **96** [KJ636126] ($n = 1$) = *C9J*; **97** [KJ636127] ($n = 5$) = *C5BEFIJ*; **98** [KJ636128] ($n = 1$) = C5C; **99** [KJ636129] ($n = 1$) = *C5A*; **100** [KJ636130] ($n = 1$) = *C5G*; **101** [KJ636131] ($n = 1$) = *C5D*; **102** [KJ636132] ($n = 4$) = *C12GI* + *C14C* + *C15B*;**103** [KJ636133] ($n = 4$) = *C12CDFH*; **104** [KJ636134] ($n = 2$) = *C14BE*; **105** [KJ636135] ($n = 2$) = *C14FH*; **106** [KJ636136] ($n = 2$) = *C8F* + *C15F*; **107** [KJ636137] ($n = 1$) = *C12J*; **108** [KJ636138] ($n = 2$) = *C13AH*; **109** [KJ636139] ($n = 1$) = *C13G*; **110** [KJ636140] ($n = 1$) = *C13F*; **111** [KJ636141] ($n = 3$) = *C13CDE*; **112** [KJ636142] ($n = 1$) = *C13B*; **113** [KJ636143] ($n = 2$) = *A2AF*; **114** [KJ636144] ($n = 14$) = *A1DE* + *A2HM* + *A3ADEG* + *C1ABCDF* + *C4D*; **115** [KJ636145] ($n = 1$) = *C4E*; **116** [KJ636146] ($n = 1$) = *A3F*; **117** [KJ636147] ($n = 21$) = *A1AGH* + *A2CDEIJLNO* + *A3BCH* + *C1HIJKL* + *C2IJ*; **118** [KJ636148] ($n = 2$) = *C2CF*; **119** [KJ636149] ($n = 1$) = *C4H*.

Number of 16S haplotypes (number of individuals): 30(1), 12(2), 5(3), 2(4), 3(5), 1(6), 1(7), 1(9), 1(10), 1(14), 1(15), 1(21). Totals: 59(174).

16S haplotypes at multiple stations: 68/77/89/102/114/117.

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