DISSERTATION ZUR ERLANGUNG DES DOKTORGRADES DER NATURWISSENSCHAFTEN
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Comparative diversification potential of an old and a young lineage of freshwater crabs on two Caribbean islands explained at the population level.

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Introduction

Insular systems

Insular systems have always been a preferred ”model system“ for scientists to study the processes of evolution (Whittaker & Fernández-Palacios, 2007) This is no wonder, as the study of an insular fauna contributed greatly to the development of the original theory of evolution (Darwin, 1860). Not only “real” islands like the Galapagos Islands, Hawaii, Madagascar or Australia represent insular systems, but also isolated habitats like deep sea hydrothermal vents (Van Dover, 2000) or isolated mountains like Mt. Kilimanjaro can act as insular systems. Size is often the main feature in which insular system differ from mainland habitats. Others are the isolated character and the different composition of fauna and flora on islands or in other insular systems. In these simpler and enclosed environments, scientist search for answers to their questions about evolution (Grant, 1998). Species on islands tend to differ from mainland relatives in some features: they are known to have reduced dispersal capabilities (Cody & Overton, 1996), change their size respectively to mainland representatives (Case, 1978; Lomolino, 1985), increase their

Figure 1. Satellite picture of the West Indies showing the Greater Antilles and Lesser Antilles.

variation among populations (Howarth & Mull, 1992) or explore new ecological niches (Roughgarden, 1995).

In general, islands tend to harbour a lower amount of species, not only downright because of their smaller area, but also per area unit. On the other hand, species found on islands are often unique, i.e. endemic to certain islands. These two factors render island species more prone for extinction (Whittaker & Fernández-Palacios, 2007). Therefore, many islands and other insular systems are considered biodiversity hot-spots and deserve special care and attention regarding conservational efforts.

Geological history of the Greater Antilles

The Caribbean islands consist of the four Greater Antillean islands, Jamaica, Cuba, Hispaniola and Puerto Rico, the Leeward Antilles and the Lesser Antilles (Figure 1). The islands are also known as the West Indies based on the geographic mistake made by their European discoverer. The arc formed by these islands delimits the Caribbean Sea. Two different scenarios exist for the geological history of the Caribbean. One assumes a generation from the Proto-Caribbean Plate in the so-called Galapagos geological hotspot around 100 mya ago in the Mid Cretaceous. This newly formed plate then moved northeast towards its present position. The second theory states a birth of the Caribbean islands between the North American and South American plates during the Mid Jurassic (160 mya) as result of their western movement. From there, it moved into the Proto-Caribbean Basin because of its overall slower western movement as neighbouring plates (Buskirk, 1985). Although both models postulate this scenario at the beginning of the Cenozoic, the geological history of the region is very complex and scientist do not agree, which parts or islands were above sea level at which time (Hedges, 1996). The slower moving Caribbean Plate collided with the Bahaman Plate, which is attached to the North American one. This resulted in volcanism, subduction and the opening of the Cayman Trough, the deepest part of the Caribbean Sea. Along with faulting, folding and uplifts, volcanism played a certain role in the generation of the islands and their mountain ranges. Presently, volcanic activity is only evident in the Lesser Antilles. Although called the Caribbean Plate, this plate consists of many different terranes and so do the Caribbean islands. For example Cuba probably was formed out of three different geological blocks (J

Pindell & Dewey, 1982), with one of these blocks being deemed unique for the West Indies. This western part shows more similarities with the North American plate (Graham, 2003). On the opposite end of the island, the eastern side was likely connected to the northern part of Hispaniola and Puerto Rico, as they belonged to the same magmatic arc till 30 mya (Iturralde-Vinent, 1994) or even 20 mya ago (Sykes et al., 1982; Pindell & Barrett, 1990). Independently from the islands of the Greater Antilles, the Lesser Antilles were formed through volcanic activity which started more or less at a time, when volcanic activity came to an end in the Greater Antilles and persists until today (Wadge, 1994). It is provoked, by the subduction of the Atlantic Plate under the Caribbean Plate, due to the differences in westward movement of the two pates. Similar to Cuba, Hispaniola is also formed out of several different terranes. One land block was aggregated from the Bahaman Bank. The northern and central part of Hispaniola fused around 45 mya ago and at that time were also connected to the western part of Cuba. This connection resulted in a similar composition of animal and plant genera (Graham, 2002) on the two islands. In the Early Miocene, the southern part of the island collided with the rest. This collision stopped the northeast movement of southwest Hispaniola and Jamaica. These two land blocks were separated from the rest of the early Greater Antilles. The opening of the Cayman Trough and the resulting stretching of the seafloor pushed Jamaica and the southwestern land block of Hispaniola northeast. During this drifting phase, the island of Jamaica became submerged for around 20 mya starting in the late Eocene. The limestone and karst formations which cover large parts of Jamaica are a result of these submarine epochs. The uprise of Jamaica, which started in the late Miocene, lifted the island again over the sea level (Draper & Lewis, 1990; Robinson, 1994). The newly emerged island of Jamaica was then available for new biological colonisations. This resulted in plenty of endemic animal and plant species. The exact colonisation pathways for Jamaica and the other islands of the Greater Antilles are intensively discussed and several opposing theories exist (Buskirk, 1984; Iturralde-Vinent & MacPhee, 1999; Hedges, 2001). The Greater Antillean island, Puerto Rico, reached its present position around 35 mya ago. The island lost its connection with Hispaniola in the Miocene (Graham, 2003) and was separated from the Virgin island due to sea level changes resulting from glacial events in the Quaternary. These changes in water level also altered the amount and distribution of

land mass on Puerto Rico, whereas the central mountain range, Cordillera Central, is the result of Eocene volcanism, uplift and later deformation followed by erosion.

Endemism in the West Indies

Apart from being a geologically very complex and interesting region, the Caribbean is also considered a biodiversity hotspot of the world (Mittermeier et al., 2004). Biodiversity can be defined as the number of species which can be found in a certain habitat or ecosystem. As a consequence of the isolated character of islands, these are often characterised by a high level of endemism. Factors like distance from the main land, size of the island and time since colonisation are important in the process of evolutionary divergence. As colonist find unoccupied habitats, sometimes quite different to their original one (Carson & Templeton 1984; Templeton 1980), adaptive radiation can take place, which results in high number of species unique to certain islands. Good examples for well studied adaptive radiation on islands are the finches from the Galapagos Islands (Grant, 1999) or the Hawaiian fruit flies

Figure 2. Satellite picture of Puerto Rico. Main island and the two smaller islands Vieques and Culebra

(Kambysellis & Craddock, 1997). These high numbers of species endemic within the limited available space on islands often results in classification as biodiversity hotspots (Mittermeier et al., 1998). Within the Caribbean hotspot, it is especially the islands of the Greater Antilles which harbour a high degree of endemic flora and fauna. These islands cover more than 90% of the 229 549 square kilometres of terrestrial surface in the Caribbean. They also present the highest elevation with 3071m above sea level, the Pico Duarte on Hispaniola (Orvis & La Pelona, 2003). Very different vegetation occurs on the islands: from cactus shrubs, savannahs over evergreen bushland, to freshwater swamps,

mangrove forests or lowland rainforests, which are now mostly deforested. In higher elevation, seasonal forest and mountain cloud forest occur (Beard, 1955). From the around 13 000 endemic plant species of the Caribbean islands (Daviset al., 1997), nearly half of them are endemic to single islands and around 25% endemic to Cuba. Of the roughly 2500 plant genera, around 10 percent are endemic and there is one plant family, the Goetziaceae, which can only be found in the West Indies (Davis et al., 1997). Even 50 of the 500 species of mosses are endemic (Delgadillo et al., 1995). Among the vertebrate species, frogs show more than 99% endemism (164 out of 165). Most of these are endemic to certain islands. The reptiles also bear a high percentage of endemism with around 94% (Hedges, 1996). This includes some interesting species radiations, one of them belonging to the genus Anolis with 150 endemic out of 154 species (Roughgarden, 1995). Of the nearly 148 mammalian species, only 29 are non-endemic. In the islands' freshwater systems 74 species of fish can be found, of which 71 are endemic, some of them even inhabiting single lakes (Hedges, 1996). The smallest percentage of endemism in Caribbean Vertebrata occurs in birds. Although one family of birds is endemic, only 35 percent of the 425 present species are restricted to the West Indies. The Caribbean

Figure 3. Satellite picture of Jamaica.

Islands not only have a high degree of endemism, they are also inhabited by some very diminutive species. On Cuba, the World's smallest bird, the bee hummingbird (*Mellisuga helenae*) and the tiniest tetrapod of the Northern Hemisphere occur (Estrada & Hedges, 1996). One can also find the smallest lizard, *Sphaerodactylus ariasae* (Hedges & Thomas,

2001) and the World's smallest snake, *Leptotyphlops carlae* in the West Indies (Hedges, 2008).

Also the invertebrate fauna of the Caribbean islands has developed a huge amount of endemic species, even if they are not documented as thoroughly as the vertebrate one. According to Woods (2001), the diversity of invertebrates known from the West Indies is only a small fraction of the really present diversity. He also remarked that the species groups, which are known, tend to be the result of adaptive radiation. As an example, only thirteen species of ostracods were known from Jamaica (Figure 3), which all lived in ponds and most of them abundant in the neotropics. Little and Hebert, 1996 then discovered several new species of ostracods, all living in bromeliads. They described eleven new species of which ten are only found on Jamaica. Similar to this, the number of endemic millipedes of the genus *Anadenobolus* from Jamaica had to be increased from one to three (Bond & Sierwald, 2002). The terrestrial mollusc fauna from Jamaica also has a high percentage of endemic species. Nearly 90%, that is 505 species out of 562, are only found on this island (Rosenberg & Muratov, 2006).

The species *Epilobocera sinuatifrons*

The freshwater crab *Epilobocera sinuatifrons* (A. Milne Edwards, 1866) belongs to the family Pseudothelphusidae and is the only freshwater crab of Puerto Rico (Figure 2) with a complete freshwater life cycle. It is endemic to the Caribbean islands Puerto Rico and Saint Croix (Chace & Hobbs, 1969; Villalobos-Figueroa, 1982; Covich & McDowell 1996). Its closest relatives are supposed to be the endemic freshwater crabs of Hispaniola (Pretzmann, 1974) *Epilobocera haytensis* (Rathbun, 1893) and *Epilobocera wetherbeei* (Rodríguez & Williams, 1995). *Epilobocera sinuatifrons* has a trapezoidal carapace with one anterolateral tooth. Adult individuals can grow to a carapace width of up to 150 mm, maturity is reached with a size of around 30 mm carapace width (Zimmerman & Covich, 2003). There is no dimorphism between the two genders, but between the two claws, as one is normally smaller and more acute and the other larger and blunter. The species has a direct development and females carry relatively large eggs, from which juvenile hatch while the eggs are still carried by the mother. After hatching, the juveniles stay with the mother for some time before they are released into suitable habitats, but do not moult

during that time. These habitats can vary greatly. Crabs can be found in rivers of very different composition. From small headwater creeks to large lowland streams, from riverbeds with mainly boulder and rocky composition to sandy and silty ones. According to Zimmermann and Covich (2003) the average flow velocity has an influence on the abundance of juvenile crabs, which tend to prefer higher velocities. Juveniles are often found hiding under rocks, wooden debris or in leaf litter, whereas large adults prefer burrows in sandy or muddy river walls. Due to their terrestrial movement capabilities, which is not only used to find food along river banks, the crabs can also be found far from any freshwater drainage system. In the Bosque Estatal de Guajataca, forest crabs were found inhabiting rock rubble in karst-sinkholes thriving in natural crevices and burrows which are probably connected with subterranean water (personal observation, Fig.4). They are also abundant in several cave systems throughout the island (Schubart & Rivera, personal observation). *Epilobocera sinuatifrons* is omnivorous, whereby a high percentage of the normal diet is made out of palm seeds and fruits, other freshwater invertebrates and terrestrial snails (Covich & McDowell, 1996; March & Pringle, 2003). The regular diet of juvenile crabs is unknown (Henry et al., 2000). Unlike its Hispaniolan relative, *Epilobocera haytensis*, *E. sinuatifrons* is no longer a regular component of local human diet, but is more endangered by commercial land use through deforestation and river regulation.

Figure 4. *Epilobocera sinuatifrons* observed in karst sinkholes in the Bosque Estatal de Guajataca, Puerto Rico. Picture C.D. Schubart.

The species *Sesarma dolphinum*, *Sesarma windsor* and *Sesarma meridies*

Sesarma dolphinum Reimer, Schubart & Diesel, 1998, *Sesarma windsor* Türkay & Diesel, 1994, and *Sesarma meridies* Schubart & Koller, 2005 are three of the currently known six river crab species of the genus *Sesarma* on Jamaica. They form part of the outcome of an adaptive radiation of the Sesarmidae on this island (Schubart et al., 1998) which led to the endemic species of the genera *Sesarma* and *Metopaulias*. From a marine ancestor, which colonised Jamaica approximatly 4.5 mya ago, after its rise over sea level, several river and terrestrial freshwater species evolved. These species are nowadays completely independent from the sea and stay their whole life cycle in their freshwater habitat, unlike most other Sesarmidae, which are often found in intertidal environments. The six river species known are *S. ayatum* Schubart, Reimer & Diesel, 1998, *S. bidentatum* Benedict, 1892, *S. windsor* Türkay & Diesel, 1994, *S. fossarum* Schubart, Reimer Diesel & Türkay, 1997, *S. dolphinum* and the recently described *S. meridies* Schubart & Koller, 2005. *Sesarma verleyi* Rathbun, 1914 is known from cave systems and inhabits cave freshwater pools and underground rivers, which are quite common on western and central Jamaica due to the limestone cover of the island. From the three true terrestrial species *S. cookei* Hartnoll, 1971 and *S. jarvisi* Rathbun, 1914 live in rock rubble on the forest floor, whereby the second species breeds in empty snail shells. The third terrestrial species, *Metopaulias depressus* Rathbun, 1896, is special, not only because of its habitat, the leaf axils of bromeliads, but also because of its brood care behaviour (Diesel, 1989). A similar brood care behaviour is also known and described for

Figure 5. *Sesarma dolphinum*. Picture C.D. Schubart.

Sesarma jarvisi (see Diesel & Horst, 1995). *Sesarma dolphinum* is the limnic species from the western tip of the island and inhabits the streams around the Dolphin Head, the mountain from which its name is derived. The closest relative is the river crab *S. fossarum*. *S. windsor* and *S. meridies* inhabit the rivers of central Jamaica, whereas *S. meridies* can be found as far east as the Rio Magno, one of the headwaters of the Rio Cobre. In central Jamaica, rivers draining north are populated by *Sesarma windsor* and those draining south by *Sesarma meridies*. These crabs can grow to a size of 27mm carapace width. It is not known, at which size they reach maturity. Their normal habitat expands from the headwaters of rivers down to hilly lowlands, but can not be found in complete unstructured lowland streams or brackish water. The lower river parts are also inhabited by *Armasis roberti* (A. Milne Edwards, 1840). Like the other limnic species of Jamaica, *Sesarma dolphinum*, *S. windsor* and *S. meridies* can be found hiding under stones in the river bed or its close vicinity or in burrows in the river banks. They have not been reported from the wider proximity of rivers. They still have larval development, but an abbreviated one, having quite large eggs, only two zoal stages and a megalopa stage. Both zoeal stages are lecithotrophic and the megalopa is a facultative lecithotrophe (Anger & Schubart, 2005). Although this abbreviated development, which can be found in all endemic freshwater crabs of Jamaica, is similar to their closely related mangrove dwelling species *Sesarma curacaoense* Rathbun, 1897, it does take place in the adult freshwater habitat rather than in a marine one. It is suspected that this early life history was already present in the ancestor which colonised the island and played a role in the rapid radiation of the endemic sesarmid crabs (Anger, 2005).

Population genetics

The basic principle of the heritability of traits was known to humans long before it was defined as a scientific subject. From the early beginnings of civilisation, humans have bred their domestic animals and plants. They were aware that variation exists among different individuals of a species and that this variation is inherited from one generation to the next. Not until the rediscovery of Georg Mendel's work and his laws (Mendel, 1901) this "knowledge" became the scientific field of genetics (Henig, 2000). The integration of Charles Darwin's theory of natural selection (1859) into Mendel's work by Sewall Wright (1931), J.B.S. Haldane (1932) and R.A. Fisher (1930) founded the discipline of population

genetics. The discipline aims to measure the amount and pattern of genetic variation, which can be found in interbreeding individuals of a species. In this way it quantifies gene flow, genetic drift, determining mating systems, mutation and natural selection within a certain population or subpopulation (Templeton et al., 1995). Early methods to infer this, included the study of easily recognisable and quantifiable traits like coloration, morphology, chromosomal composition or blood groups. Although variation in those traits can be quite important, these methods did not allow an estimation of the complete amount of genetic variation one can find in natural populations. With the application of protein electrophoresis (Lewontin & Hubby, 1966) the field of population genetics experienced a boost in the 1970s. The study of allozyme variations allowed the recognition of much more variation in the proteome of the investigated species. It also had the advantage that it is a relatively easy method and suitable to nearly all species. With the method by Sanger et al. (1977), direct sequencing of DNA was increasingly used by scientists interested in population genetics. Although the original method of sequencing was work and time consuming, modern methods of automated sequencing like pyrosequencing (Ronaghi et al., 1998) enable the sequencing of 100 million basepairs in only seven hours. This way the fundamental variation in the genome under research can be recognised. The recognition of the high amount of variation in genetic data led to the

Figure 6. Picture of a typical headwater river system from Jamaica depicting the regular habitat of the riverine species of the genus *Sesarma* from Jamaica.

theory of neutrality by Kimura (1968). The Neutral Theory as proposed by Kimura, stated that most of the detected variation in the molecular data has no impact on selection. It assigned the same relative fitness to all genotypes found (i.e. they are neutral in reference to each other). This does not imply that this variation has no effect at all. But in combination, the final fitness outcome of all alleles is the same. Most new mutation never become fixed, as their deleterious character leads to their quick elimination. Another important theory arising from evidence of growing molecular data bases is the coalescence theory (Kingman, 1982). Early views of population genetics were directed towards the future: how will the detected amount and the structure of genetic variations influence the evolutionary success? As DNA sequencing became more and more popular and accessible for researchers, the view changed to a retrospective one: which evolutionary processes have been at work to form the observed present situation? This change of view and reconstruction algorithms in combination with more and more molecular DNA data culminated in a very powerful statistical theory for population genetics, the coalescence theory. The various alleles found in a population originally emerged from a single ancestral allele. Moving backward, two alleles descend from an ancestral allele, they coalesce at this point. The allele in which all coalesce is called the most recent common ancestor (MRCA). The MRCA is the original copy of an allele from which all following copies of the population emerged. An established form of presenting the relationship of sequence data and their coalescence event into a MRCA are gene trees (Hedrick, 2005). The topology of the gene tree describes the relationship of two sister taxa. If the MRCA was present till the split of two sister taxa they are called monophyletic. The taxa are called paraphyletic, when the most recent common ancestor existed before their divergence. Therefore, some lineages can occur in more than one taxon. This is also referred to as “incomplete lineage sorting”. In recent years, different network methods have been developed to display gene genealogies (Posada & Crandall, 2001). The coalescence theory has produced a framework how to interpret data mined with molecular methods. It is a very dynamic field and has innervated population genetics. It was also improved and more factors of evolutionary significance, like gene flow (Beerli & Felsenstein, 2001) or dynamic population size (Harvey & Steers, 1999), were incorporated into the coalescence approach.

The mitochondrial DNA

In the past 30 years, the mitochondrial genome (mtDNA) has been used by more and more scientists to answer questions of phylogeny, phylogeography, population size and population structure. This way, they were able to get new insights, which would not have been possible with traditional methods, because, for example, phenotypic plasticity hindered them. Mitochondrial molecular markers have thus become an everyday useful and trusted tool. Surely the mitochondrial genome has some properties which are very useful, but it has also some restrictions one has to keep in mind when working with this tool. In several features it is quite different from nuclear DNA and some of those features were the reasons for its “success” as molecular marker. Unlike the diploid nuclear DNA, it occurs with several copies in one cell and the usually haploid maternally inherited mtDNA genome consists of about 16 000 base pairs, roughly a 1/10 000 of the size of a nuclear genome (Wolstenholme, 1992). With the publication of universal primers (e.g. Kocher, 1989) it became relatively easy to amplify mtDNA in nearly all animal species. As mtDNA is in most cases only transferred by females from one generation to the next and as it occurs in haploidy, its effective population size is only a quarter of the one of nuclear DNA. This also means that possible new alleles are fixed much faster in mtDNA. However, this four times higher effective population size in nuclear DNA is a value which is true in theory, but rarely reached in reality, as the effective population size is also shaped by the reproductive success or the distribution of a certain genome. Therefore, even if the smaller effective population size for mtDNA in comparison to nuclear DNA is true in general, this can even be reversed for certain species or events (Ballard & Whitlock, 2004). Another effect which can disrupt these numbers is paternal leakage (heteroplasmy). This exception from the normal solely maternal inheritance has been described, although in low frequency, in several species (Kondo et al., 1990; Kvist et al., 2003). It has been shown that this frequency can be increased in species where hybridisation happens recurrently (Kvist et al., 2003). Paternal leakage also makes recombination in mtDNA possible (Innan & Nordborg, 2002). Under normal circumstances animal mtDNA does not show recombination (Birky, 2001). This is one of the features used in phylogenetic research, as most computational methods need this assumption. The lack of recombination also means that anything impacting one part of the mtDNA will also impact other parts. Therefore no independent results from different mtDNA regions can be achieved. In recent times,

studies have been published, which show that recombination is possible in mtDNA (Dowton et al., 2003), although the rate by which this happens seems to be very different from species to species.

Mitochondrial and nuclear DNA differ in their mutation rate. It has been shown that the mutation rate for crustacean mtDNA lies between 1.7% and 2.6% per million years for the COI gene (Schubart et al., 1998). Brown et al. (1979) also postulated a mutation rate of around 2% per Myr for primates and that the mutation rate for the nuclear DNA was only a tenth of the mitochondrial one. This rapid rate of evolution of mtDNA makes single individuals suitable as OTUs, „operational taxonomic units“ (Avise, 2000). Additionally, the mutation rate of individual genes in the mitochondrial genome also vary. The rate of pairwise sequence divergence in Jamaican sesarmid crabs is only 0.65% per Myr for the 16S rRNA gene, but 1.66% per Myr for the COI gene (Schubart et al., 1998). Therefore, mitochondrial molecular markers are effective tools to infer gene flow or population history over a range of temporal and spatial scales (Sunnucks, 2000). The two mentioned markers are used for different questions. While 16S rRNA is often used to

Figure 7. Picture of a typical river system from Puerto Rico depicting the regular habitat of the species *Epilobocera sinuatifrons*.

infer macroevolution (Schubart et al., 2000), the cytochrome oxidase I gene is more used in studies concerning population genetics (Bilodeau et al., 2005).
There exists the danger of pseudogene amplification. Pseudogenes or numts (nuclear copies of mitochondrial DNA) are copied fragments from mitochondrial genes translocated to the nucleus. These are detected in more and more species including humans (Bensasson et al., 2001) and crustaceans (Schneider-Broussard, R., 1997; Nguyen et al., 2002). As numts are probably no longer coding, they can accumulate mutations at a different rate from the actual coding copy in the mitochondria. Therefore, studies which include but do not recognise the pseudogenes are prone to construct flawed phylogenies (Wallace et al., 1997).

Introgression

Introgression is the distribution of genes between either species or normally separated populations via hybridisation and backcrossing (Avise, 1994). Hybridisation has been reported for many plant and animal species as has introgession, the later is sometimes difficult to detect with molecular methods. The extent to which the introgressing gene is present in a species or population can vary from very low frequencies to a complete replacement by the alien gene (Bernatchez et al., 1995). One reason for this can be selection, if the hybridising species or population occupy similar environments and the mtDNA of one is better adapted. This adapted mtDNA would be successfully distributed by direct selection as long as no other selecting effects are present. Another reason for fixation of alien genes can be drift, if it creates a population with fixed deleterious alleles. This can happen in small populations, which then would have a lower average fitness as other population or closely related species. Selection can then again support the introgression of genes in this less fit population (Lynch, 1997). This results in a reasonable probability for introgression in species with hybridisation or population with secondary contact. This probability is greater for mtDNA than it is for nuclear genes. So when phylogenies are constructed solely with mtDNA markers the results can falsely be interpreted as incomplete lineage sorting or ancestral polymorphism (Ballard & Whitlock, 2004). Some authors also point out that the mitochondrial DNA is probably not always a neutral marker (Ballard, 2004). They found evidence for direct selection (De Stordeur,

1997) and indirect selection (Burton et al., 1999). Therefore it is suggested to test for neutrality when mtDNA is used to infer phylogeny or population structure and history.
Mitochondrial DNA is a powerful tool and has strengthened evolutionary studies immensely in the last decades. Despite all its great use, its possible shortcomings need to be considered. Therefore, phylogenetic and population studies should not employ this tool solely, but combine it with other, nuclear markers. In this combination possible flawed effects can be recognised and the information of mtDNA can still be used to gain new insights.

Aim of this thesis

The aim of this thesis is to identify dispositions and processes which lead to the highly different numbers of endemic, freshwater-dependant crab species on the two Greater Antilles Jamaica and Puerto Rico. From Puerto Rico only one endemic freshwater crab is described, the species *Epilobocera sinuatifrons*. On Jamaica several endemic species of the family Sesarmidae are present, which are all the result of an adaptive radiation (Schubart et al., 1998). In the effort to understand possible reasons for the observed low species diversity in the genus *Epilobocera* on Puerto Rico, its genetic population structure shall be studied. Therewith the presence of potential cryptic species is investigated. In comparison, the genetic population structure of *Sesarma dolphinum* from Jamaica is analysed to explore the diversity within this recently described species and examine potential reason for the detected structure. To confirm the species status of the recently separated species *Sesarma meridies* and *S. windsor*, the population structure of both species is investigated. Thereby, the degree of gene flow shall be quantified or hybridisation detected. In the species *Epilobocera sinuatifrons* and *Sesarma dolphinum* additional morphometric analysis shall be performed to investigate, if possible genetic differences are corresponded by phenotypic differentiation. With genetic population structure as a central point of this thesis, two mitochondrial genes COI and ND1 are sequenced as they provide suitable levels of variation. To compensate for possible shortcomings of the sole application of mitochondrial markers, the nuclear ITS1-5.8S-ITS2 complex is additionally used.

Chapter I: Morphometrics

Material and methods

Sample collection

Sampling sites for *Epilobocera sinuatifrons* in Puerto Rico were chosen by initial classification of freshwater streams into five geographic regions. These five regions were the vicinities of El Yunque National Forest in the northeast of the island, the provinces of Yabucoa, Maunabo, Patillas, Arroyo and Guayama in the southeast, the region around San German in the southwest, the vicinity of Guajataca in the northwest and the streams around central Monte Guilarte in the central-western part of the island. Initial analyses revealed that further sampling would be necessary in order to better determine an apparent west-east gradient of haplotypes. Therefore, a second collecting trip in 2006 covered streams between the Monte Guilarte region and the Carite forest along the Ruta Panoramica. This road runs along the southern slopes of a mountain range which stretches from east to west in south-central Puerto Rico. In this way, streams with

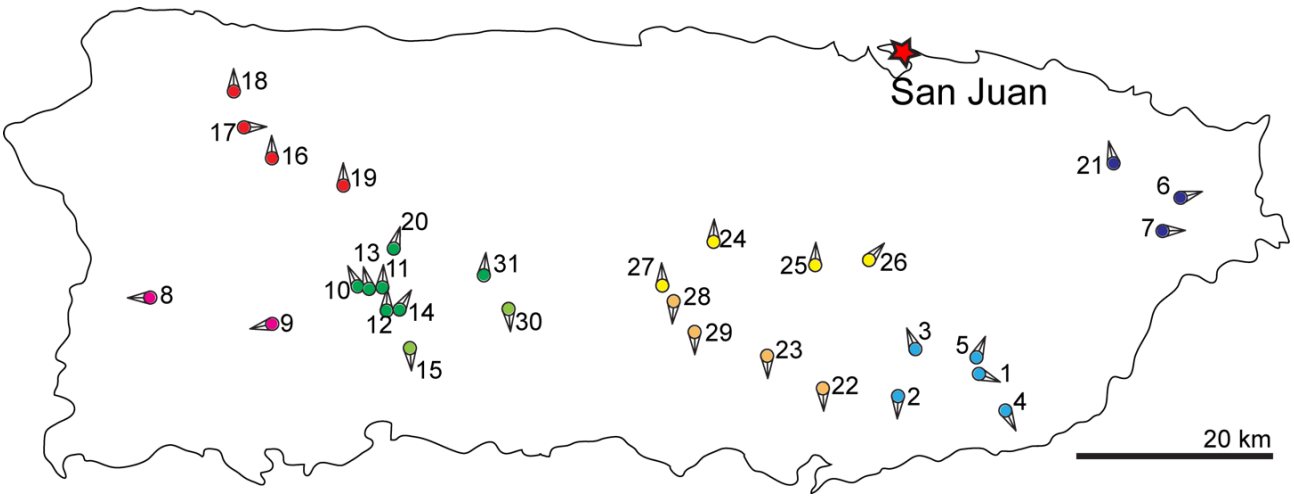


Figure 8. Map of Puerto Rico showing sampling sites position and number where individuals of *Epilobocera sinuatifrons* for this study were collected. Arrows pointing in the direction of drainage at the sampling sites.

geographically close headwaters, but belonging to either southern or northern drainage systems were sampled. Overall, in three collecting trips (including preliminary sampling by C.D. Schubart and R. Diesel in 1997) 31 different sites all over Puerto Rico were sampled. In Figure 8, all the sites are shown, also indicating the direction of flow of the streams.

Crabs were exclusively caught by hand or with hand nets. We aimed for at least one large male voucher specimen per sample site.

Animals of the genus *Sesarma* were collected from the western parishes of Jamaica and the central parishes Trelawny, Manchester, and Clarendon, the distribution ranges of *Sesarma dolphinum* Reimer J, C.D. Schubart & Diesel, 1998, respectively *Sesarma windsor* Türkay & Diesel, 1994 and *Sesarma meridies* Schubart & Koller, 2005. From the northeastern border of the distribution range of *Sesarma dolphinum*, which is the Flint River, to the southern border, which is marked by the Deans Valley River, the headwaters of all major water-systems were checked for the presence of specimens. Over a time period of nine years, from 1997 till 2005 more than 100 individuals from 14 different sampling sites were collected. In the distributing range of *Sesarma windsor* and the recently described *Sesarma meridies* overall 15 localities were sampled. In the years 1995 to 2003 around 112 individuals of the two species were collected. All sampling sites are shown in Figure 9. As far as possible, a minimum of five individuals per spot was collected. Therewith a sampling size sufficient enough for statistical analysis was aimed for. All animals were collected by catching them by hand and nets.

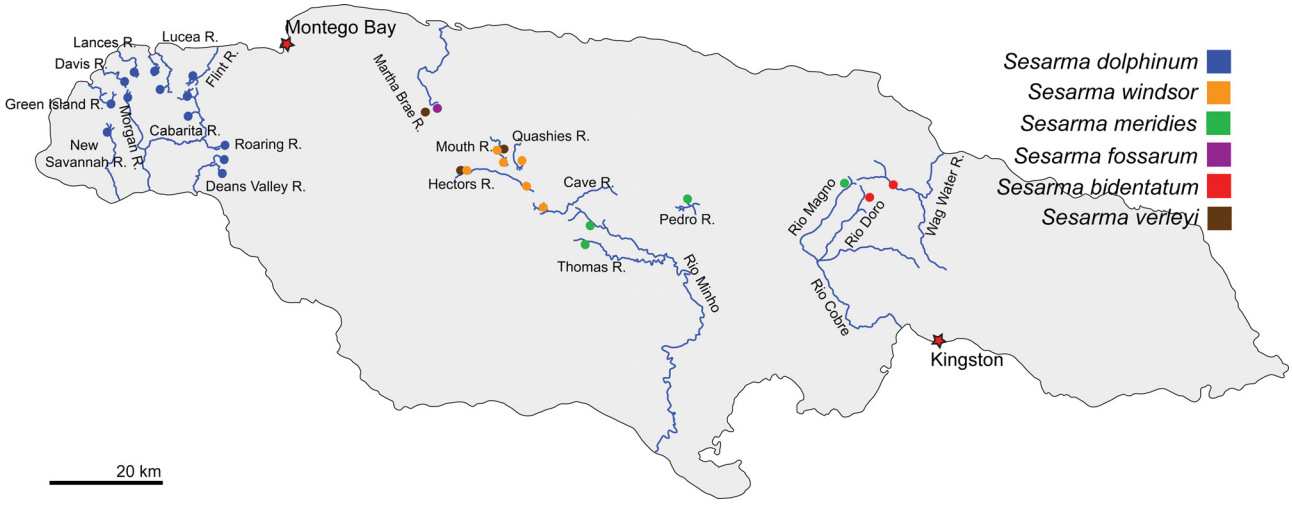


Figure 9. Map of Jamaica showing selected rivers and sampling sites where species for this study were collected.

From each sampling site the coloration of at least one live individual was recorded by means of digital imaging. To ensure minimum damage to the genetic material crabs were cooled down and killed by placing them in commercial refrigerator for some time. They

were then preserved in 95% ethanol. Single legs or claws which were lost by the animals during the catching process were stored in 95% ethanol immediately.

Morphometrics

Morphometric data were collected to detect phenotypic manifestation of possible genetic differences. A mechanical caliper gauge with a digital display was used to take measurements. From the collected individuals of *Sesarma dolphinum* 92 specimen were complete enough so that the following measurements could be taken: the carapace width was measured at two separate positions, first at the widest part including the exorbital tooth (CWf) and second at the back end of the carapace (CWb). Carapace length (CL) was measured along the center-line and carapace height (CH) in the center of the carapace at the highest spot. At the carapace also the forehead (FW) width between the two eyestalks and the length of the exorbital tooth (ET) were measured. Three different measurements were taken from the claws, the height (PrH) and length (PrL) of the propodus and the length of the dactylus (DaL). From the pereiopods the third and fourth were measured. The length (3L, 4L) and width (3W, 4W) of the merus of these two walking legs were recorded. Finally, the pleon (PlW) was measured at its widest part.
From the Puerto Rican freshwater crab *Epilobocera sinuatifrons* 111 individuals were analyzed. Here the following characters were recorded; the width of the carapace measured at the widest point including anterolateral tooth (CW), the length of the carapace measured at the central carapace (CL), body height (CH), the frontal width as the distance between the inner orbits (FW), and the dorsal length (3L, 4L) and width (3W, 4W) of the meri from the third and fourth pereiopod. Additionally, the length of the dactyli (DaL) and the height (PrH) and length (PrL) of the propodi of both chelae were measured, recording which of the claws was smaller. During all measurements extra care was taken not to squeeze the individuals, nor to measure claws or legs which have been regenerated recently or to measure damaged dactyli. To minimize probable errors due to allometric growth (Reuschel & Schubart, 2006) only individuals over a certain size were measured. From the *Sesarma dolphinum* dataset only individuals with CL larger than 12mm and from the *Epilobocera sinuatifrons* dataset only individuals with CL larger than 20mm were analyzed. All measurements were logarithmically transformed to further minimize the effect of possible allometric growth. Measurements were tested for normal distribution using the

one-sample Kolmogorov-Smirnov test. Those which showed normal distribution were included in a discriminant function analysis. The variable which had the greatest weight on the outcome of the discriminant function analysis was calculated. The discriminant function analysis was then redone without this variable to assure that the observed differences are not the result of a single factor. Based on the outcome of the Nested Clade Analysis (NCA) the different populations were also compared via t-tests. All calculations were performed in SPSS version 16 (SPSS Inc, Chicago IL).

Results

Epilobocera sinuatifrons

According to the Kolmogorov-Smirnov test, 10 out of the 15 measured characters in the *Epilobocera sinuatifrons* morphometric dataset showed normal distribution and were used for the analyses. The measurements of the interorbital distance, the carapace height and all measurements from the larger chelae were not normally distributed. The 30 different collection points were pooled into five groups (Centre [Guilarte State Forest], Northeast [El Yunque National Forest], Northwest [Bosque de Guajataca], Southeast [Carite] and Southwest [San German]) based on the initial sampling strategy as specified above. With these groupings, the Wilk`s Lambda for the discriminant model resulted in a value of 0.488 and a significant p-value ($p \leq 0.034$). Even though the test revealed significant differences between the five groups, the corresponding classification matrix shows an overall weak correct classification of 44.9% (Table 1), with some very low correct classifications down to 30.3%. This result is reflected in the

Table 1. Percentage of correct classification based on the morphometric classification function for five geographic groups of *Epilobocera sinuatifrons*. Overall correct classification of 44.9%.

	predicted group membership				
population	**1**	**2**	**3**	**4**	**5**
1 south east	30.3	42.4	12.1	3	12.1
2 north east	20	60	6.7	6.7	6.7
3 south west	0	16.7	50	16.7	17.7
4 north west	23.5	11.8	23.5	35.3	5.9
5 center	0	16.7	11.1	5.6	66.7

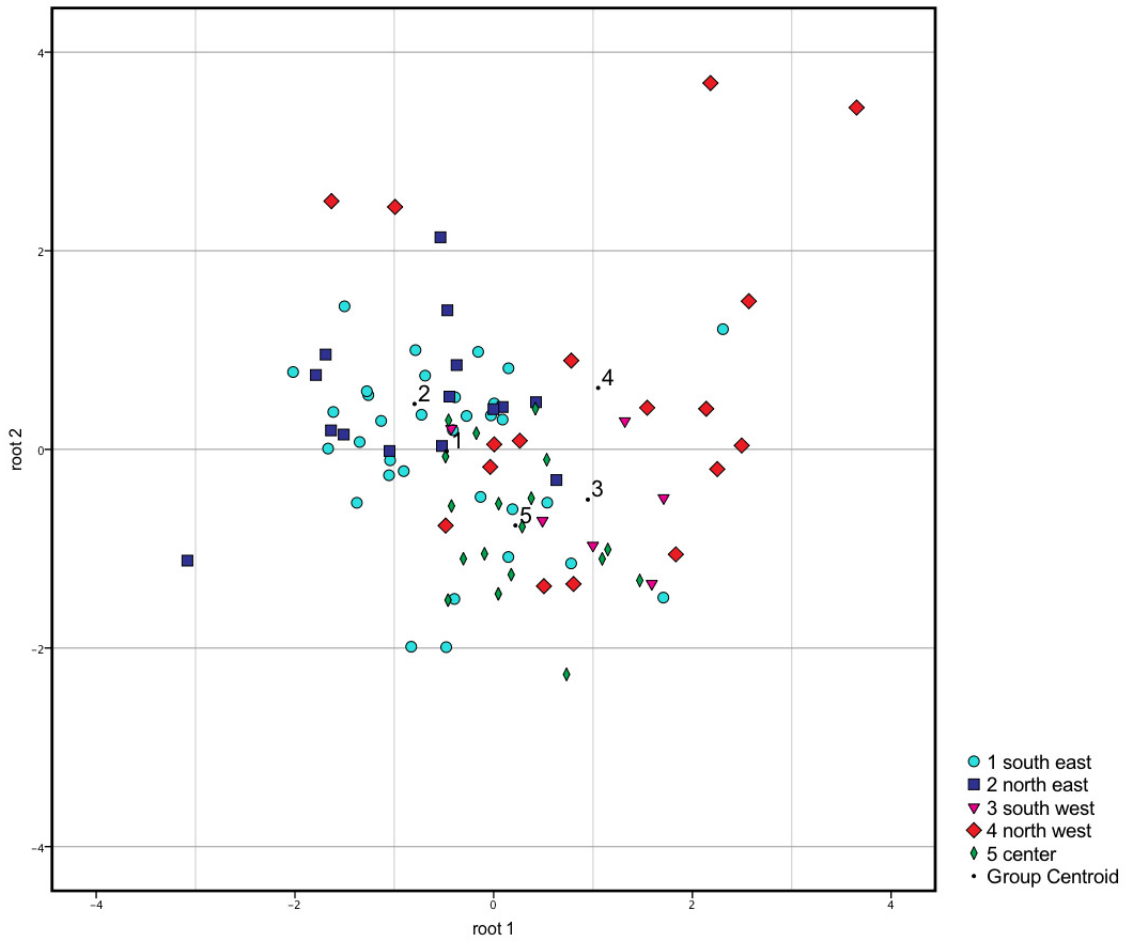


Figure 10. Canonial analysis showing discrimination by morphometric measurements between five geographic groups of *Epilobocera sinuatifrons* from Puerto Rico; plot of the first discriminant function (root 1) against the second (root 2)

scatterplot of the first two canonical functions (Figure 10). The measurements of the carapace length turned out to have the strongest impact on the outcome of the discriminant analysis. When the analysis was redone without those measurements the Wilk`s Lambda for the discriminant model resulted in a value of 0.546 but was no longer significant ($p \leq 0.66$). Because of the low correct classification and the strong influence of the carapace length, we performed a second discriminant analysis with a pooling into just three groups. All sampling points were now classified in either West, Center or East. These three groups again showed significant

Table 2. Percentage of correct classification based on the morphometric classification function for three geographic groups of *Epilobocera sinuatifrons*. Overall correct classification of 64.4%.

	predicted group membership		
population	**1**	**2**	**3**
1 east	66.7	22.9	10.4
2 center	10.5	84.2	5.3
3 west	34.8	21.7	43.5

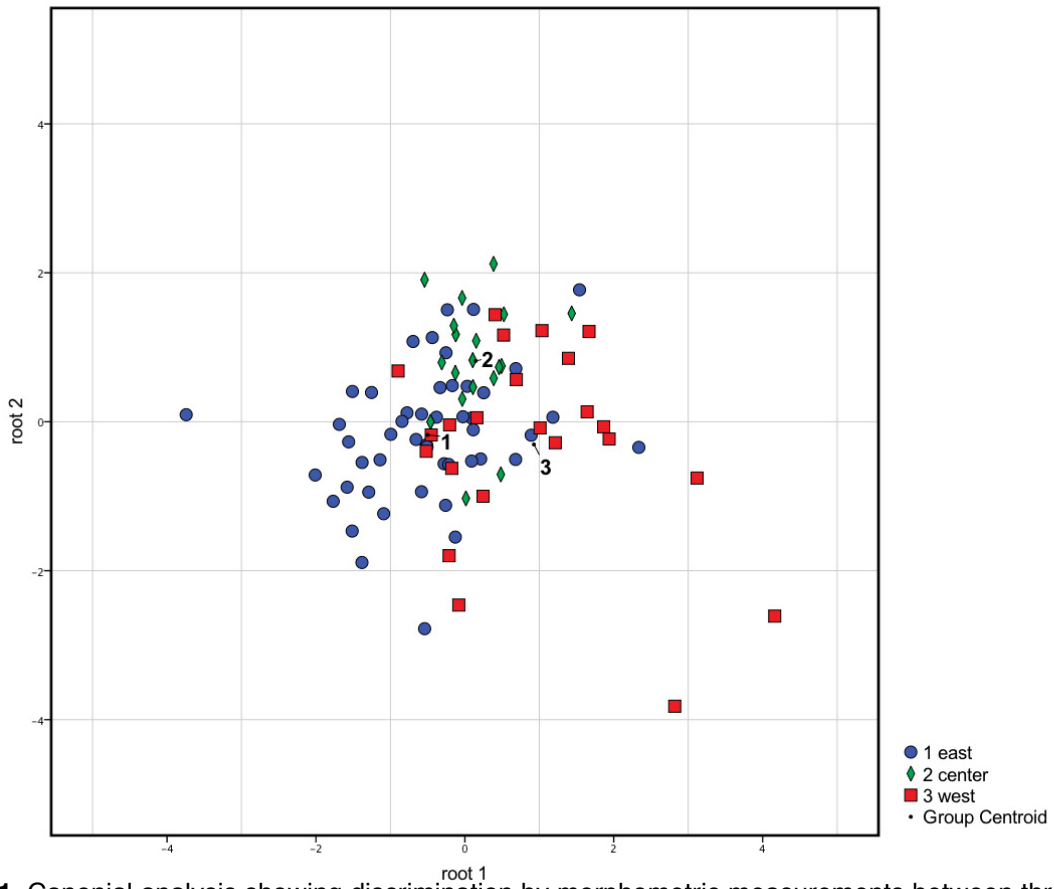


Figure 11. Canonial analysis showing discrimination by morphometric measurements between three geographic groups of *Epilobocera sinuatifrons* from Puerto Rico; plot of the first discriminant function (root 1) against the second (root 2)

differences (Wilk`s Lambda 0.614; p<0.005) but now an increased correct classification was reached with an overall value of 64.4% (Table 2). This is reflected in the scatterplot shown in Figure 11. In this case the height of the smaller propodus had the strongest impact on the analysis, but the recalculation of the discriminant analysis without this measurements still resulted in significant differences (Wilk`s Lambda 0.683; p≤0.024). In contrast, when the sampling points were pooled into Northern, Central and Southern points, no significant differences were found (Wilk`s Lambda 0.779; p≤0.557) and the corresponding classification matrix (Table 3) only revealed 52.2% overall correct

Table 3. Percentage of correct classification based on the morphometric classification function for three geographic groups of *Epilobocera sinuatifrons*. Overall correct classification of 52.2%.

	predicted group membership		
population	**1**	**2**	**3**
1 north	48.7	25.6	25.6
2 center	22.9	54.3	22.9
3 south	25	18.8	56.2

placement. The morphometric data show, that there are some morphometric differences in a west-east direction in *Epilobocera sinuatifrons*, but less and not significant differences in a south-north direction. However, all morphometric differences are not very pronounced and do not allow consistent distinction of morphotypes.

Sesarma dolphinum

In the *Sesarma dolphinum* dataset, not all single collection sites had enough individuals for statistical analyses. Based on the preliminary genetic results from the NADH subunit 1 data and the drainage systems of the corresponding collection sites, samples were pooled into nine groups. The two sampling points from the upper Cabarita River formed one group. Furthermore all sampling points from the Green Island River and all sampling points

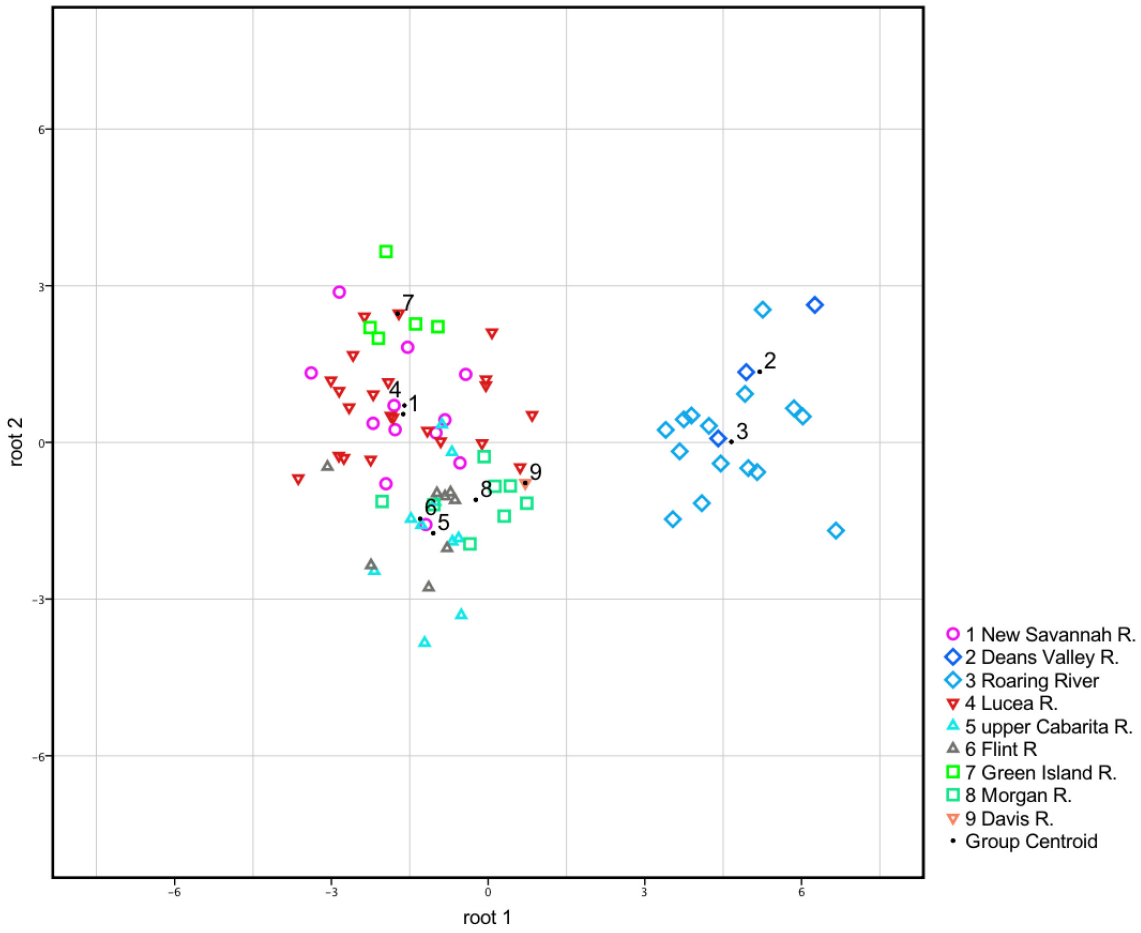


Figure 12. Canonial analysis showing discrimination by morphometric measurements between nine populations of *Sesarma dolphinum* from western Jamaica; plot of the first discriminant function (root 1) against the second (root 2)

from the Deans Valley River region were pooled together to one group each. Sampling points from the two Lucea rivers and the sampling point near the village of Askenish, draining into West Luvea River, comprised another group. Although genetically very similar, I did not pool animals from the Roaring River with those from Deans Valley River. From the 15 characters measured, all showed normal distribution in the Kolmogorov-Smirnov test. The Wilk`s Lambda for the overall model is 0.015 with $p \leq 0.001$, which indicates a very good discrimination, also shown in the overall correct classification of 71.4%. In Figure 12, a two-dimensional plot of the first two canonical values is shown.

Table 4. Percentage of correct classification based on the morphometric classification function for nine populations of *Sesarma dolphinum*. Overall correct classification of 71.4%.

	predicted group membership								
population	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1 New Savannah R.	75	0	0	8.3	0	0	8.3	8.3	0
2 Galloway R.	0	66.7	33.3	0	0	0	0	0	0
3 Roaring R.	0	26.7	73.3	0	0	0	0	0	0
4 Lucea R.	9.1	0	0	68.2	0	0	18.2	4.5	0
5 upper Cabarita R.	10	0	0	10	40	40	0	0	0
6 Flint R.	0	0	0	0	37.5	62.5	0	0	0
7 Green Island R.	0	0	0	0	0	0	100	0	0
8 Morgan R.	0	0	0	0	0	0	0	100	0
9 Davis R.	0	0	0	0	0	0	0	0	100

These two canonical values explain 85.1% of the variables found in the dataset. In the plot, two separate clusters are clearly visible. One cluster contains only samples from the southeastern distribution range of *Sesarma dolphinum*, namely from the Deans Valley River system and the Roaring River. In the second cluster, the different sampling sites are not as differentiated but certain sites form distinct groups. This picture is reflected in the classification matrix (Table 4). This matrix indicates, what percentage of each site are placed in the correct group according to the discriminant analysis. Although the two southeastern sites do show less than absolute correct placement, the wrongly placed samples can be found in the other group and vice versa. Similar to this, the Flint River site has only 40% correct placement, but another 40% are placed in the upper Cabarita group. Vice versa the 37.5% of the upper Cabarita which are wrongly placed are all found in the Flint River group. This is interesting, because the Flint River group belongs to different

river systems, draining to the northeast, whereas the upper Cabarita drains to the south and is connected to the Roaring River, but the sampling points are geographically very close to the Flint system. The three sites, Green Island River, Davis River and Morgan River, have all correct placement at 100% respectively. They also belong to different drainage systems and are geographically quite close. Overall, the populations show good to very good classification by means of their morphometric characters.

Chapter II: *Epilobocera sinuatifrons* - population genetics

Material and methods

Molecular methods

DNA extraction was performed using a modified Puregene method from Gentra System. For the extraction muscle tissue from the last pereiopod was used. After the extraction the DNA pellet was resuspended in 20 µl of TE buffer and the concentration was ascertained on agarose gel. Dilutions of the DNA solution were made to a final concentration of 1ng/µl of DNA. From this dilutions 1µl was used for polymerase chain reactions. We used three different genetic markers, the mitochondrial cytochrome subunit 1 (CO1), the mitochondrial NADH-Dehydrogenase subunit 1 (ND1) and the nuclear ITS1-5.8S-ITS2 (ITS) region. Not every marker was used with all three species and species complex respectively. The primer used for CO1 were the universal primer CO1472 (Folmer et al.), the COL6b (ACA AAT CAT AAA GAT ATY GG) and COH6 (TAD ACT TCD GGR TGD CCA AAR AAY CA) (Schubart & Huber, 2006) and for ND1, NDL4 (5‘-AAAAGKCTAATTRTTTTGTG-3‘) and NDH2 (5‘-GCTAAATATATWAGCTTATCATA-3‘) which produced a 807 bp ND1 fragment in *E.sinuatifrons*. For the ITS region the ITSL1 (5‘-GGA AGT AAA AGT CGT AAC AAG G-3‘; White et al., 1990) and ITSH1 (5‘-TTC AGT CGC CCT TAC TAA GGG AAT CC-3‘) primer were used. The resulting fragments had a length between 1700 bp and 1800 bp in *E.sinuatifrons* due to several microsatellite-like repeat motives. For PCR a standard 25µl reaction was set up containing 2.5µl of 10x buffer, 2.5µl of 1.25 mM dNTPs, 0.5µl of both primer (20mM), 2µl of 25mM $MgCl_2$, 1µl of 0.5 U/µl TAQ and 15µl of double-distilled water. 40 cycles were applied at an annealing temperature of 48°C for the CO1 and ND1 primer and 50°C for the ITS primer. The CO1 and ND1 PCR product was cleaned using QuickClean (GenScript, Piscataway NJ) and sequenced on an ABI-PRISM 310 (Applied Biosystems, Carlsbad CA). To prepare for cloning the ITS PCR product was treated with an A-Addition kit from Quiagen (Quiagen GmbH, Düsseldorf) to add an A overhang. Then cloning was performed using the TOPO-TA cloning kit from Invitrogen (Invitrogen Corporation, Carlsbad CA). 2µl PCR product was added to a mix of 2µl ddH_2O, 1µl salt solution and 1µl TOPO vector. This mix was incubated for 30 minutes at room

temperature. Chemical competent TOP10 One Shot® *E.coli* cells were thawed on ice and 2μl of the TOPO cloning reaction was added. They were incubated on ice for 30 minutes before heat shocked for 30 seconds at 42°C and immediately returned on ice. 250μl SOC medium was added and reaction tubes were shacked horizontally (200rpm) at 37°C. After 1 hour 25μl of the cells were spread even on prewarmed LB plates containing 50 µg/ml ampicillin. Plates were incubated over night at 37°C. Colonies which had successfully included the vector with the PCR product were picked and transfered to 50μl of ddH_2O. This solution was denaturised for 10 min at 96°C and 1μl was used for a PCR with 35 cycles and 55°C as annealing temperature to check if the correct fragment was cloned. This PCR product was cleaned with PCR Cleanup Millipore plates (Millipore Corporation, Billerica MA) and thereafter cycle-sequenced in both directions using 1/16th of Big Dye v3.0 reaction and standard protocols. The sequencing was performed on an automated ABI 3730 machine.

Computational methods

All sequences obtained were proofread for possible errors made by the computerized analysis provided with the sequencers. We used ChromasLite (http://www.technelysium.com) to read chromatograms and edit possibles errors. The corrected sequences for CO1 and ND1 were aligned completely by eye using Bioedit (Hall, 1999) due to the lack of indels. For the ITS sequences an alignment was created with the ClustelW plugin of Bioedit. Based on this priory alignment the alignment was manually checked as the microsatellite regions were not always correctly recognized from the automated alignment. For further analysis all alignment files were converted to the nexus file format.

The CO1 and ND1 datasets were analyzed using maximum parsimony (MP) and maximum likelihood (ML) optimality criteria. These computations were done with PAUP 4 version beta 10 (Swofford, 1998). For the maximum parsimony analysis gaps were considered as fifth state and all characters were weighted equal. Trees for MP were constructed using heuristic search with TBR branch swapping adding 10 random taxon and 2000 bootstrap pseudo-replications to infer confidence values. To calculate the best fitting model for the maximum likelihood analysis, the program MODELTEST 3.7 (Posada & Crandall, 1998) was used. With ML the search was also heuristic, with 10 random taxon

addition and again 2000 bootstrap pseudoreplicates were used to estimated the phylogenetic confidence of the obtained tree. Finally a 50% majority rule consensus tree was generated. The model of evolution calculated was also used for a Bayesian Inference (BI) analyses. MrBayes v.3.1.2 (Huelsenbeck & Ronquist, 2001) was used with four MCMC chains run for 3,000,000 generations and sampling every 1000 generations. Two independent runs were performed. The posterior probabilities of the phylogeny were determined for the remaining trees after possible random and suboptimal trees were excluded with a burn in phase of 60,000 generations. Finally consensus trees were constructed.

To be able to analyze the ITS dataset some preprocessing was necessary. One part of the informational account of ITS sequences is the higher number of indel events (Simmons, 2000). The indels are created by microsatellite like position in the ITS1 - ITS2 region and the noncoding character of this region. To render the indels utility for tree search methods the simple indel coding method (Simmons, 2000) was used, which was calculated with the program GapCoder (Young, 2003). Further computation was done in MEGA 4 (Tamura et al., 2007). We constructed a Neighbor joining (NJ) tree with the Maximum Composite Likelihood algorithm and 5000 pseudo-replication. A original, a 50% condensed and a radiation tree were generated. For the Bayesian Inference analyses first the model of evolution was defined. Due to the high number of sequences only two MCMC chains were run. Two independent runs for 3,000,000 generations with sampling every 1000 generations, 60,000 generation burn in phase and calculation of consensus trees were performed. For the *Epilobocera sinuatifrons* data the closely related species *Epilobocera haytensis* from the island Hispaniola and *Epilobocera gilmanii* from Cuba were chosen as outgroup.

The nexus file of the CO1 and ND1 dataset was furthermore used to constructed a statistical parsimony network using the algorithm outlined in Templeton et al. 1992 and implemented in the TCS software package (Clement et al., 2001), which is currently available in version 1.21. Based on the obtained haplotype network of the ND1 data a nested clade analysis (NCA) was performed (Templeton et al., 1995; Templeton, 2004) to test the null hypothesis of no association between the geographic distribution of the haplotypes. The haplotype network was converted into a nested statistical design using the instruction given in Templeton and Sing (1993) and in Crandall and Templeton (1996).

To test for an association between the genetic composition and the geographic distribution of the haplotypes, two distances were calculated. First, the clade distance Dc, which estimates how geographically widespread a clade is and second, the nested clade distance Dn, which measures the relative distribution of a clade compared to the other clades in the same higher clade level. All calculations were done in the application GEODIS 2.5 (Posada et al., 2000) using 1,000,000 permutations and direct distances. The direct distances option was favored over river distances as all species in this study are freshwater species with no marine form, which would be necessary to connect certain rivers. Additionally, *Epilobocera sinuatifrons* has considerable terrestrial dispersal capability (Covich, Rivera & local Puerto Ricans, personal observations). The direct distances between the single sample locations were measured in GoogleEarth (http://earth.google.com). To infer the historical events that caused the observed genetic population structure we used the most recent inference key (November 2005) from Templeton (http://darwin.uvigo.es/software/geodis.html).

The ITS data set was also analyzed using a network method. Again the preprocessed data obtained with GapCoder was utilized. The greater amount of variation within the ITS data set did not allow to used the statistical parsimony algorithm of the TCS software package to calculated a network. Therefore, the software Splitstree version 4 was used (Huson, 1998). This software package allows the computation of all kinds of evolutionary networks. We used it to construct minimum spanning networks of the gapcoded ITS sequence data. To measure the genetic differentiation between the populations F_{ST} values were calculated using analysis of molecular variance (AMOVA) in Arlequin ver. 3.0 (Excoffier et al., 2005)

Results

Cytochrome oxidase subunit 1

With the first mitochondrial marker, the cytochrome subunit 1, I was able to amplify a 624 basepair long fragment. When preliminary data from this marker were analyzed, some inconsistencies were recognized. A small part of the sequences showed low genetic differences, but the majority was all equal. This was even true for individuals which came

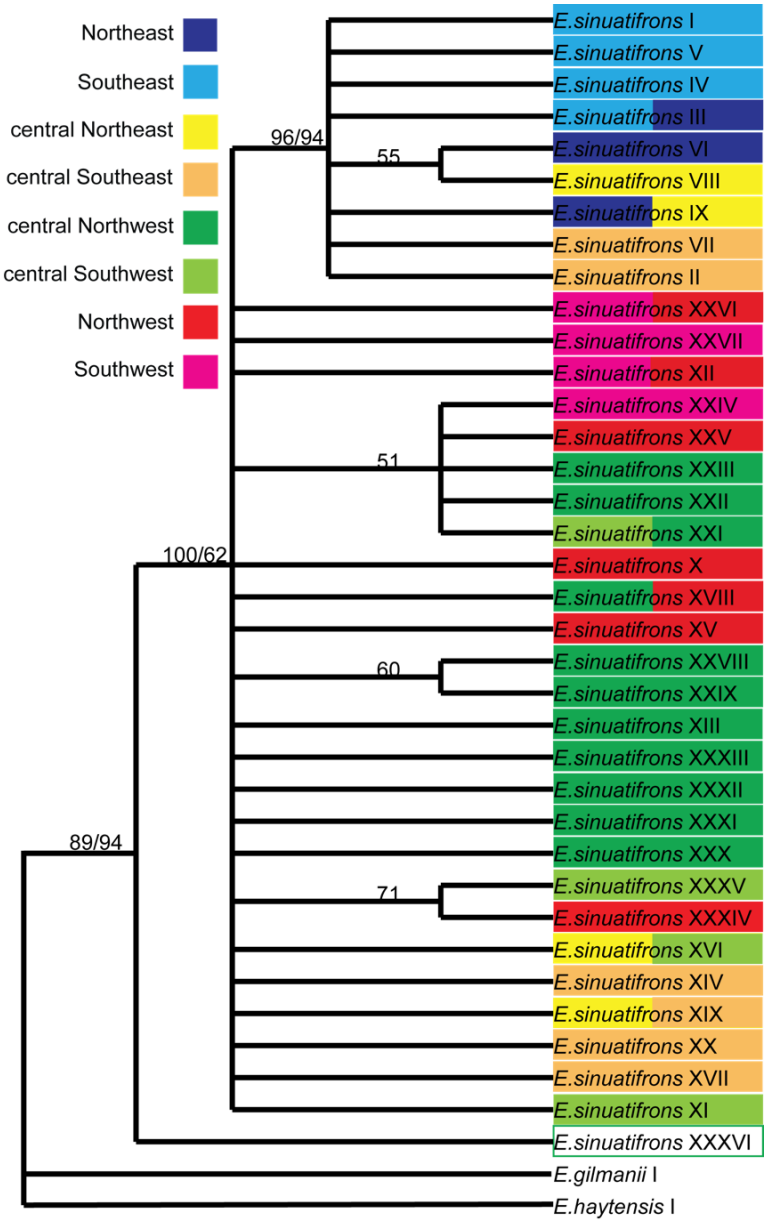


Figure 13. Bootstrap 50% majority-rule consensus tree of phylogenetic relationships within the species *Epilobocera sinuatifrons* with *E.gilmanii* and *E.haytensis* as outgroup. Maximum parsimony and maximum likelihood (with F81+I+G model of evolution) topologies. Confidence values from 2000 bootstrap replicates (MP/ML) based on 807 basepairs of the ND1 mitochondrial gene; only bootstrap values above 50 are shown. Coloration of haplotypes according to map from Figure 8.

from distant sampling sites and already showed genetic variability in the preliminary NADH subunit 1 data. Additionally in some sequences doublepeaks were regonized. No evidence for possible contamination could be determined. Different primer combinations for the cytochrome subunit 1 region rendered similar results as the original primers. Based on these results we suggested the presence of pseudogenes (Gusmão et al., 2000). As there are no absolutely reliable methods to eliminate possible interference of pseudogenes and I had already promising results from the ND1 without any indication of pseudogenes, I concentrated my efforts on the second mitochondrial marker.

NADH subunit 1

From 55 individuals, including the two outgroup species, 807 basepairs of the NADH subunit1 gene were amplified. Among those we found 36 different haplotypes and the ND1 sequence dataset consisted of 204 variable sites of which 76 were parsimony informative. Using the Akaike information criterion (Akaike, 1974) MODELTEST selected the F81+I+G as best fitting model for the maximum likelihood analysis (-Ln=2334.95) with the following

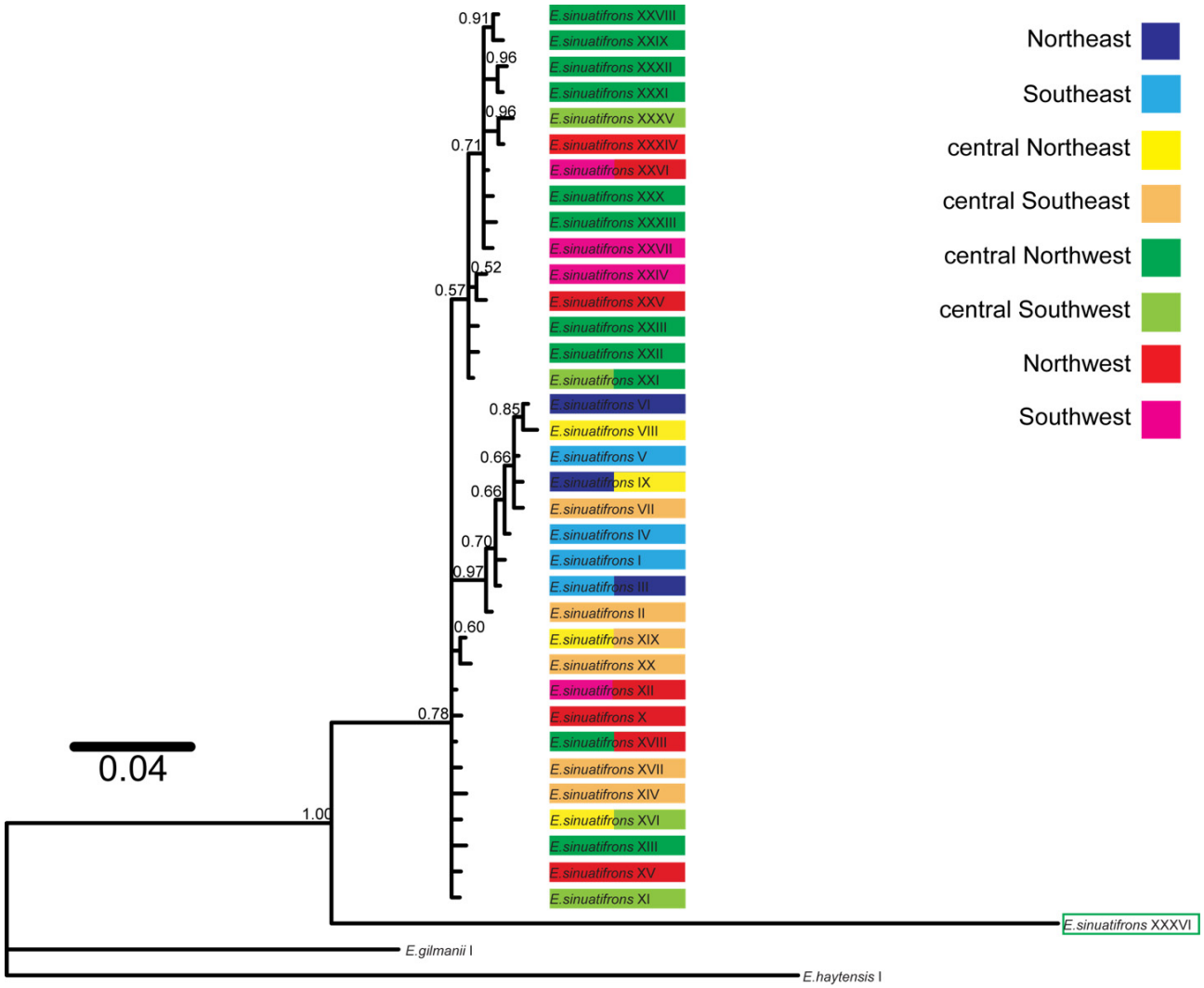


Figure 14. Bayesian inference tree of phylogenetic relationships within the species *Epilobocera sinuatifrons* with *E.gilmanii* and *E.haytensis* as outgroup. Bayesian inference topology with F81+I+G model of evolution. Posterior probabilities based on 807 basepairs of the ND1 mitochondrial gene; only values above 0.50 are shown. Coloration of haplotypes according to map from Figure 8.

base frequencies; A=0.3018, C=0.0974, G=0.1576, T=0.4431. It revealed a proportion of invariable sites of 0.596 and a gamma distribution shape parameter of 1.99. The maximum parsimony analysis resulted in one most parsimonious tree with the same tree topology as the maximum likelihood tree. The combined 50% consensus tree for both methods is shown in Figure 13. The Bayesian Inference tree is shown in Figure 14. The combined MP and ML tree shows a very homologous picture at the dichotomy level. There are only two visible graduations. First, haplotypes I to IX form a distinct clade that is discrete from the rest of the tree with high bootstrap support. All individuals in which these haplotypes were found were collected in the eastern part of Puerto Rico at sampling points 1 to 6, 21 to 23 and 25. Second, the haplotype XXXVI, which was only found in one individual, is

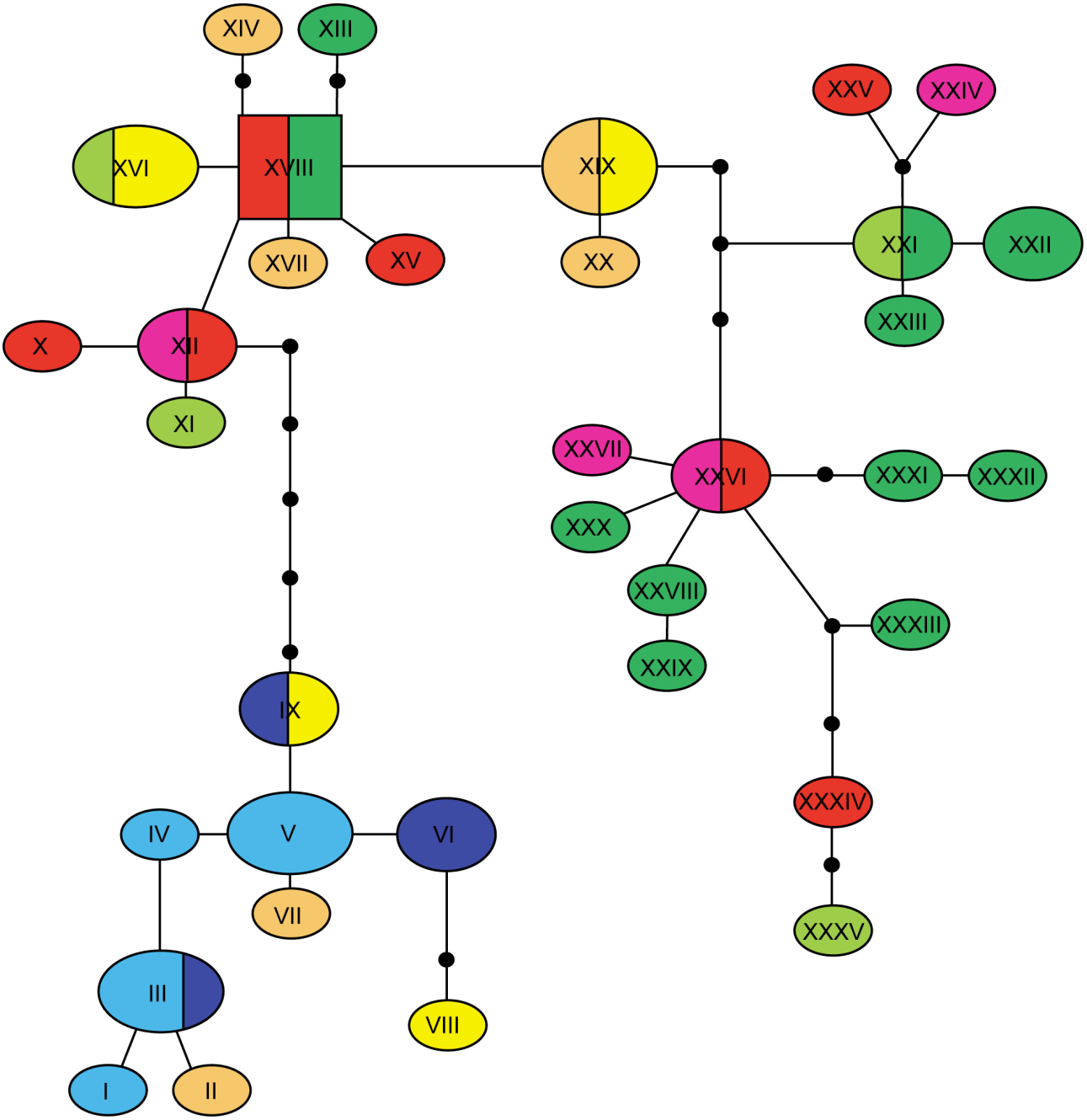


Figure 15. Statistical parsimony network constructed with TCS of *Epilobocera sinuatifrons* (N=55) from a 807-basepair fragment of the ND1 gene. Each line represents one substitution; dots on the lines indicate additional substitutions separating two haplotypes. Coloration according to the sample sites in Figure 8. The size of the circle is representative for the frequency of the haplotypes (small: N=1; medium: N=2–3, square: ancestral haplotype).

basal to and quite distinct from all the other haplotypes of *Epilobocera sinuatifrons*, but still separated from the two outgroup species. This juvenile specimen was found during the preliminary sampling in 1997 in a small pool of water at Monte Guilarte. The distribution of haplotypes in the Bayesian Inference tree is similar to the one in the combined MP and

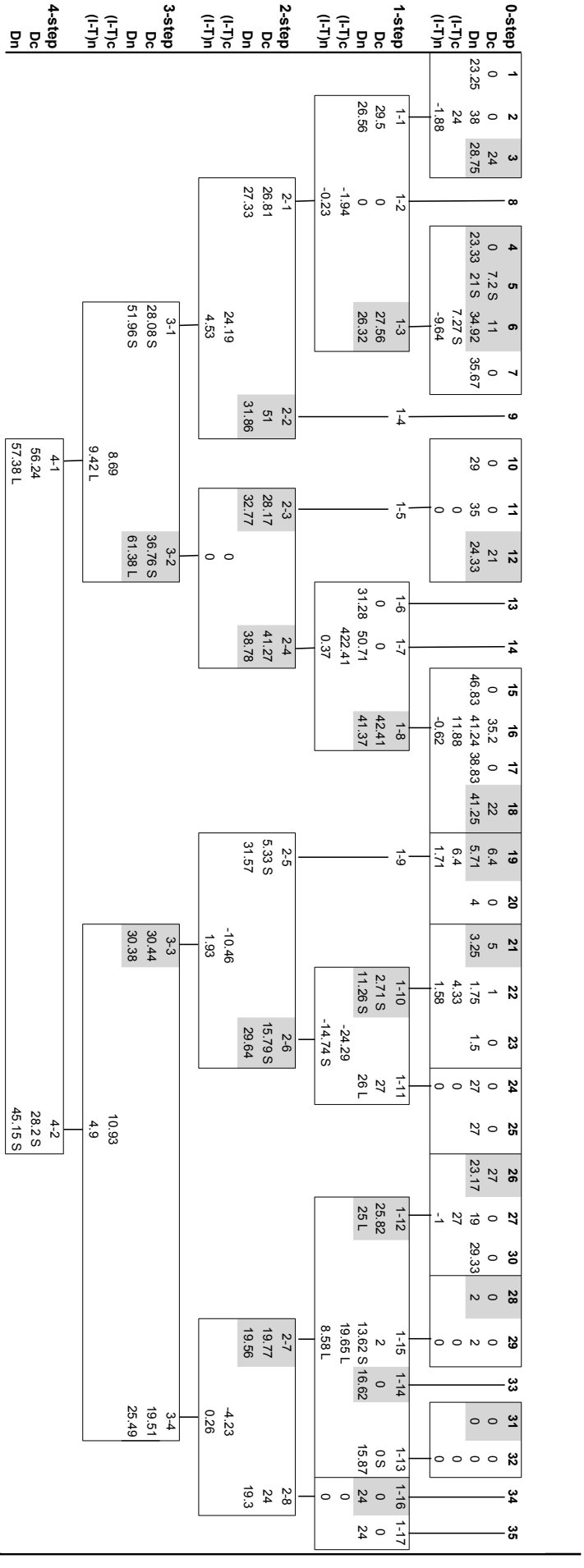


Table 5. Nested clade distance analysis of ND1 haplotypes observed in *Epilobocera sinuatifrons*. The nested design is given in Figure 16 D_c and D_n are clade and nested clade distances, respectively (for details see Templeton et al, 1995). Interior vs. tip contrasts for D_c and D_n are indicated with 'I-T' in the corresponding clade. Interior clades are identified by shading. The S and L superscripts refer to significantly small and large values at the 0.05 level, respectively. Significance of values is based on permutation analysis using 1,000,000 resamples.

ML tree. In addition it shows one clade with Bayesian posterior probabilities higher 0.5, containing haplotypes XXI to XXXIII, which are all from the western part of the island.

All 55 sequences obtained for the NADH subunit 1 dataset were also used for the statistical parsimony network. From the 36 haplotypes of *Epilobocera sinuatifrons* only the haplotype XXXVI, corresponding to the juvenile specimen from Monte Guilarte, and the two outgroups could not be connected to the network using a 95% confidence interval (Figure 15). Haplotype XVIII was selected as ancestral state by the software due to its central position in the network (high number of directly connected haplotypes). Together with the haplotypes X to XX it most likely forms the ancestral group. These haplotypes were found in individuals from all over the island with only specimen from the easternmost locations missing. Accordingly in this ancestral group, individuals from the western and central-western collection points were most prominent. From this ancestral group, two other groups branched off. The first, which consists of the haplotypes I to IX, is connected to the ancestral haplotype XVIII via five missing steps. Only individuals collected in the eastern parts Puerto Rico show these haplotypes. It is dominated by individuals from the two collection areas from the east coast of the island. The second group is subdivided into two separate groups, but the distance to the main group is shorter (2-3 missing haplotypes). A smaller subgroup consisting of the haplotypes XXI to XXV is found mainly in specimen from the western and the central-western part. The other subgroup consists of haplotype XXVI to XXXV. A large percentage of individuals from the central collection sites shows haplotypes found in this group, but again specimen from all western collection areas are found therein. In general, no clear separation of individuals from north or south drainages is recognizable. Like in the morphometric data, again a different gradient from east to west is clearly recognizable, but the separation between the single groups is more visible. To further examine how this genetic pattern is

Table 6. Nested Contingency Results for *Epilobocera sinuatifrons* (ND1) based on 1,000,000 permutations in GeoDis. Inferences were made using Templetons (2005) revised key.

Clade	Inference chain	Inferred pattern
1-3	2-11-12-NO	Contiguous Range Expansion
2-6	19-20-2-11-12-NO	Contiguous Range Expansion
2-7	2-3-4-NO	Restricted Gene Flow with Isolation by Distance
3-3	19-20-2-11-12-NO	Contiguous Range Expansion
4-1	2-11-12-NO	Contiguous Range Expansion
total	2-11-17-4-NO	Restricted Gene Flow with Isolation by Distance

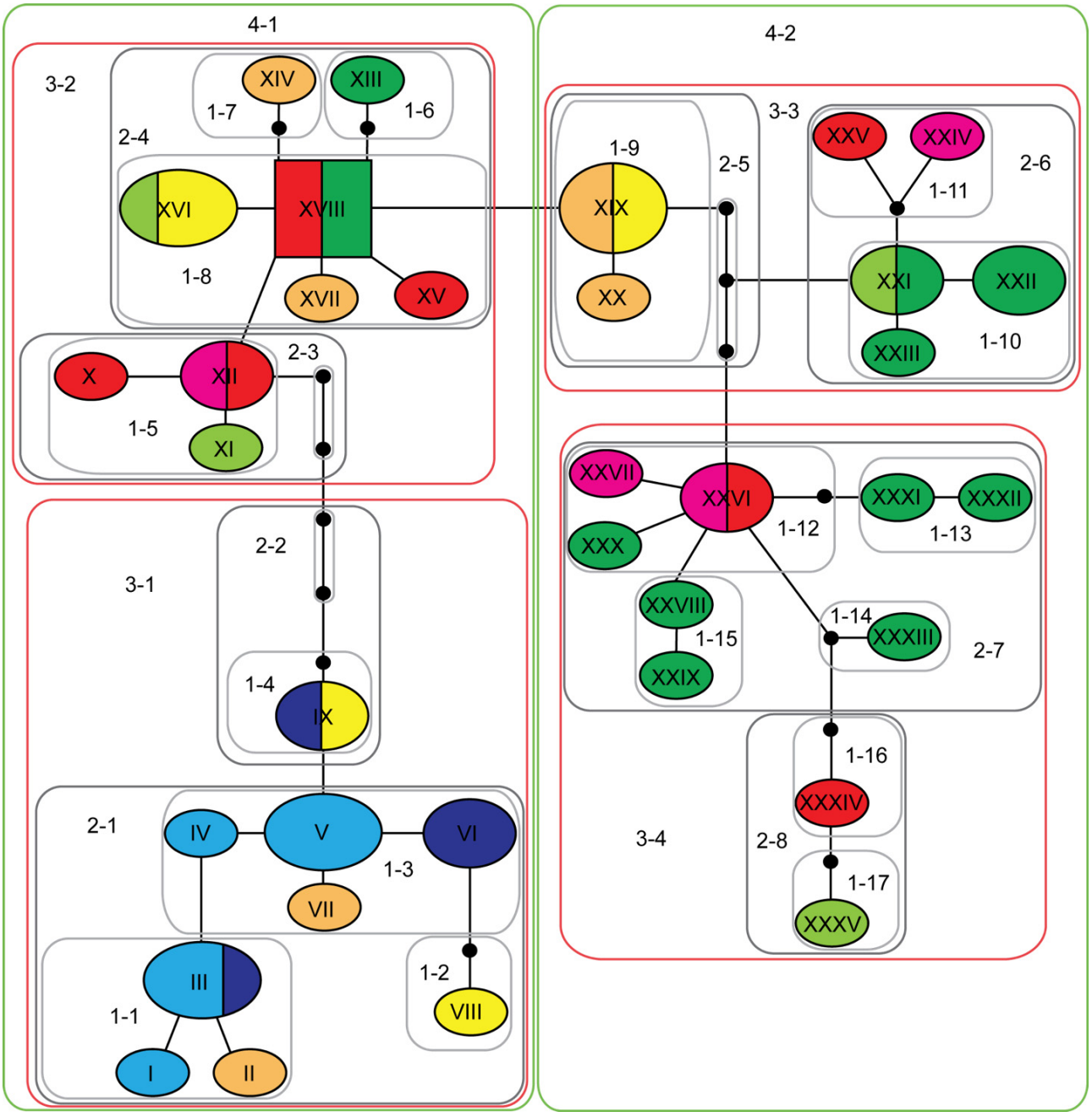


Figure 16. Statistical parsimony network constructed with TCS of *Epilobocera sinuatifrons* (N=55) from a 807-basepair fragment of the ND1 gene and the corresponding nesting design for the Nested Clade Analysis. Each line represents one substitution; dots on the lines indicate additional substitutions separating two haplotypes. Coloration according to the sample sites in Figure 8. The size of the circle is representative for the frequency of the haplotypes (small: N=1; medium: N=2–3, square: ancestral haplotype). Light grey lines enclose the 1-step clades (1-n), dark grey lines enclose 2-step clades (2-n), red lines enclose 3-step clades (3-n) and green lines enclose 4-step clades (4-1 and 4-2).

associated with the geographic distribution of the haplotypes and to conclude which historical events have lead to this situation we performed a nested clade analysis. The NCA grouped the 35 0-level clades into 17 1-level clades. The 1-level clades resulted in

eight 2-level clades, four 3-level clades and two 4-level clades, which completed the total cladogram. The complete nesting design is shown in Figure 16 and Table 5 states the within-clade and nested-clade distances. Only 22 out of these clades showed genetic and geographic differences and seven of those had significant low or high within-clade or nested-clade distances or interior to tip within-clade or nested-clade distances. The lowest level clade with such significant values is clade 1-3 from the south eastern end of the island, of which inference resulted in a contiguous range expansion. The same historical

Table 7. Pairwise F_{st} values for all regional population groups of *Epilobocera sinuatifrons* based on haplotype frequencies of the ITS1-5.8S-ITS2 dataset. Significant levels are shown in lower left part of the matrix (calculated from 10,000 permutations).

n=clones k=individuals	Northeast (n=26)	Southeast (n=20)	central Northeast (n=51)	central Southeast (n=11)	central Northwest (n=64)	central Southwest (n=14)	Northwest (n=32)	Southwest (n=20)
Northeast (k=5)		0.04791	0.1457	0.05604	0.29893	0.14284	0.2483	0.24963
Southeast (k=5)	p<0.05		0.20209	0.11553	0.36262	0.19359	0.3164	0.30637
central Northeast (k=4)	p<0.0001	p<0.0001		0.06758	0.12528	0.05943	0.08537	0.09546
central Southeast (k=3)	p<0.05	p<0.05	p<0.0001		0.18947	0.07233	0.1394	0.14952
central Northwest (k=11)	p<0.0001	p<0.0001	p<0.0001	p<0.0001		0.11075	0.03316	0.05676
central Southwest (k=2)	p<0.01	p<0.01	p<0.01	p<0.05	p<0.0001		0.04794	0.07015
Northwest (k=6)	p<0.0001	p<0.0001	p<0.0001	p<0.01	p<0.01	p<0.01		0.02573
Southwest (k=5)	p<0.0001	p<0.0001	p<0.0001	p<0.01	p<0.01	p<0.01	p<0.05	

event explains also the genetic and geographic composition of clade 2-3, which includes 3 haplotypes, one from the southwestern, one from the southern drainage system of the central-western area and a haplotype which is shared by individuals from both western collection areas. Clade 3-3 with haplotyes from all central and the western areas and clade 4-1, which includes the east coast group and the ancestral group, were also interpreted as contiguous range expansion. For clade 2-7, restricted gene flow with isolation by distance

is the most likely historical process. In this clade, only haplotypes from the Monte Guilarte area with north drainage and western specimen are found. The same process is also responsible for the total cladogram composition. It seems that genetic exchange is common within areas and is not restricted to drainage systems, but this does not expand to the whole island. Especially the eastern part of the island is genetically well separated.

ITS1-5.8S-ITS2

From 40 individuals of *Epilobocera sinuatifrons* we amplified the ITS1-5.8S-ITS2 complex with an average basepair number of around 1620. In total, we obtained 238 clones, whereby the number of clones per individuals varied between one and seventeen. After the incorporation of all indel positions in the dataset via simple search method, the number of aligned sites increased to 1765. In total, we found 236 alleles with 729 variable sites, of which 258 were parsimonious informative. This implies that in most cases more than two alleles were present in our sequence data. The constructed neighbor joining trees are shown in Figure 17 (original and 50% condensed) and Figure 18 (radiation tree). Figure 19 shows the phylogeny estimated by Bayesian Inference. In Figure 20 the minimum spanning network constructed from this data is displayed. Overall, the ITS dataset does not give a clear picture of geographic distribution of alleles but certain trends already seen in the morphometric and mitochondrial data can be confirmed. The Bayesian Inference tree does not show any differentiation but in the neighbor joining trees clones from individuals from the eastern part of the island are concentrated on the top part of the original and condensed tree and accordingly on the top branch of the radiation tree. They form no separate clade but most other clones clustering with them are from adjacent geographic areas. Also a considerable amount of clones from individuals from Monte Guilarte clustered together in a basal clade. Again, this clade does not consist of geographically clearly allocated alleles, but with one exception the other clones are again from bordering sampling points.

In the minimum spanning networks three major groups can be recognized. A western, an eastern, and a central group respectively including alleles from the neighboring regions, and standing in a triangular relation to each other. The trend of restricted gene flow between the eastern part of Puerto Rico and the rest of the island is further supported by

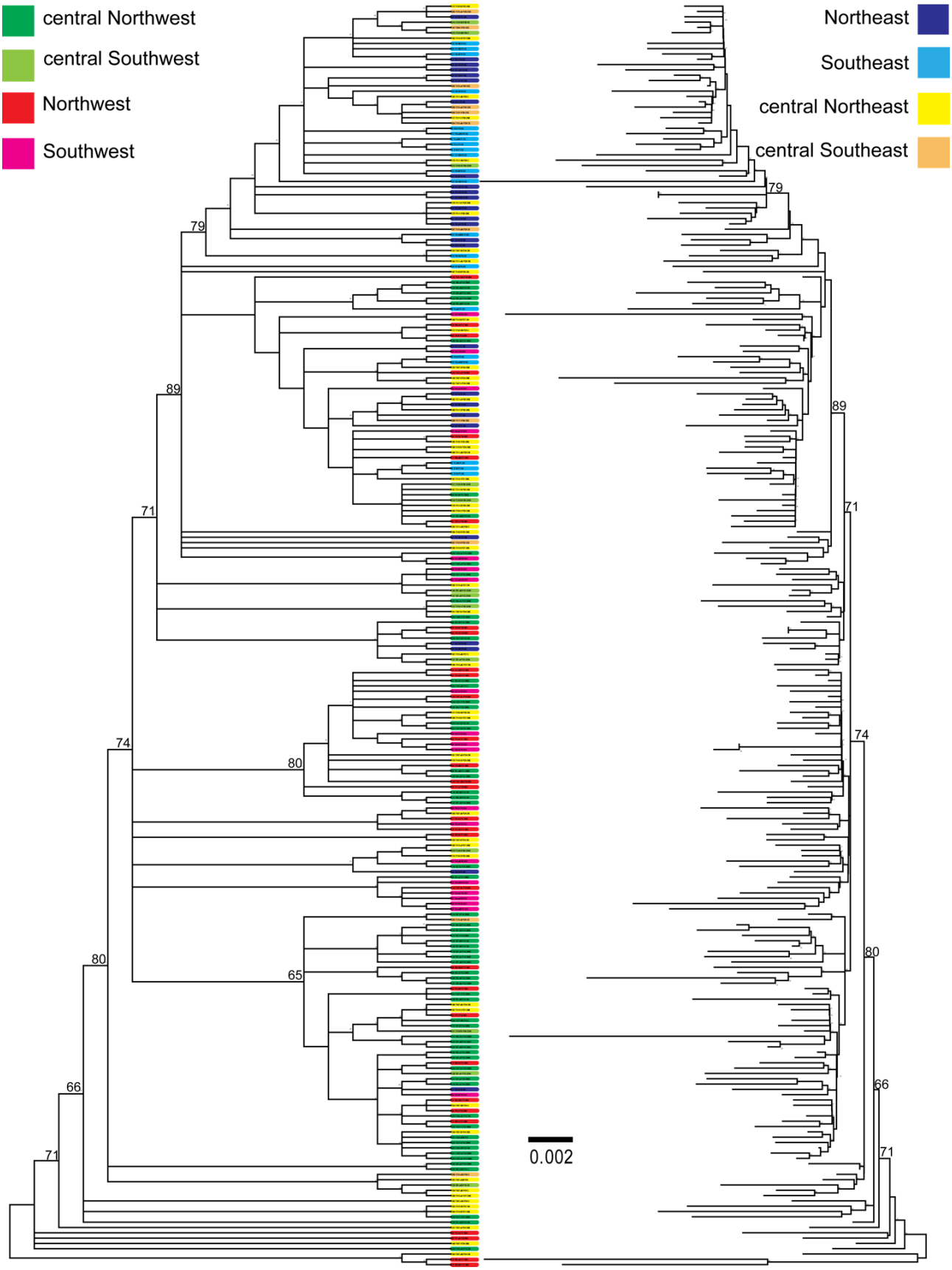


Figure 17. Original and 50% condensed neighbor joining tree of phylogenetic relationships within the species *Epilobocera sinuatifrons*. Confidence values from 5000 bootstrap replicates (Maximum Composite Likelihood algorithm) based on 1765 gapcoded basepairs of the ITS1-5.8S-ITS2 nuclear complex; only bootstrap values above 50 are shown. Coloration of haplotypes according to map from Figure 8.

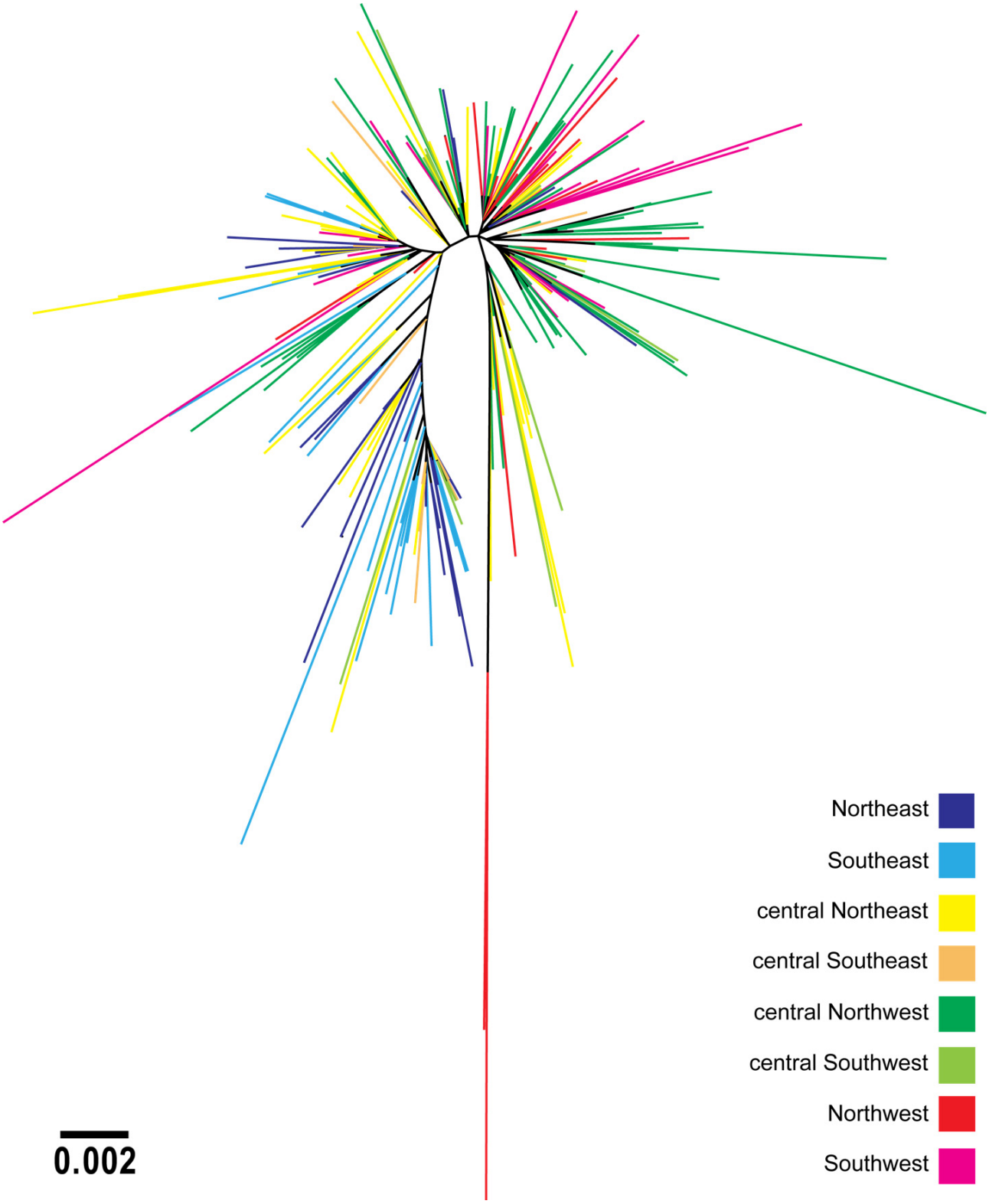


Figure 18. Radiation neighbor joining tree of phylogenetic relationships within the species *Epilobocera sinuatifrons* based on 1765 gapcoded basepairs of the ITS1-5.8S-ITS2 nuclear complex; only bootstrap values above 50 are shown. Coloration of haplotypes according to map from Figure 8.

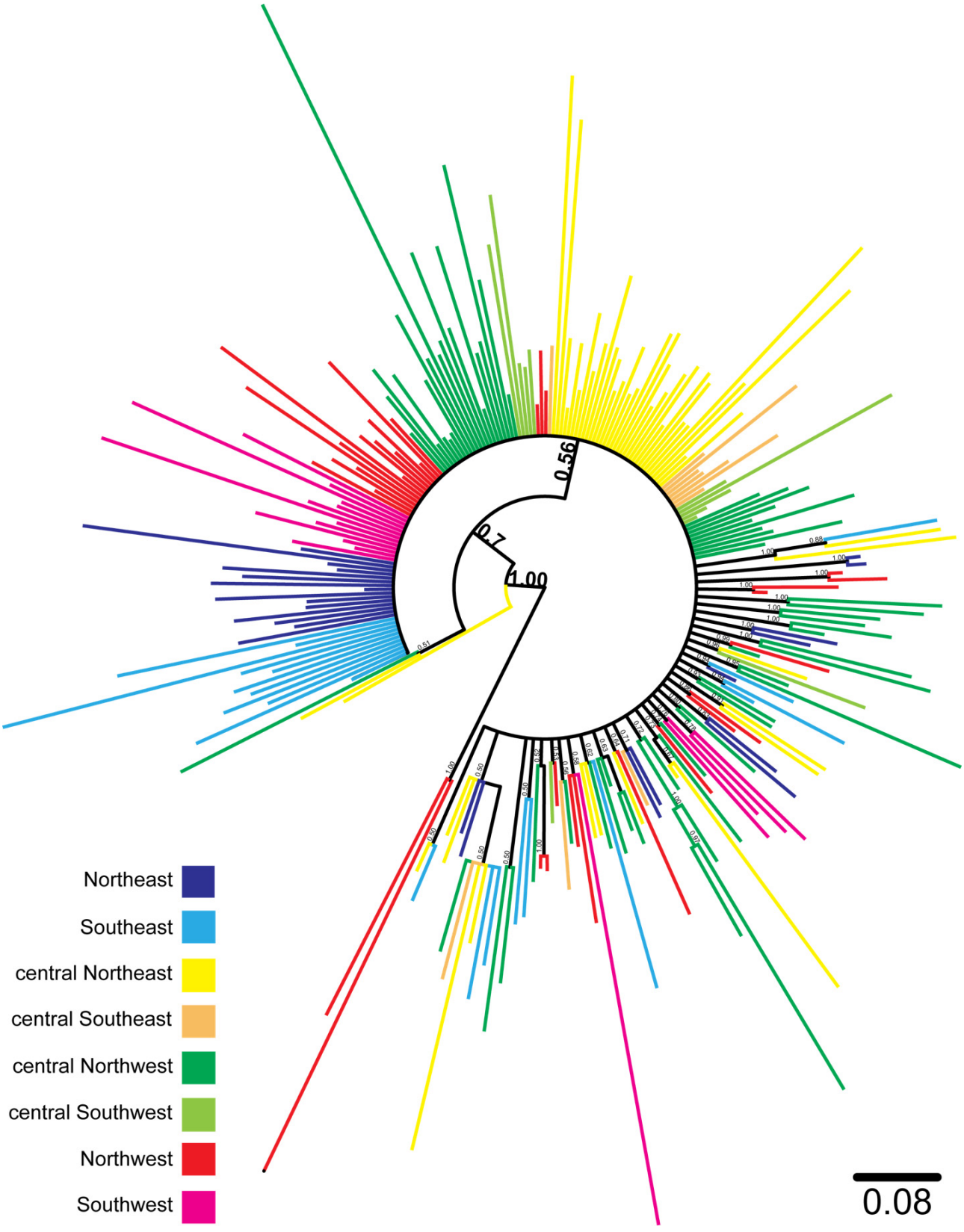


Figure 19. Bayesian inference tree of phylogenetic relationships within the species Epilobocera sinuatifrons. Bayesian inference topology with F81+I+G model of evolution. Posterior probabilities based on 1765 gapcoded basepairs of the ITS1-5.8S-ITS2 nuclear complex; only values above 0.50 are shown. Coloration of haplotypes according to map from Figure 8

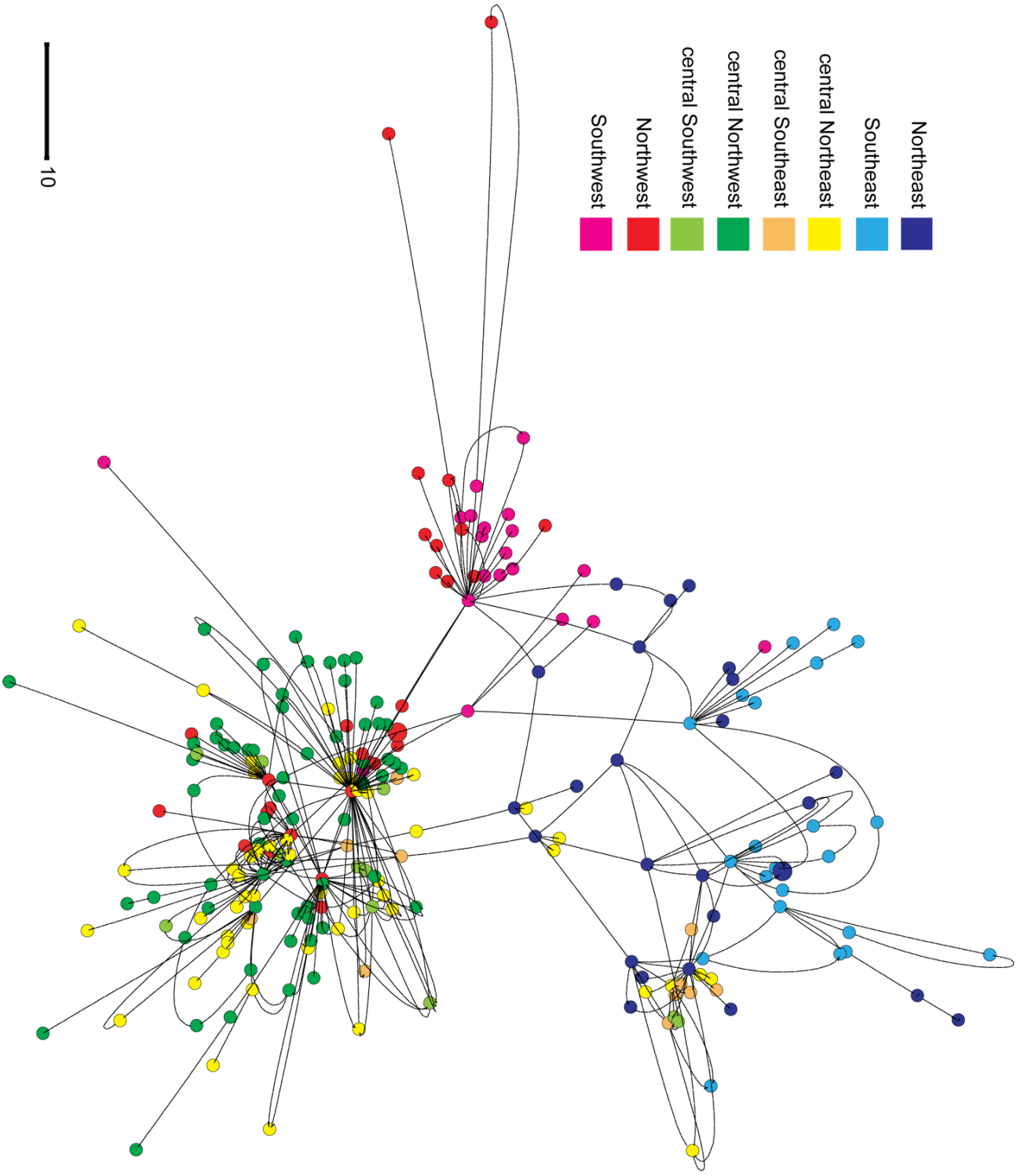


Figure 20. Minimum spanning network based on 1765 gapcoded basepairs of the ITS1-5.8S-ITS2 nuclear complex of the species *Epilobocera sinuatifrons*. Coloration of haplotypes according to map from Figure 8.

the F_{st} values from the AMOVA (Table 7), as only the two eastern regions show higher values.

Chapter III: *Sesarma* - population genetics

Material and methods

The molecular and computational methods described in chapter II: *Epilobocera sinuatifrons* - population genetics, were also applied for the *Sesarma* datasets.

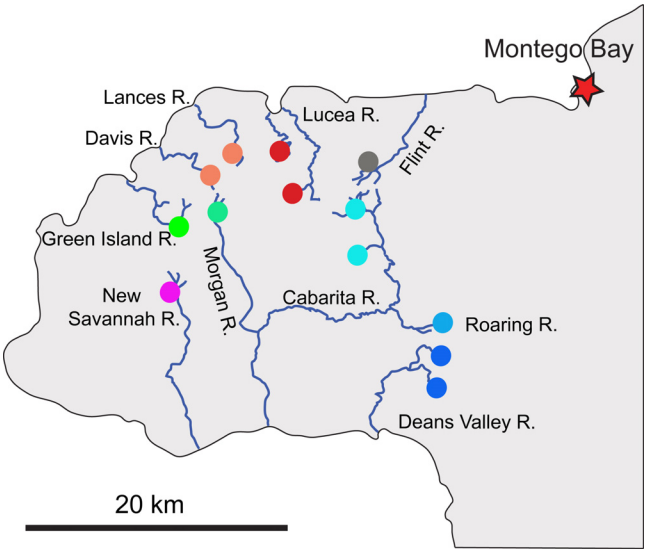


Figure 21. Map of western Jamaica showing selected rivers and sampling sites where species of *Sesarma dolphinum* were collected.

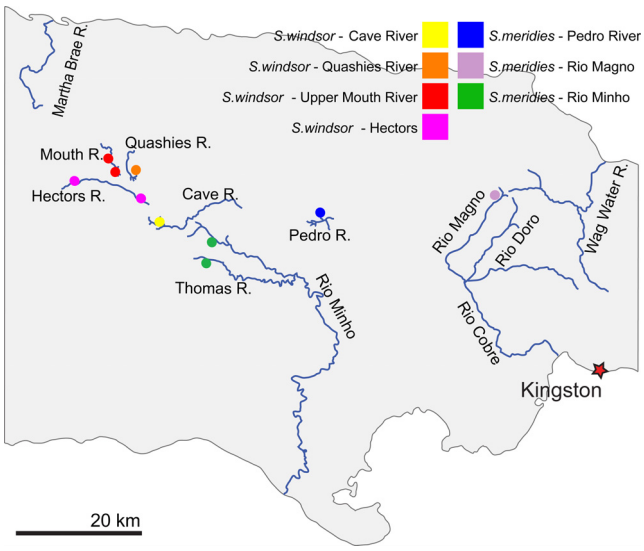


Figure 22. Map of central Jamaica showing selected rivers and sampling sites where species of *Sesarma windsor* and *Sesarma meridies* were collected.

Results

Cytochrome oxidase subunit 1

The presence of pseudogenes was suggested for the first mitochondrial marker, the cytochrome subunit 1, in *Epilobocera sinuatifrons* and it was therefore not used any further. That being the case I also concentrated my efforts on the second mitochondrial marker for the *Sesarma* dataset.

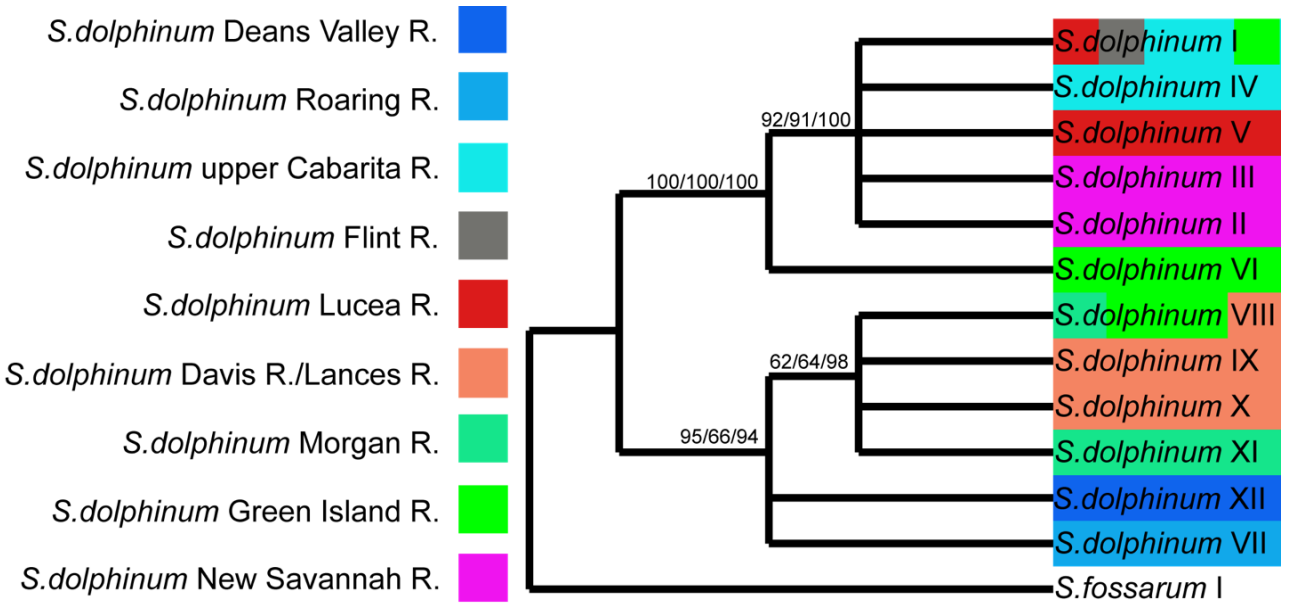


Figure 23. Bootstrap 50% majority-rule consensus tree of phylogenetic relationships within the species *Sesarma dolphinum* with *Sesarma fossarum* as outgroup. Maximum parsimony, minimum evolution and Bayesian inference (with GTR+I model of evolution) topologies. Confidence values from 2000 bootstrap replicates (MP/ME) based on 842 basepairs of the ND1 mitochondrial gene; only bootstrap values above 50 are shown. Coloration of haplotypes according to map from Figure 21.

Sesarma dolphinum

NADH subunit 1

For the NADH subunit 1 dataset a fragment of 842 bp was successfully sequenced. I obtained 12 different haplotypes from 37 individuals, not including the outgroup. The maximum parsimony analysis revealed the following results: overall, 56 positions were variable and 17 characters were parsimony informative, resulting in one most parsimonious tree with a length of 58 steps, a RI of 0.987 and a CI of 0.983. Modeltest suggested the model GTR+I as best fitting one (-Ln = 1404.15) using the Akaike information criterion. Base frequencies were A=0.2870, C=0.0855, G=0.1795 and T=0.4480 and the proportion of invariable sites was 0.4153. The maximum parsimony, the maximum likelihood and the Bayesian inference analyses all resulting in the same tree topology (Figure 23). The 50% consensus trees show two separated clades, which are supported by high confidence values and Bayesian posterior probabilities. The haplotypes which are comprised in clade-I can all be found in individuals of *Sesarma dolphinum* from the northern part of its distribution range, ranging from the Flint River and upper Cabarita River over Lucea rivers and Lances River to the Green Island and Flamstead River. The clade also includes two haplotypes from the New Savannah River (H4, H5). Haplotypes

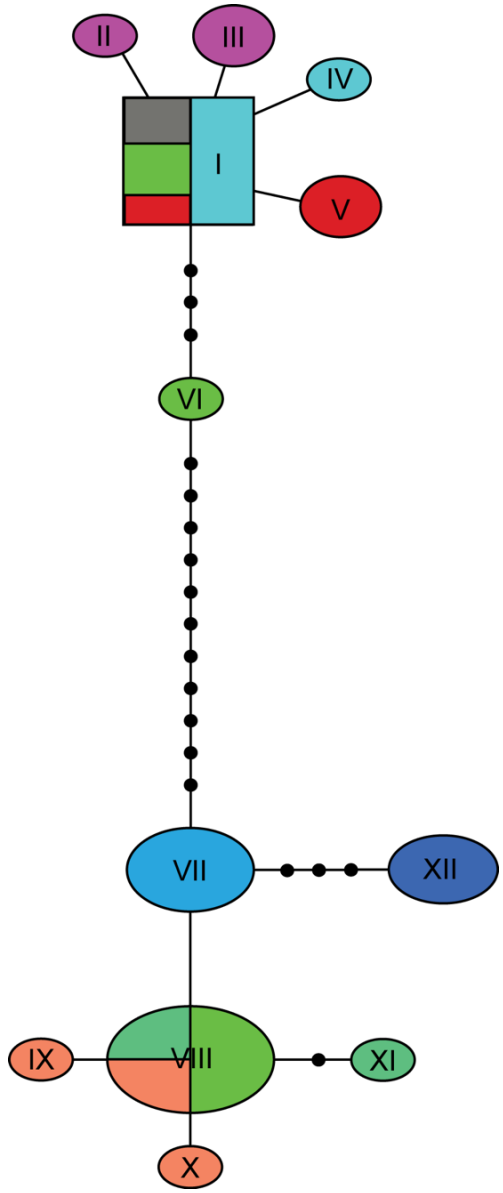


Figure 24. Statistical parsimony network constructed with TCS of *Sesarma dolphinum* (N=37) from a 842-basepair fragment of the ND1 gene. Each line represents one substitution; dots on the lines indicate additional substitutions separating two haplotypes. Coloration according to the sample sites in Figure 21. The size of the circle is representative for the frequency of the haplotypes (small: N=1; medium: N=2–3; large: N=4-5; huge: N>10; square: ancestral haplotype).

found in clade-II can be found in samples of two different origins. They are either from areas not covered in clade-I, mainly the Deans Valley (H12) and the Roaring (H7) Rivers, or the samples are from the northwest, namely the Green Island, Flamstead and Lances Rivers. This second group therefore overlaps geographically with clade-I.

The statistical parsimony network (Figure 24) calculated for the NADH subunit 1 dataset displays a similar picture as the trees, but in a more detailed way. The length of the fragment (842 basepairs) was the same but 40 individuals were used, which did not increase the number of unique haplotypes (12). The outgroup could not be connected to the network when we applied a 95% confidence interval. Again, two major groups can be identified, but the relationship between the different haplotypes is better resolved. TCS suggests haplotype H1 as the ancestral haplotype. This haplotype can be found in samples from the Flint River system, the Lucea rivers and the upper Cabarita River. Also two individuals from the Green Island River carried this haplotype. The four haplotypes which differ from the ancestral haplotype H1 by only one or two substitutions are the haplotypes H2 to H5. Haplotypes H4 and H5 can only be found in samples from the New Savannah River system, haplotype H3 is only present in the Lucea rivers and haplotype H2 was shown by one sample from the upper Cabarita River. Haplotype H6 encountered in one individual from the Green

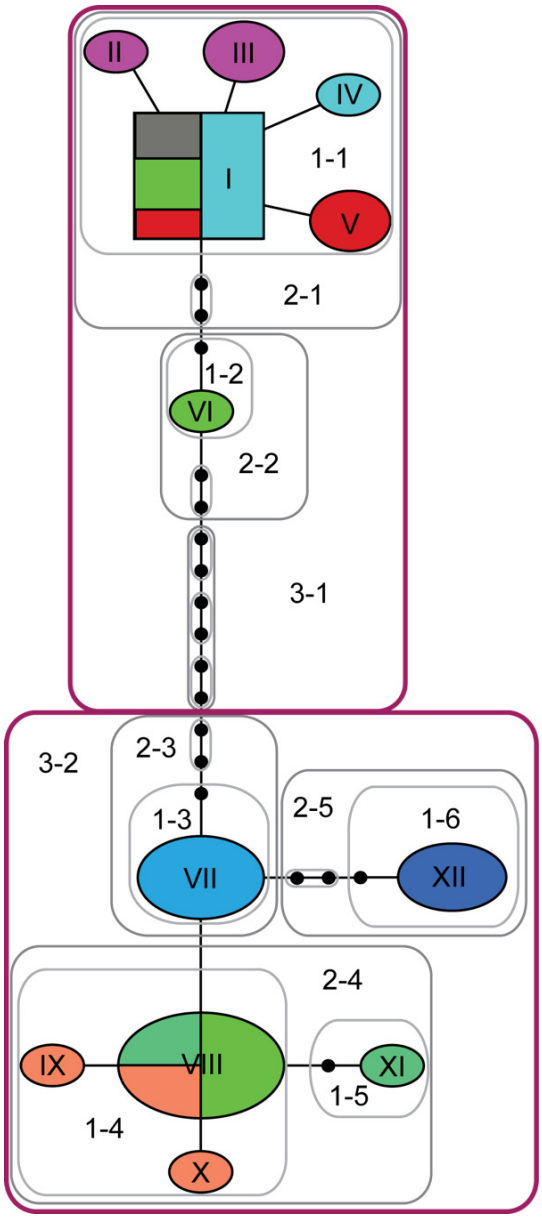
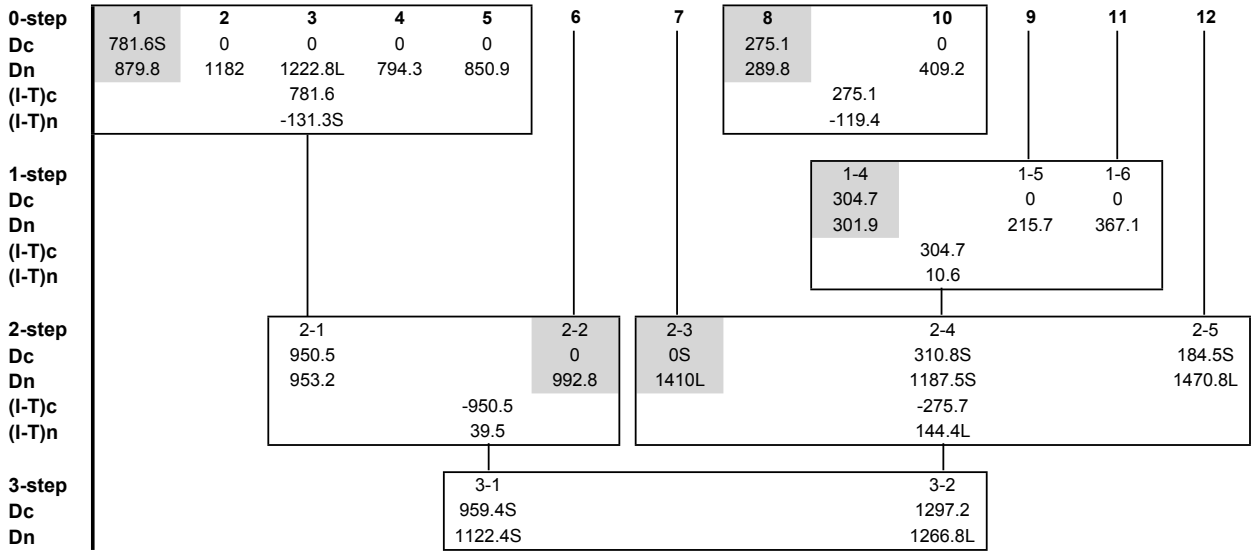


Figure 25. Statistical parsimony network constructed with TCS of *Sesarma dolphinum* (N=37) from a 842-basepair fragment of the ND1 gene and the corresponding nesting design for the Nested Clade Analysis. Each line represents one substitution; dots on the lines indicate additional substitutions separating two haplotypes. Coloration according to the sample sites in Figure 21. The size of the circle is representative for the frequency of the haplotypes (small: N=1; medium: N=2–3; large: N=4-5; huge: N>10; square: ancestral haplotype). Light grey lines enclose the 1-step clades (1-n), dark grey lines enclose 2-step clades (2-n) and red lines enclose 3-step clades (3-1 and 3-2)

Island River, differs from the first major group by four substitutions and from the second major group by 12. In the second major group, haplotype H8 has the highest frequency with 25 percent of all sequenced individuals belonging to this haplotype. It contains samples from the Green Island River, the Morgan River and the Lances River. Also the three of the four haplotypes which are connected to haplotype H8 by either one or two substitutions belonging to individuals collected in these river systems. These are haplotype H9 from the Lances River and haplotypes H10 and H11 from Morgan River. All these river systems are from the northwestern part of the study area. The fourth haplotype which is connected to H8 by one substitution, H7, was only found in the five samples from the Roaring River. The Roaring River is located in the southeastern distribution area of *Sesarma dolphinum*. The Deans Valley River lies even further southeast and marks the border between *Sesarma dolphinum* and *Sesarma fossarum*. The six samples from that river carry this own haplotype H12. From the closest haplotype H7 they are separated by four substitutions and therefore can be seen as a third group of the network.

This network displays the present state of the genetic relations between the different populations of *Sesarma dolphinum*. However, it does not tell us how these genetic relationships originated. To investigate such a

Table 8. Nested clade distance analysis of ND1 haplotypes observed in *Sesarma dolphinum*. The nested design is given in Figure 25. D_c and D_n are clade and nested clade distances, respectively (for details see Templeton et al, 1995). Interior vs. tip contrasts for D_c and D_n are indicated with 'I-T' in the corresponding clade. Interior clades are identified by shading. The S and L superscripts refer to significantly small and large values at the 0.05 level, respectively. Significance of values is based on permutation analysis using 1,000,000 resamples.



question a nested clade analysis was performed. The twelve haplotypes from the statistical parsimony network built the 0-level clades. Based on these 0-level clades, seven 1-level clades, five 2-level clades and two 3-level clades were constructed, which formed the total cladogram. At the 1-level, two clades show geographic association, but only the clade 1-1 has significant values for the within-and nested-clade distances and the interior to tip within- and nested-clade distances. The analysis of clade 1-1 with the inference key resulted in an inconclusive outcome. From the 2-level clades, only one has geographic associations, but does not show any significant values. At the 3-level, again two clades have geographic associations, whereas clade 3-1 displayed significant values. Analyzing these values with the inference key, gives three

Table 9. Nested Contingency Results for *Sesarma dolphinum* (ND1) based on 1,000,000 permutations in GeoDis. Inferences were made using Templetons (2005) revised key.

Clade	Inference chain	Inferred pattern
1-1	2-11-17-NO	Inconclusive Outcome
3-2	19-20-2-11-12-13-14-NO	Long Distance Colonisation and/or Past Fragmentation
total	2-3-4-NO	Restricted Gene Flow with Isolation by Distance

scenarios how the present state could come into place: either by past fragmentation, or by long distance colonization, or by a combination of these two possibilities. The total cladogram also infer geographic associations and the analysis produced significant values for the within- and nested-clade distances. This time, the inference key suggested one outcome: the present state of the *Sesarma dolphinum* populations under research is the result of a restricted gene flow with isolation by distance. The nesting design is shown in Figure 25, the analysis of the geographic associations in Table 8 and the results of the inference key in Table 9.

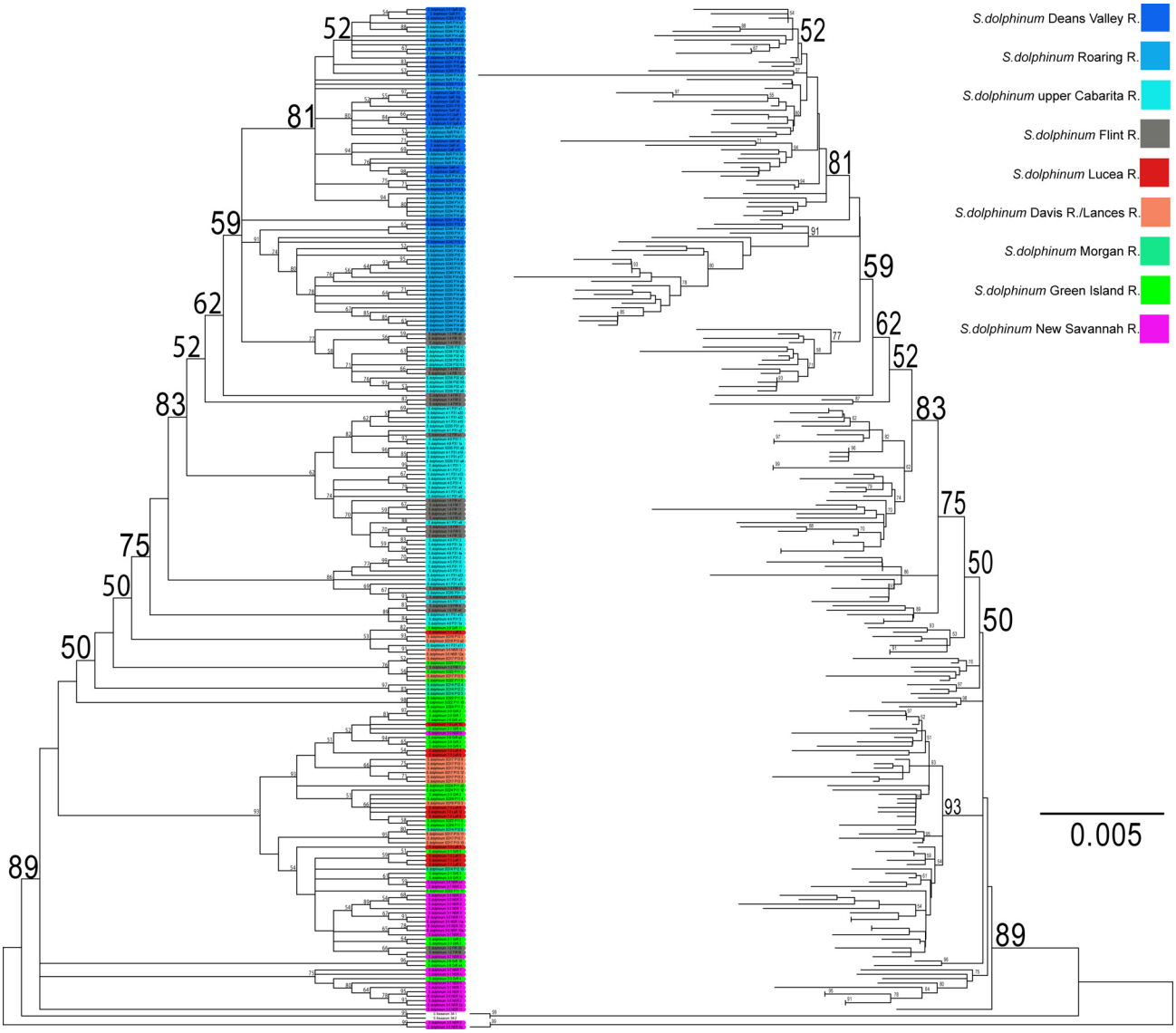


Figure 26. Original and 50% condensed neighbor joining tree of phylogenetic relationships within the species *Sesarma dolphinum*. Confidence values from 5000 bootstrap replicates (Maximum Composite Likelihood algorithm) based on 1408 gapcoded basepairs of the ITS1-5.8S-ITS2 nuclear complex; only bootstrap values above 50 are shown. Coloration of haplotypes according to map from Figure 21.

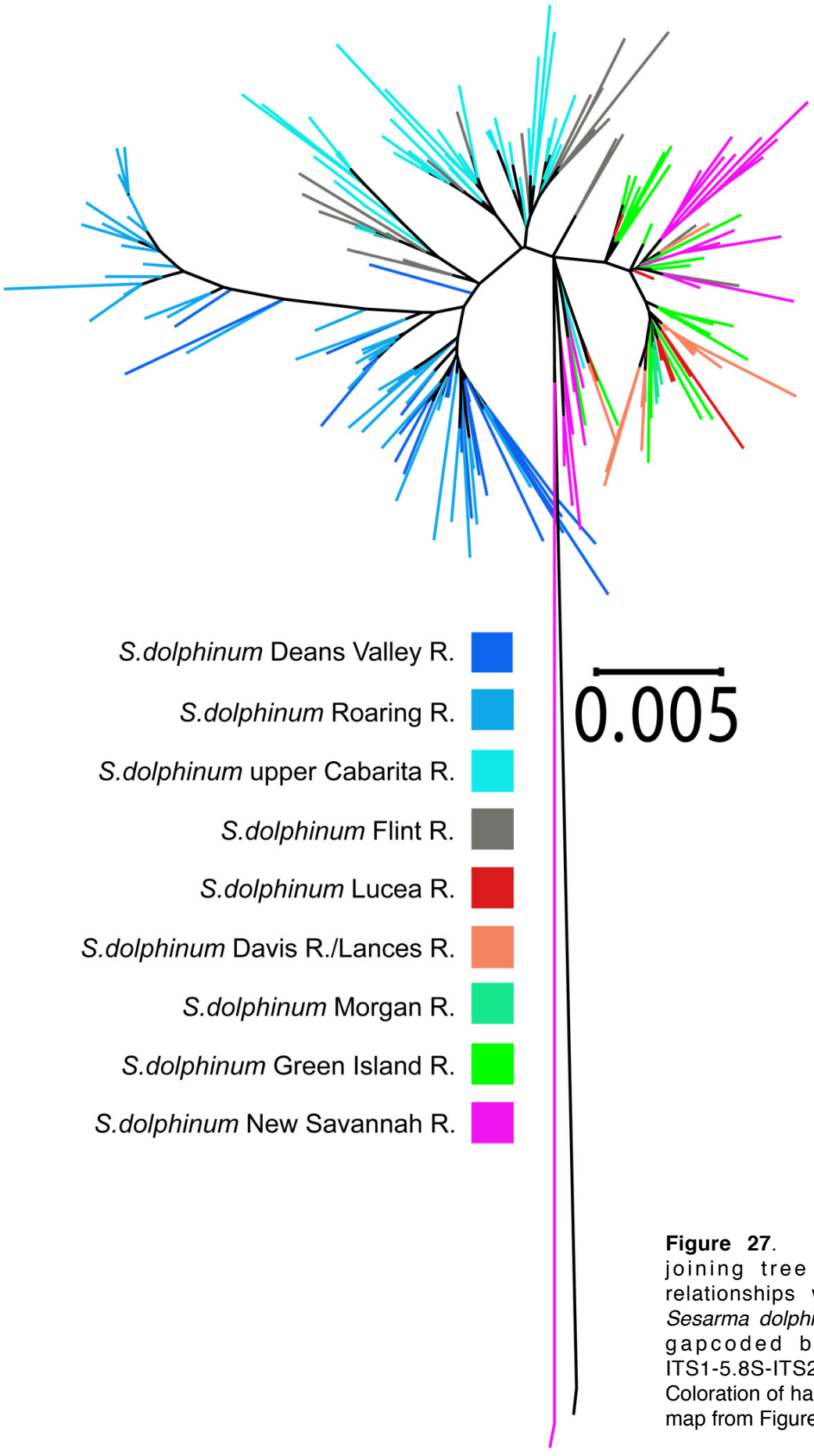


Figure 27. Radiation neighbor joining tree of phylogenetic relationships within the species *Sesarma dolphinum* based on 1408 gapcoded basepairs of the ITS1-5.8S-ITS2 nuclear complex. Coloration of haplotypes according to map from Figure 21.

ITS1-5.8S-ITS2

For the ITS1-5.8S-ITS2 complex 232 clones from 32 samples of *Sesarma dolphinum* were sequenced. The numbers of clones per individual varied between 3 and 15, with an approximate length of the sequences of 1350 basepairs. This places this dataset in an intermediate position considering the length of ITS sequences (Harris et al., 2000) found in crustaceans. Two of the clones had large inserts with over 300 basepairs and were excluded from the analysis. After the conversion of the alignment data with Gapcoder we obtained 1408 sites and 223 haplotypes. Only 10 haplotypes occurred more than once and no haplotype more than twice. The number of variable positions is 415 and 173 positions are parsimony informative. The neighbor joining original and 50% condensed trees are displayed in Figure 26 and the 50% consensus tree from the Bayesian approach

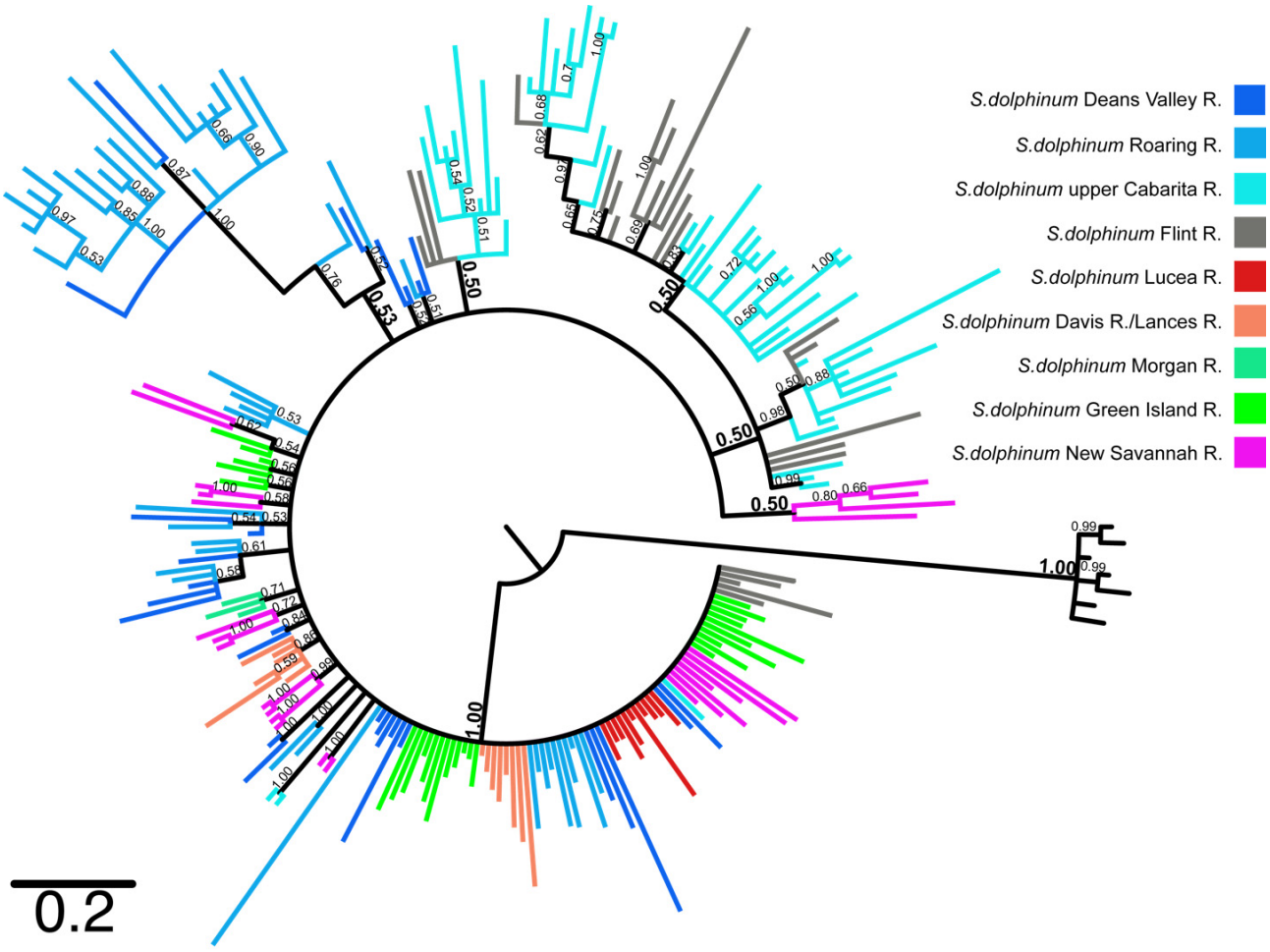


Figure 28. Bayesian inference tree of phylogenetic relationships within the species *Sesarma dolphinum*. Bayesian inference topology with F81+I+G model of evolution. Posterior probabilities based on 1408 gapcoded basepairs of the ITS1-5.8S-ITS2 nuclear complex; only values above 0.50 are shown. Coloration of haplotypes according to map from Figure 21

is shown in Figure 28. The neighbor joining tree can be divided into five clades. The clones which can be found in clade I are all from individuals from the Deans Valley River or the Roaring River. Although clade-I consists of two subclades, there is no further geographic separation of the clones. The sister clade to clade-I is clade-II and only bears clones from three individuals, of which two being from the Flint River and the third is from the upper Cabarita River. All clones from these individuals are found in clade-II. In the clades III and

Table 10. Pairwise F_{st} values for all population of *Sesarma dolphinum* based on haplotype frequencies of the ITS1-5.8S-ITS2 dataset. Significant levels are shown in lower left part of the matrix (calculated from 10000 permutations).

n=clones k=individuals	Flint R. (n=24)	Lucea R. (n=4)	Davis R. (n=14)	Morgan R. (n=5)	Green Island R. (n=29)	New Savannah R. (n=17)	Galloway R. (n=29)	Roaring R. (n=44)	upper Cabarita R. (n=48)
Flint R. (k=3)		0.37514	0.42624	0.36819	0.31784	0.21282	0.4424	0.32427	0.04277
Lucea R. (k=2)	p<0.0001		0.07404	0.11646	0.01120	0.20766	0.62805	0.52456	0.363
Davis R. (k=2)	p<0.0001	p<0.05		0.11129	0.0792	0.18119	0.63125	0.50048	0.40927
Morgan R. (k=2)	p<0.0001	p<0.05	p<0.01		0.05366	0.11531	0.58909	0.46086	0.35564
Green Island R. (k=4)	p<0.0001	p<0.05	p<0.01	p<0.1		0.13191	0.55874	0.46254	0.3258
New Savannah R. (k=4)	p<0.0001	p<0.0001	p<0.01	p<0.01	p<0.0001		0.42403	0.33825	0.23344
Galloway R. (k=5)	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001		0.17929	0.40655
Roaring R. (k=6)	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.01		0.30156
upper Cabarita R. (k=5)	p<0.05	p<0.0001	p<0.0001	p<0.01	p<0.0001	p<0.0001	p<0.0001	p<0.0001	

IV only clones from northeastern samples can be found. All clones from the remaining samples pool together in clade-V, without any further recognizable segmentation. Two clones from a New Savannah River individual are found to be even more basal than the sisters species and outgroup *Sesarma fossarum*. The same overall picture is depicted if the calculated neighbor joining tree is shown as radiation tree (Figure 27). The clones from clade V form a separate basal branch. Again the individuals from the New Savannah River, the Morgan, Green Island, the Lances, both Lucea rivers and the Flint River show distinct patterns, but some of the shorter branches only consist of clones from individual rivers, indicating a beginning genetic separation. The branch on the tip of the radiation tree consist only of clones from the Roaring and the Deans Valley River. No further distinction

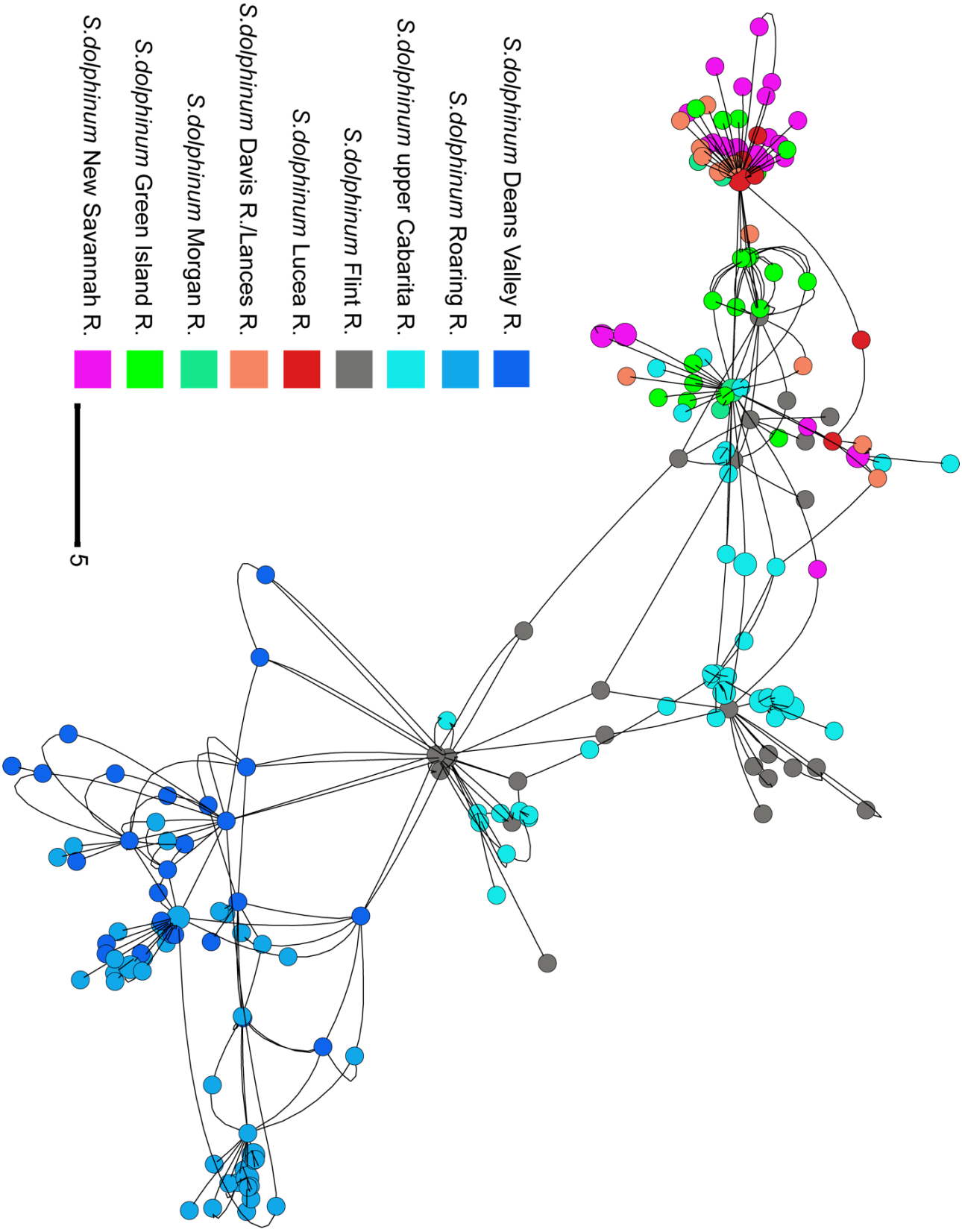
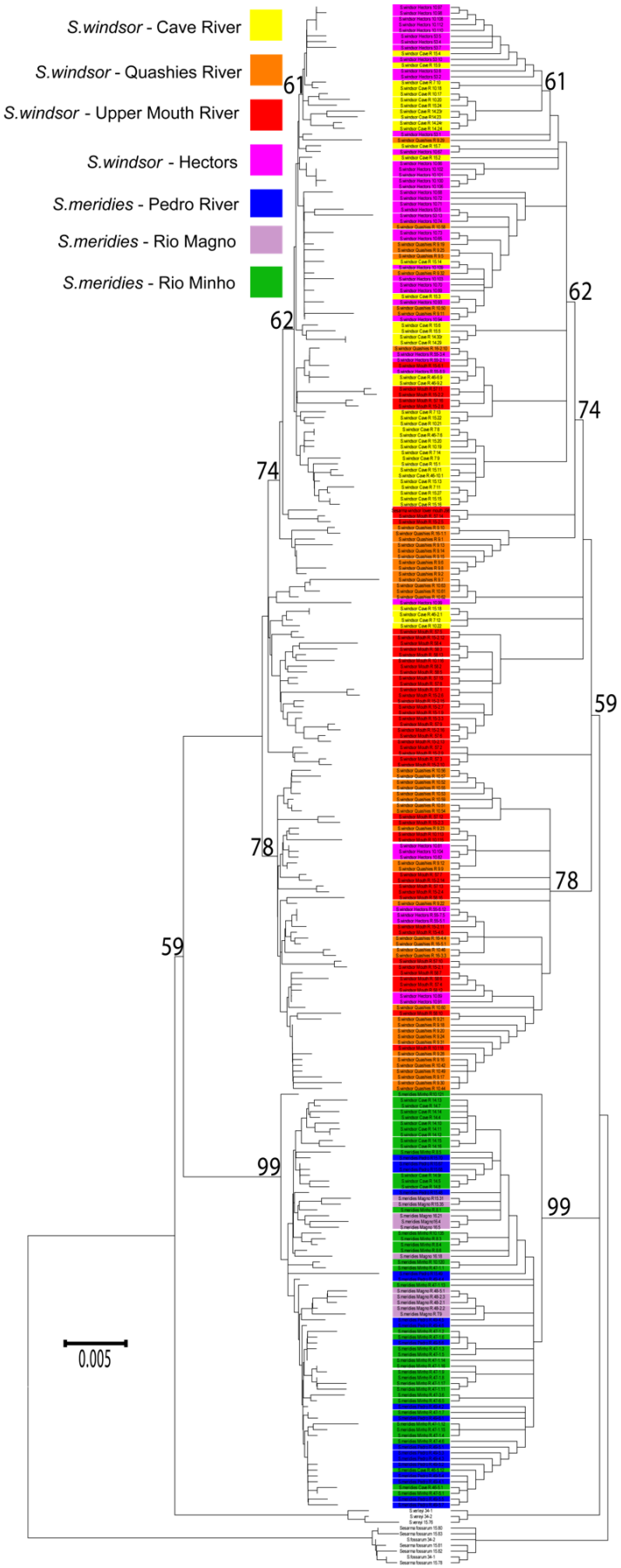


Figure 29. Minimum spanning network based on 1408 gapcoded basepairs of the ITS1-5.8S-ITS2 nuclear complex of the species *Sesarma dolphinum*. Coloration of haplotypes according to map from Figure 21.



between the two river systems is recognizable. Between the basal branch and the more derived branch, three smaller groups split off. They are completely made up of clones from either the Flint River or the Upper Cabarita system. This is interesting as, although belonging to drainage systems which drain in opposite directions, they are in close geographic proximity to each other and form the geographic connection between the clones from clade V and the clones from the Deans Valley-Roaring River area.

The topology of the Bayesian inference tree supports the overall picture of the neighbor joining trees. Again clones from the Flint River and upper Cabarita system cluster together. With the exception of five clones from the Flint River, they are all found on only two clades, which show

Figure 30. Original and 50% condensed neighbor joining tree of phylogenetic relationships within and between the species *Sesarma windsor* and *Sesarma meridies* with *Sesarma verleyi* as outgroup. Confidence values from 5000 bootstrap replicates (Maximum Composite Likelihood algorithm) based on 1427 gapcoded basepairs of the ITS1-5.8S-ITS2 nuclear complex; only bootstrap values above 50 are shown. Coloration of haplotypes according to map from Figure 22.

Bayesian posterior probabilities of 0.50. The clones from the Deans Valley River and the Roaring River are found again together on the same clades, but in the Bayesian Inference tree they form more clades and some are also found in basal positions.
The intermediate position of the Upper Cabarita River system together with the Flint River is also very nicely depicted in the minimum spanning network constructed from the gapcoded ITS data (Figure 29). Again, the Deans Valley and Roaring River clones pool together as do the clones from the individuals from all western rivers, including both Lucea rivers. The clones which form the connection between those two pools consist only of the ones from individuals found in the northeastern area of the distribution range of *Sesarma dolphinum*.

The pairwise F_{st} values between all populations of *Sesarma dolphinum* from the AMOVA are listed in Table 10. The indication of highly restricted gene flow between the Deans Valley - Roaring River and the all other populations is further supported. The unrestricted gene flow between the Flint River and the upper Cabarita is also shown in the low F_{st} values. The results from the AMOVA also show ongoing gene flow between the Lucea rivers, Davis River, Morgan River and the Green Island River. But gene flow from these rivers to the Flint River and the upper Cabarita River is reduced. The New Savannah River population occupies an intermediate position.

Sesarma windsor and *Sesarma meridies*

ITS1-5.8S-ITS2

I used the ITS1-5.8SrRNA-ITS2 complex to look into the genetic population structure of *Sesarma windsor* and *Sesarma meridies* from central Jamaica. Overall, from both species we obtained suitable sequences from 31 individuals and 260 clones. The length of the ITS gene varied between 1200 basepairs and 1300 basepairs. After gapcoding, the length of the aligned data was 1427 basepairs. The 223 haplotypes have 314 polymorphic sites of which 142 are parsimony informative. Most haplotypes occurred only once and the most common haplotype was found eleven times. The Neighbor Joining original tree and the 50% condensed tree are shown in Figure 30. Figure 31 shows the same NJ tree as

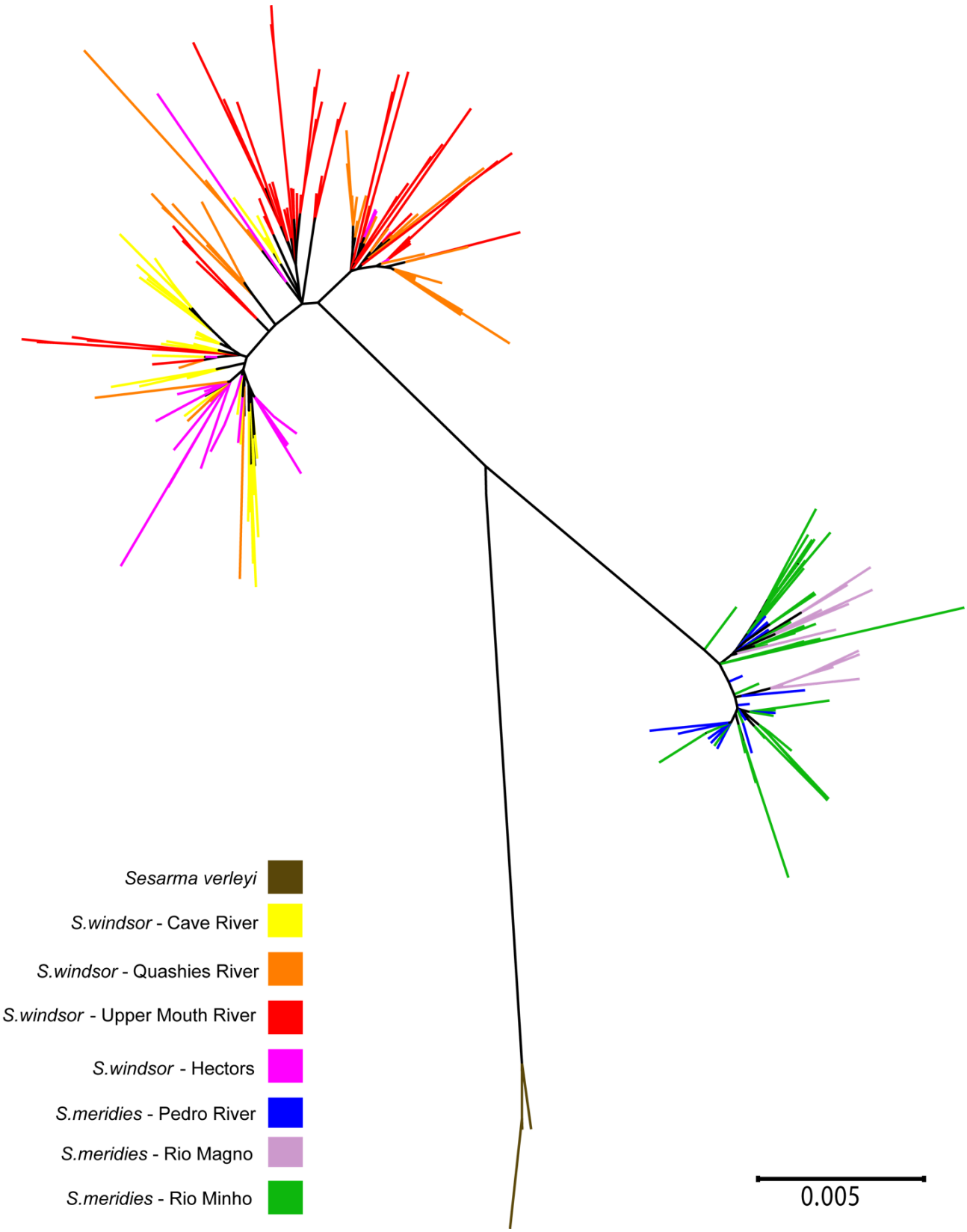


Figure 31. Radiation neighbor joining tree based on 1427 gapcoded basepairs of the ITS1-5.8S-ITS2 nuclear complex of the species *Sesarma windsor* and *Sesarma meridies* with *Sesarma verleyi* as outgroup. Coloration of haplotypes according to map from Figure 22.

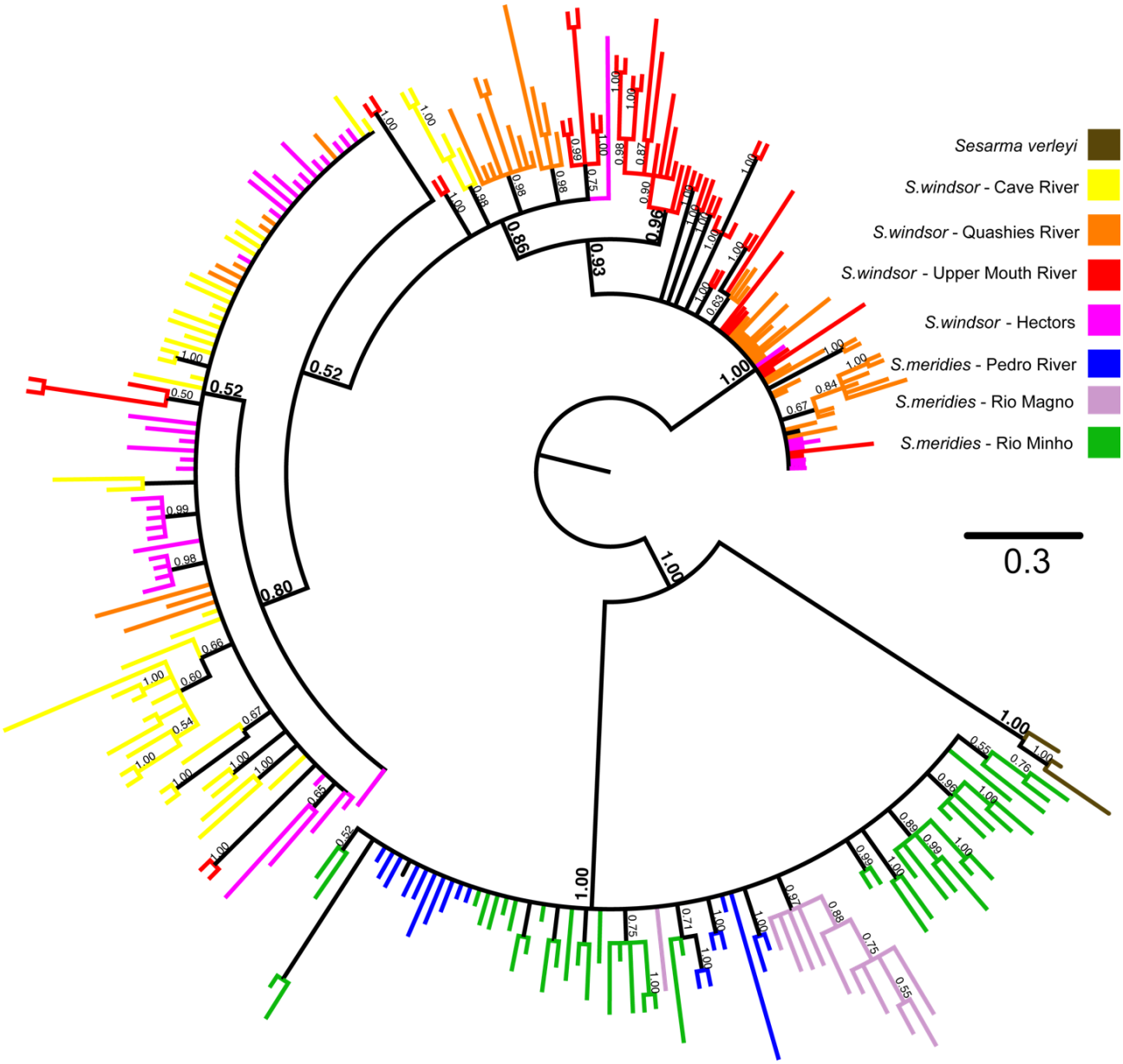


Figure 32. Bayesian inference tree of phylogenetic relationships within and between the species *Sesarma windsor* and *Sesarma meridies* with *Sesarma verleyi* as outgroup. Bayesian inference topology with F81+I +G model of evolution. Posterior probabilities based on 1427 gapcoded basepairs of the ITS1-5.8S-ITS2 nuclear complex; only values above 0.50 are shown. Coloration of haplotypes according to map from Figure 22

radiation tree. In both figures, the two species are well separated. Although there are two major clades and branches, respectively, the bootstrap values for this basal separation are not too high. In the *Sesarma meridies* clade no subdivision into the different river systems is recognizable. The different river systems are not grouped together in smaller clades, but display a scattered across the tree. Even the clones from individuals from the Rio Magno, its clones form a single branch on the NJ radiation tree but the rest is scattered with clones from the other sampling points. Also in the *Sesarma windsor* clade no strict separation is

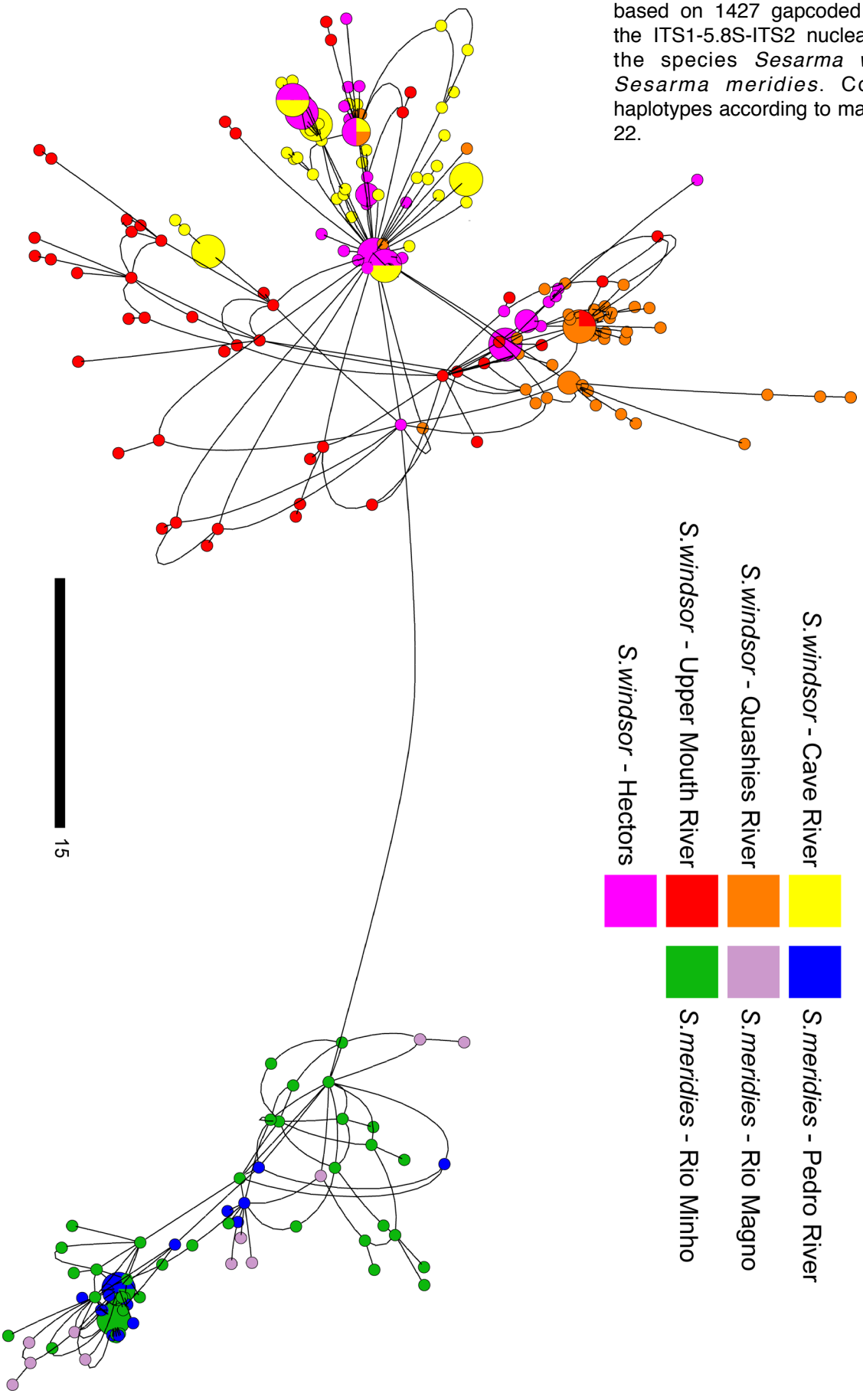


Figure 33. Minimum spanning network based on 1427 gapcoded basepairs of the ITS1-5.8S-ITS2 nuclear complex of the species *Sesarma windsor* and *Sesarma meridies*. Coloration of haplotypes according to map from Figure 22.

visible. Although there is no strict division, some of the clades are dominated by certain river systems. This trend is clearly pictured in the NJ radiation tree, as some branches only contain clones from a single sampling point. Other clades or branches are mostly composted of clones from a certain river a set of rivers.

The phylogeny estimated by the Bayesian inference method is shown in Figure 32. The two species are separated with a Bayesian posterior probability of 1.00. In this tree some differentiation is visible in the *Sesarma meridies* clade. With the exception of a single clone the Rio Magno clones form a single clade with a posterior probability of 0.97. Again in the *Sesarma windsor* clone no rigid separation into the different rivers is displayed, but certain clades are dominated by certain populations.

In Figure 33 the minimum spanning network constructed from the ITS1-5.8S-ITS2 complex data is shown. The two species are in two separated network clouds which are connected by a single line of missing haplotypes. The overall picture is similar to the one from the NJ trees and the Bayesian inference tree. Within *Sesarma meridies* no strict division of the

Table 11. Pairwise F_{st} values for all population of *Sesarma windsor* and *Sesarma meridies* based on haplotype frequencies of the ITS1-5.8S-ITS2 dataset. Significant levels are shown in lower left part of the matrix (calculated from 10000 permutations).

n=clones k=individuals	*S.meridies Rio Minho (n=28)*	*S.meridies Pedro R. (n=19)*	*S.meridies Magno R. (n=11)*	*S.windsor Cave R. (n=42)*	*S.windsor Hectors R. (n=43)*	*S.windsor Quahies R. (n=52)*	*S.windsor Mouth R. (n=51)*
S.meridies Rio Minho (k=6)		0.19341	0.53127	0.80596	0.8315	0.77479	0.70757
S.meridies Pedro R. (k=3)	p<0.0001		0.48769	0.80741	0.83586	0.75119	0.70184
S.meridies Magno R. (k=3)	p<0.0001	p<0.0001		0.80419	0.83458	0.74416	0.69193
S.windsor Cave R. (k=8)	p<0.0001	p<0.0001	p<0.0001		0.15558	0.31952	0.27955
S.windsor Hectors R. (k=5)	p<0.0001	p<0.0001	p<0.0001	p<0.0001		0.26282	0.2535
S.windsor Quahies R. (k=5)	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001		0.15865
S.windsor Mouth R. (k=4)	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	

different river system is recognizable. The same is more or less true for *Sesarma windsor*, but here certain parts of this network however, are dominated by specific river systems or sets of rivers.

The strict separation of the two population is reinforced by the high F_{st} values of the AMOVA shown in Table 11. The F_{st} values also support restricted gene flow between the Rio Magno and the other populations of *Sesarma meridies*. The pairwise F_{st} values of the *Sesarma windsor* population indicate beginning restricted gene flow corresponding to the distance between the different rivers.

Discussion

Epilobocera sinuatifrons

The islands Jamaica and Puerto Rico form part of the Greater Antilles and are of similar size. The composition of their habitats for freshwater crustaceans in regarding of river size, composition, distribution and average water current velocity, as well as available alternative freshwater habitats (e.g. caves and bromeliad leaf axils) is also similar. Interestingly, on the island of Jamaica, at least ten different species of freshwater crabs belonging to the family Sesarmidae are present, whereas Puerto Rico only harbors a single freshwater crab species. *Epilobocera sinuatifrons* from Puerto Rico belongs to an old lineage of freshwater crabs, the family Pseudothelphusidae (Rodríguez & Magalhães, 2005) which has representatives on all islands of the Greater Antilles with the exception of Jamaica (Rodríguez & López, 2003). The Jamaican freshwater crabs are the result of a relatively recent adaptive radiation, after the colonization of the island by a marine ancestor and are therefore much younger species (Schubart et al., 1998). This striking difference in species number gives rise to the question, if either the high number of species on Jamaica are overestimated or the Puerto Rican ones underestimated. The existence of cryptic speciation has been reported from several limnic crustaceans (Daniels et al., 2003; Daniels et al., 2006; Shih et al., 2006; Taylor et al., 1998) and could be an explanation for the low species number on Puerto Rico. Williams (1983) indicated a relationship between island size and species number, whereby the two factors geographical isolation and new niche utilization play an important role for the species richness.

In this study the population structure of the Puerto Rican freshwater crab is analyzed by using three different methods. I studied phenotypic differences in the populations of *Epilobocera sinuatifrons* and genetic population structure with one mitochondrial marker (ND1) and one nuclear region (ITS1-5.8SrRNA-ITS2). While the differences between populations turned out to be smaller than expected some restricted gene flow between easter and western population was found with all three methods.

The morphometric analysis of the different populations of *Epilobocera sinuatifrons* followed the initial geographic subdivision of this study. The grouping was reduced to three areas,

east, west and center after the initial outline revealed very low variability. In the plot of the first two canonial functions some clustering according to geography becomes visible. The percentage of correct classified cases with only three groups raises to 64.4%. The eastern and central group also show high correct classification with 66.7% and 84.2%. The values become significantly lower, if the sampling points are subdivided into northern, central and southern groups. The Cordillera Central mountain range divides most of Puerto Rico`s drainage systems in either northern or southern rivers. However, it seems that the drainage direction and location on either northern or southern slopes of the mountain range has less effect on the population structure than a west to east gradient. Especially the easternmost populations can be separated from those of the rest of the island, if the morphometric differences are looked upon. No further significant differentiation is possible with this dataset. It is not evident from the morphometric data alone, how this separation was established, if via drainage rearrangement, via terrestrial movement or if phenotypic differences are due to different environmental conditions.

Two genetic markers were used to further investigate if the established morphometric differences are the result of restricted gene flow. The combined maximum parsimony maximum likelihood and the Bayesian inference trees of the ND1 mitochondrial gene show similar results (Figure 13 & Figure 14). In all trees haplotypes I and III to VI can be found together in one clade with high confidence values. These haplotypes are only found in individuals from the northeastern and southeastern sampling spots. The other haplotypes in this clade are from both central-eastern populations, while haplotype IX is shared by individuals from the northeastern and the central northeastern region. The remaining clades in both trees with high bootstrap values or posterior probabilities include haplotypes from the central-western and western sampling sites. The variation found in the morphometric data is therefore not an entirely phenotypic phenomenon and also here most differentiation is found in a west-east as opposed to a north-south pattern. The distribution of the remaining haplotypes does not allow recognition of further geographical structure in the trees with the exception of the haplotype XXXVI. This haplotype is found between the two outgroup species *Epilobocera gilmanii* and *Epilobocera haytensis* and all other *Epilobocera sinuatifrons* haplotypes. It was only found in a single juvenile individual, which was collected in the Guilarte National forest during the preliminary field trip by C.D.

Schubart and R. Diesel in 1997. Although this region, including the vicinity of the original collection site of this juvenile individual was intensely sampled, no further specimen showed this haplotype. Its position in the tree would indicate that it belongs to a yet undescribed species. Morphological differences are not recognizable mainly because of the juvenile state of the sample. Any further assumption on its exact nature, phylogenetic relationship and habitat are pure speculation. Unfortunately it could not be excluded entirely that the different state of this haplotype is the result of improper storage of the collected material after sampling which might have lead to DNA degeneration (Wandeler et al., 2003).

In the parsimony network constructed with the software TCS 1.21 (Clement et al., 2001) the relationship between the haplotypes and their geographic appearance is better resolved. Again the separated position of the eastern haplotypes is clearly visible. With the help of a Nested Clade Analysis (Figure 16) I investigated the historical events which lead to the observed haplotype distribution. The nested clade analysis compares the genetic structure of a set of samples with its geographical distribution and infers the evolutionary processes which lead to the observed structure. This analysis is done for every clade on every level, therefore it is possible to infer the evolutionary history of the sample set from historical events to recent one. In the eastern group only one clade (1-3) showed significant values and the inference key suggests contiguous range expansion. Contiguous range expansion is also suggested for the clade 2-6, consisting of haplotypes found in all four western sampling regions. Within the same level, for clade 2-7, again with haplotypes from the western part of the island restricted gene flow with isolation by distance is inferred. The group containing the suggested ancestral haplotype is quite inconsistent in its geographical origin. It is separated from the eastern group by five missing haplotypes and by three from the western group. It contains haplotypes which are found in populations from nearly all parts of Puerto Rico. Only haplotypes from the most eastern sampling sites are missing. This strong combination of haplotypes from most parts of Puerto Rico suggests that the distribution into separate population is either a rather young phenomenon or disrupted by regular gene flow between the populations. The Nested Clade Analysis produced no significant values for this intermediate group. Only the next higher clade, which combined this group with the eastern clade resulted again in contiguous range expansion. The overall cladogram is also characterized by contiguous

range expansion. The results from the parsimony network and the Nested Clade Analysis suggest, that there is gene flow restriction between the different river populations of *Epilobocera sinuatifrons*, but only significant over very long distances and most pronounced in the eastern populations.

The results from the nuclear ITS1-5.8SrRNA-ITS2 gene complex support the theory of relatively regular gene flow between the populations of *Epilobocera sinuatifrons*. The Bayesian inference tree does not show any structuring (Figure 19) and the Neighbor joining trees reveal only minor structuring (Figure 17 & Figure 18): In the radiation tree only a higher amount of clones from eastern individuals are visible on the tip of the branchesand mostly missing from the base of the branches. Except from this quite gradual difference, no further separation is recognizable. It therefore bolsters the isolated position of individuals from the eastern part of the island, which is also shown by the pairwise F_{st} values in Table 7. This partial isolation is even more visible in the minimum spanning network of the ITS data (Figure 20). Here a separation into three groups, corresponding to west, center and east, is present. The clones from individuals from the most eastern sampling points group together as do the ones from the most western sampling points. The third group contains clones from the complete Cordillera Central mountain range. The separation into those three groups is relatively clean, as only a few clones are misplaced. This result corresponds to the results from the discriminant analysis, where the same three groups could be distinguished. The uniformity of the central sampling points suggest, that there is recent gene flow along the mountain range with no separation between northern or southern drainage systems.

Combining the results from the three different approaches (morphometrics, mitochondrial and nuclear markers), the existence of cryptic species can be ruled out. Gene flow must be recent or ongoing throughout most of the island, resulting in only weak isolation by distance between distant populations. One possibility to explain gene flow between riverine species are geological events like changes in river capture. Through faulting and erosion activities streams are disconnected from their original drainage system and rearranged to a new river. This has been shown to be the fact for freshwater fish (Hurwood & Hughes, 1998; McGlashan & Hughes, 2001). In their analysis of the population structure

of *Epilobocera sinuatifrons* using the cytochrome oxidase subunit 1 Cook et al. (2008) suggested recent drainage rearrangement as primary reason for the observed gene flow between rivers. Their analysis of the population history by means of NCA revealed contiguous range expansion for the western part of the island, similar to the results in this study and ruled out terrestrial dispersal among rivers due to insignificant F_{st} values in the pairwise analysis between populations. In contrast to this I find very low and significant F_{st} values in the nuclear marker, especially between the western populations of *Epilobocera sinuatifrons*.

Although juvenile *Epilobocera sinuatifrons* have a strong dependency on freshwater habitats (March & Pringle, 2003), adults have a higher terrestrial dispersal ability and can therefore reach new drainage systems independent from geological events. *Epilobocera sinuatifrons* is known to leave the freshwater habitat to feed on adjacent forest floors (Zimmerman & Covich, 2003) and the crabs can also be found on the forest floor in great distances from any surface river (personal observation): several individuals were active at night in limestone sinks in the Guajataca National Forest. The water collected in burrows and in sinks in this karstic environment was apparently sufficient to support the freshwater requirements of *Epilobocera sinuatifrons*. Also female specimens carrying juvenile crabs were observed in this sinkholes. These individuals also showed no differences in their genetic nor morphometric data to individuals from neighboring rivers. The ability to obtain oxygen from air as mentioned by Rodríguez (1986) further supports the theory of longer terrestrial movement by *Epilobocera sinuatifrons*. This potential for terrestrial dispersal was probably already present during the colonization of the Greater Antilles by the Pseudothelphusidae (Rodríguez & Magalhães, 2005) as it also reported from the Hispaniolan relative to *E. sinuatifrons*, *Epilobocera haytensis* (see Rivera & Schubart, submitted) The Pseudothelphusidae colonized the West Indies in the Late Eocene to Early Oligocene (30-50 mya) via island-land bridges from northern South America (Iturralde-Vinent & MacPhee, 1999) and were already well adapted to the limnic environments present in the Caribbean.

Sesarma

The colonization of land and subsequent radiation of crabs into different habitats, as seen in the land-living crabs of Jamaica, is a fascinating example of adaptive radiation in the sense of Darwin (1860) and Schluter (2000). They have been studied intensively over the last 15 years (Schubart et al., 1998a; Schubart & Koller, 2005) and in this time frame, five new species have been described increasing the number of known freshwater crabs from Jamaica to ten. All five species belong to the ecotype of mountainous river crabs, where previously only one species, *Sesarma bidentatum* was known. The description of the new species was based mainly on morphological traits and only recent studies incorporated molecular methods (Schubart et al., 1998b; Schubart & Koller, 2005). Although the expert will be able to differentiate between the single species and sometimes even between populations (Reimer et al., 1998) for the untrained eye these freshwater crabs look similar. The mountain headwater streams they inhabited by them are also very similar and in some cases lie in near proximity. Even if their distribution ranges do not overlap, different headwater tributaries to the same river may be inhabited by different species, as in the case of *Sesarma meridie*s and *Sesarma bidentatum* in the Rio Cobre system (unpublished). Such condition can lead to hybridization which should be detected by molecular analysis of the different species (Sang & Zhong, 2000). Hybridization has been reported to happen frequently in African freshwater species (Barnard, 1935, 1950) and is more likely to happen in closely related species (Smith, 1992). Therefore an analysis of the Jamaican crab diversity using molecular methods is necessary to confirm that the reported species richness is indeed present and to determine the mechanism responsible for its formation and maintenance.

The present study concentrated on three species of the ten different freshwater crabs of the family Sesarmidae from Jamaica; *Sesarma dolphinum,* which inhabits the streams around the Dolphin Head in westernmost Jamaica and from there to the southern region of the Westmoreland province; *Sesarma windsor* which is found in the rivers of central Jamaica draining to the north and *Sesarma meridies* from the rivers of central Jamaica draining south and the more eastern Rio Magno from the Rio Cobre system.

The population structure of the two species *Sesarma windsor* and *Sesarma meridies* and their relationship to each other was investigated using the nuclear ITS1-5.8SrRNA-ITS2 complex. Considering the question if the two species from central Jamaica show signs of hybridization, no evidence for such events could be found in this study. The constructed Neighbor joining trees (Figures 30 & 31), the Bayesian inference tree (Figure 32) as also the minimum spanning network (Figure 33) display a clear separation between *Sesarma windsor* and *Sesarma meridies*. No single nuclear allele from one species is found in individuals from the other species. The recognition of the animals inhabiting the south erly draining rivers of central Jamaica as new species, namely *Sesarma meridies*, is therefore further supported by a nuclear marker complex. The fact that no sign for hybridization can be found is especially interesting considering the geographic positions of the different rivers. The localities of both species are in some cases not farther apart, or even closer, as different rivers inhabited by the same species. The distance between the headwaters of the Cave River, inhabited by *Sesarma windsor*, and Rio Minho, inhabited by *Sesarma meridies* is as small as 2 kilometers. The closest collection sites of the two different species are only 9 kilometers afar. This corresponds to the average distance between sampling sites of *Sesarma windsor*. In contrast the Rio Magno collection point of *Sesarma meridies* is nearly 30 kilometers in linear distance separated from the next closerst collection point in the Pedro River. Although no differences between these sites can be recognized in the neighbor joining trees they become visible in the Bayesian inference tree and the AMOVA analysis (Table 11), but by far not as clear as the separation between the two species.

The clearcut separation between *Sesarma windsor* and *Sesarma meridies* is even more astonishing considering the characteristics of some rivers from central Jamaica. Due to the high amount of water-soluble limestone, some rivers disappear in sinkholes only to reappear in the coastal plains. Therefore the exact drainage course of rivers is unknown as are possible underground connections between rivers. In the light of such a situation it is difficult to propose the isolation mechanisms in place between *Sesarma windsor* and *Sesarma meridies*.

The ITS data show a clear isolation between the two species of central Jamaica and that there is restricted gene flow between the different populations of *Sesarma windsor* and

Sesarma meridies. Although the distribution of clones from the different populations of *S. windsor* is not completely separated in the minimum spanning network, single populations are clustered in parts of the network. It appears that the clones from individuals from the Hectors River occupy a more intermediate position in the network, whereas Mouth River and Quashies River are further separated in the network. As the exact underground course of the rivers inhabited by *Sesarma windsor* are unknown, it is difficult to understand potential contact and gene flow between the different population.

In the Bayesian inference tree of *Sesarma meridies* nearly all clones from the Rio Magno population form a single clade and the pairwise F_{st} values indicate restricted gene flow between this river and all other populations. The Rio Magno forms part of the Rio Cobre drainage system, which is a lowland river (no freshwater crabs present), but has four main headwater rivers, the Rio Magno, the Rio Cobre, Rio Doro and the Rio Pedro. The later three are not inhabited by *Sesarma meridies*, but by *Sesarma bidentatum* the prevalent freshwater crab species in this part of Jamaica. The data at hand do not indicate any hybridization between the two species, although they inhabit the same drainage system. In the mitochondrial data provided with the original description of *Sesarma meridies* (Schubart & Koller, 2005), the Rio Magno animals show some differences in comparison with the other population of the species. Now also the nuclear data indicate restricted gene flow. The avoidance of species mixture or hybridization in the Rio Cobre is very interesting and further investigation will be necessary to determine, if it is of ecological or hydrographic nature.

The population structure of *Sesarma dolphinum* was studied with three approaches: morphometrics, population genetics with the ND1 mitochondrial gene, and population genetics with the ITS1-5.8SrRNA-ITS2 nuclear complex. Overall, the three approaches gave quite similar results and point towards the existence of at least two major differentiated metapopulations. Each method had different resolution-potential, but separated the southeastern populations from the rest.

The discriminant analysis of the morphometric data from *Sesarma dolphinum* showed two well-separated groups, one containing the individuals from the Roaring River and Deans Valley River and one formed by all other sampling sites. Morphological differences within

this species had already been recognized by Reimer et al. (1998) when they described *Sesarma dolphinum*. Daniels et al. (2003) mentioned that morphometrics can have limited use when dealing with cryptic species. As we are dealing with a rather young species (Schubart et al.,1998a) phylogenetic signal should not yet be obscured by convergent evolution in morphometrics. It has been shown that crustaceans can have a high morphological plasticity (Finston, 2000). So it could be possible that the here established significant morphometric differences are the result of different environmental conditions, like predation or nutrition factors or the result of allometric growth. By using only specimens of a certain size (carapace length > 12mm) and applying logarithmic transformation the possible negative influence of allometric growth was obviated. Based on personal observations no environmental differences could be found among the collection sites. Detailed environmental studies were not the goal of this study, but could be a worthwhile approach for further investigations of the Jamaican Sesarmidae. Apart from the main differentiation into two major morphological groups the overall correct classification of individuals in the discriminant analysis was also high, whereas falsely classified samples are often restricted to geographical neighboring groups (Table 4).

The results of the morphometric data are supported by results from molecular methods with the mitochondrial ND1 and the nuclear ITS1-5.8S-ITS2 marker. The populations Roaring River and Deans Valley River show distinct haplotypes, which are found in close relationship both in the ND1 and in the ITS trees and networks. Only their relationship to the other populations differs between the markers. In the ND1 data these two populations are connected to the haplotypes found in the Green Island River, Morgan River and Davis/ Lances River and the Deans Valley population is even separated from the Roaring River population (Figure 25). In contrast, in the ITS data the upper Cabarita River and Flint River populations occupy an intermediate position between the Deans Valley River and Roaring River and the remaining populations. The upper Cabarita River and the Flint River are geographical closer to these two populations than the Green Island River, the Morgan River and the Davis/Lances River. The picture that geographical close populations, which are from different drainage systems or even drain in opposite directions, group together or share haplotypes is repeated twice in the molecular data. The upper Cabarita River and the Flint River drain in opposite directions, but the headwaters of the two rivers are only separated by around 2000 meters. In all molecular comparisons, haplotypes and clones

from the two population group together. The Green Island River, Morgan River, Lances River and Davis River again show similar results. They drain in different direction, are not connected, but geographically close and group together according to the ND1 as well as to the ITS data.

Interestingly, the Morgan River, the upper Cabarita River and the Roaring River, which form part of the same drainage system and connect in the lowland parts of the Cabarita River can be genetically separated from each other. Each of these populations are more closely related to their respectively closest geographical population from other drainages. The overall picture is nicely depicted by Table 10 showing the pairwise F_{st} values from the ITS data for all populations. The F_{st} value for two populations is in direct correlation to the distance between the two populations (Figure 34). It also shows that gene flow is only present between closely located populations and, at the intraspecific level, highly restricted between distant populations of *Sesarma dolphinum*.

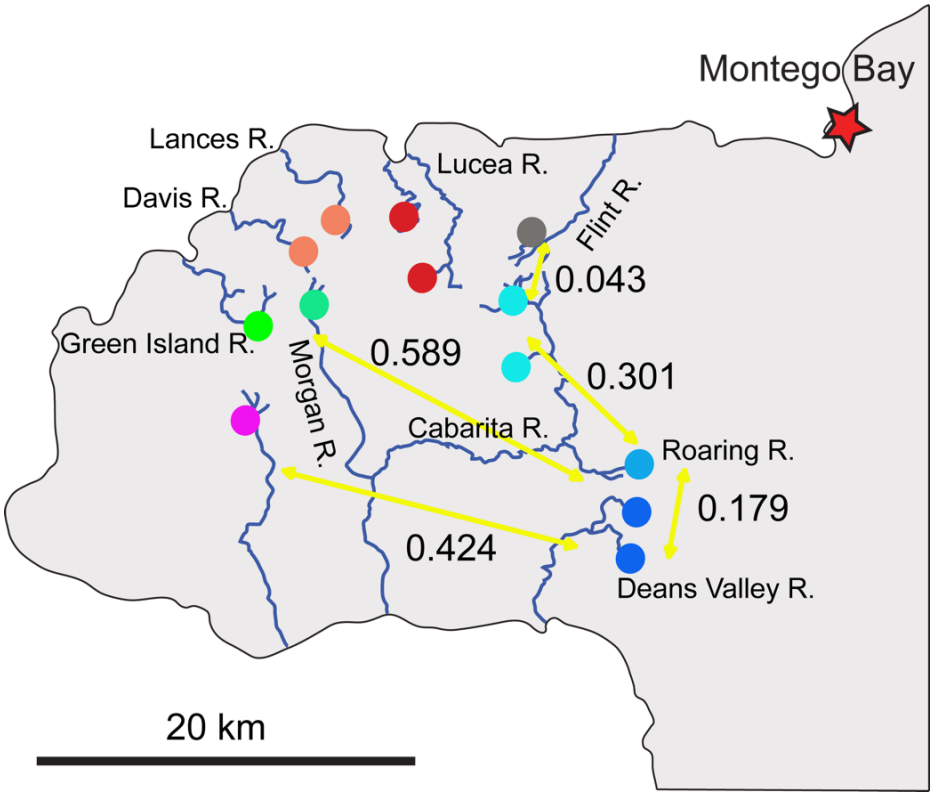


Figure 34. Map of western Jamaica showing selected rivers and sampling sites where species of *Sesarma dolphinum* were collected with selected pairwise Fst values between populations.

In the mitochondrial dataset some of the animals from the northwestern populations show haplotypes which are close to the separated southeastern Roaring River and Deans Valley River populations. Based on the different results from the nuclear marker and the morphometric data, this could be the result of introgression. After a secondary contact

between the populations, the southeastern haplotype may have been introduced and maybe even dominate in the northwestern populations. How these contacts may have come into place, can only be hypothesized: specimens from the Roaring River could have been washed down and reached the upstream Morgan River from the lower Cabarita River. From there, the closer populations in the Green Island River and the Davis River also got populated with the southeastern haplotype. The introgression is not complete, as the ancestral ND1 haplotype can also be found in the Green Island River and the southern population in the New Savannah River shows similar haplotypes to the ancestral one. A distribution from southeastern animals to other regions could have also happened by human translocation. The crabs sometimes constitute part of the locals' diet, which is indicated by the name "mountain chicken" regionally given to the crabs. This is a scenario which happens, but not too regularly, as it would result in a more diffuse distribution of haplotypes. It seems unlikely that only animals from one population have been introduced to others.

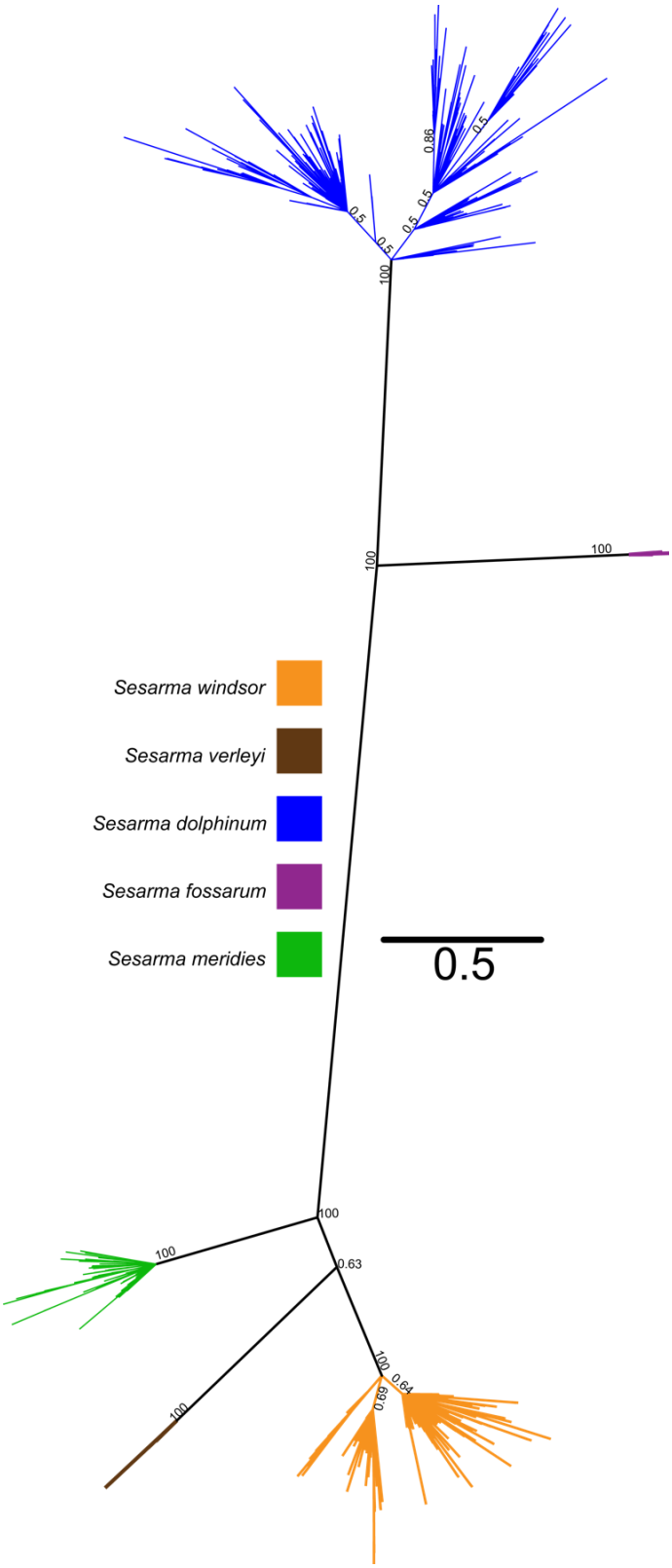


Figure 35. Bayesian inference tree of phylogenetic relationships between certain *Sesarma* species. Bayesian inference topology with F81+I+G model of evolution. Posterior probabilities based on the gapcoded ITS1-5.8S-ITS2 nuclear complex; only values above 0.50 are shown.

The Sesarmidae of Jamaica have no direct development, but still larval stages, although these stages are already abbreviated. Further abbreviation and total reduction of larval development have evolved in other freshwater decapods to complete their life cycle in freshwater and has lead to strong differentiation and restricted gene flow in these species (e.g. Shih et al., 2006). In these species, gene flow is restricted between drainage systems which are separated by geological barriers, but the within drainage dispersal is high (Daniels et al., 2003). Reuschel & Schubart (submitted) did not find any restricted gene flow in the freshwater shrimp *Xipocaris elongata*, which is found in the same habitat as the sesarmid crabs and has an amphidromous life cycle. The present data show that belonging to the same drainage system does not automatically result in gene flow, wether on a intraspecific level as in the case of the Cabarita River system nor on a interspecific level as in the Rio Cobre system. The data rather suggests, that migration mostly occurs between headwaters of the rivers. In order to accomplish local stability as here observed, there needs to be a mechanism to retain the larval stages in the parental habitat. This could be achieved via the maternal burrows as theorized by Diesel & Schubart (2000) and Anger (2005). All land living sesarmid species on Jamaica found ways to rear their offspring in small congregations of water, such as empty snail shells (*Sesarma jarvisi* Rathbun, 1914) or the leaf axils of bromeliads (*Metopaulias depressus* Rathbun, 1896). This kind of retention also asks for some kind of paternal care similar to the one observed in *Metopaulias depressus* (see Diesel, 1992). It could be speculated, if such paternal care was established early in the colonization of Jamaica by sesarmid crabs and even have acted as a key innovation during the adaptive radiation of the Jamaican Sesarmids.

The results from the morphometric analysis, the mitochondrial marker ND1 and the nuclear ITS1-5.8S-ITS2 complex all allow similar conclusions. The different populations of the recently described and young freshwater crab *Sesarma dolphinum* are already isolated from each other as are the two species *Sesarma windsor* and *Sesarma meridies* and some of their populations. This isolation is greater between geographic more distant population and being part of the same drainage system does not necessarily break the isolation nor does it lead to hybridization between closely related species. Contact between the different populations must be established via short terrestrial movement or

changes in the direction of river drainage, which may be supported by the instable geological situation of Jamaica. The limited genetic exchange could be the result of the use of maternal burrows to retain larvae in the paternal habitat.

Comparison of the two freshwater crab lineages

The ancestor of the Puerto Rican freshwater crab *Epilobocera sinuatifrons* colonized the island approximately 30 to 50 mya ago (Iturralde-Vinennt & MacPhee, 1999). This ancestor was probably already well adapted to the river habitats present on the island and therefore the pressure for further adaptation was low. In contrast, the marine ancestor of the freshwater sesarmid crabs of Jamaica colonized the island only 4.5 mya ago. Although the older lineage of freshwater crabs had comparatively more time to evolve regional endemisms, the present data only shows low morphological and genetic differentiation over the whole island. On Jamaica, on the other hand, the younger lineage underwent an adaptive radiation resulting in at least ten different species, whose species status is further consolidated by the results of this study (Figure 35). The differentiation found among populations, even in a single Jamaican species (e.g. *Sesarma dolphinum*) exceeds the differentiation found in *Epilobocera sinuatifrons* all over Puerto Rico. The driving force behind these differentiations is probably the different dispersal capabilities in the two lineages. The pronounced ability for terrestrial movement in *Epilobocera sinuatifrons* overcame most barriers to gene flow, resulting from geological events on Puerto Rico. The Jamaican freshwater crabs on the other hand avoid dispersal via drainage systems, possibly by retaining their larvae and juveniles in the paternal habitat, which lead to strong interspecific and intraspecific isolation. The species diversity on Jamaica is most likely even greater than already known, as phylogeographic data from *Metopaulias depressus* suggest the existence of an undescribed species (Heine, 2006; Rivera, 2007) and another new terrestrial species is currently described by Schubart (in preparation).

Despite of these differences, there is one thing that unfortunately the freshwater crabs from both islands have in common. Both lineage are endangered by increased land use and environmental destruction. Description of their diversity and uniqueness shall contribute to their protection.

Summary

The islands of the West Indies are considered to be a biodiversity hotspot with a high amount of endemic species for the whole region and on certain islands. In terms of freshwater crabs, the island of Jamaica harbors the greatest amount of endemic species in the Caribbean. These freshwater crabs belonging to the family Sesarmidae are the result of an adaptive radiation (Schubart et al., 1998a), which occurred after the colonization of the island by a marine ancestor approximately 4.5 mya. Currently, ten different freshwater species of *Sesarma* are known from Jamaica. On the other islands of the Greater Antilles no entirely freshwater sesarmid crab can be found. Instead, freshwater crabs belonging to the family Pseudothelpusidae are present, but in much lower species richness. The island of Puerto Rico for example is only inhabited by a single endemic freshwater crab species, *Epilobocera sinuatifrons* A. Milne Edwards, 1866.

The aim of this study was to determine the processes and mechanisms which lead to the differences in freshwater crab diversity between the otherwise similar islands of Jamaica and Puerto Rico. A further aim was to investigate, if the species number on Puerto Rico was underestimated due to possible existence of cryptic species or the species number on Jamaica overestimated and the recently described species not completely isolated. The population structure of *Epilobocera sinuatifrons*, *Sesarma dolphinum* Reimer, Schubart & Diesel, 1998, *Sesarma windsor* Türkay & Diesel, 1994 and *Sesarma meridies* Schubart & Koller, 2005 was analysed using morphometric methods, a mitochondrial and a nuclear genetic marker.

In the case of *Epilobocera sinuatifrons*, no evidence for the presence of cryptic species could be found. Furthermore, the overall differentiation between the populations is low and gene flow is ongoing and unrestricted. Only a gradient between the eastern and western part of the island is present.

The analysis of the population structure of the Jamaican sesarmids support the species status of the recently described species and no evidence for hybridization could be found with he nuclear markers. It also revealed a high diversity between the different populations of the three investigated species. The gene flow between the different populations of the species turn out to be stronger restricted with greater geographic distances between the populations. Belonging to the same larger drainage system does not necessarily allow

gene flow between populations or cause mixing or hybridization between species, suggestion isolation in the upper reaches of the rivers.

As a conclusion, the different abilities for dispersal seem to have a great impact on the observed differences of species diversity between the two freshwater crab lineages. The high ability of terrestrial movement leads to ongoing gene flow within the species of *Epilobocera sinuatifrons*. The apparent retention of larvae and juveniles in the parental habitat is probably the key innovation which lead diversification and adaptive radiation in the Jamaican sesarmid crabs.

Zusammenfassung

Die Inseln der Karibik gelten als ein Biodiversititäts-„Hotspot“ mit einer hohen Dichte an endemischen Arten, nicht nur für die gesamte Region, sondern auch auf den einzelnen Inseln. Die größte Anzahl endemischer Süßwasserkrabben beheimatet Jamaika. Sie gehören ausschließlich zur Familie der Sesarmidae und sind das Ergebnis einer adaptiven Radiation (Schubart et al., 1998a), die nach der Kolonisierung der Insel vor ca. 4,5 Millionen Jahren durch einen marinen Vorfahren stattgefunden hat. Zum jetzigen Zeitpunkt sind zehn verschiedene Süßwasserkrabben auf Jamaika bekannt. Auf den anderen Inseln der Großen Antillen finden sich keine Krabben der Familie Sesarmidae die nur auf das Süßwasser beschränkt sind. Stattdessen findet man Krabben der Familie Pseudotelphusidae, die aber eine viel niedrigere Artenvielfalt aufweisen. Zum Beispiel findet sich auf der Insel Puerto Rico nur eine einzige endemische Süßwasserkrabbe: *Epilobocera sinuatifrons* A. Milne Edwards, 1866.

Das Ziel dieser Arbeit ist es Vorgänge und Mechanismen, die zu diesem Unterschied in der Artenvielfalt bei Süßwasserkrabben zwischen diesen sonst sehr ähnlichen Inseln geführt haben, herauszufinden. Weiter sollte festgestellt werden, ob einerseits die Anzahl der Arten auf Puerto Rico als zu gering eingeschätzt werden, da vielleicht kryptische Arten vorhanden sind, oder anderseits die jamaikanische Artenvielfalt als zu hoch, da vielleicht die Arten noch nicht komplett von einander isoliert sind. Die Populationsstruktur von *Epilobocera sinuatifrons*, *Sesarma dolphinum* Reimer, Schubart & Diesel, 1998, *Sesarma windsor* Türkay & Diesel, 1994 und *Sesarma meridies* Schubart & Koller, 2005 wurde mit Hilfe morphometrischer Methoden und zweier molekularer Marker untersucht.

Bei *Epilobocera sinuatifrons* konnte keine Hinweise für das Vorhandensein kryptischer Arten gefunden werden. Vielmehr wurde eine sehr geringe Differenzierung zwischen den Populationen mit einem hohen Anteil gegenwärtigem und ungehindertem Genfluss gefunden. Nur zwischen dem westlichstem und östlichstem Teil der Insel konnte ein gradueller Unterschied festgestellt werden.

Die Analyse der Populationsstruktur der jamaikanischen Süßwasserkrabben unterstützt den Artenstatus, der als letztes beschriebenen neuen Arten; zwischen ihnen konnten mit den nukleären Markern auch keine Hinweise für eine Hybridisierung gefunden werden. Sie zeigt weiterhin eine hohe Diversität zwischen den Populationen der drei untersuchten

Sesarmiden. Der Genfluss zwischen den einzelnen Populationen ist stark eingeschränkt und direkt proportional zu ihren geografischen Abstand. Dabei führt die Zugehörigkeit zum selben Flusssystem weder zu höherem Genfluss innerhalb einer Art, noch zu Hybridisierung zwischen den Arten, was auf Isolation in den oberen Bereichen der Flüsse hindeutet.

Zusammenfassend lässt sich sagen, dass die unterschiedlichen Verbreitungsfähigkeiten der beiden Arten einen starken Einfluss auf die beobachtete Unterschiede in der Diversität in den beiden Süßwasserkrabbenlinien hat. Die hohe Fähigkeit bei *Epilobocera sinuatifrons* sich terrestrisch fortzubewegen führt zu ständigem Genfluss zwischen den Populationen. Bei den jamaikanischen Sesarmiden hingegen war deren Fähigkeit ihre Larvenstadien im elterlichen Habitat zurückzuhalten wahrscheinlich die Schlüssel-erfindung, die ihre Diversifikation und die adaptive Radiation ermöglichte.

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Ich erkläre hiermit an Eides statt, dass ich die vorliegende Arbeit ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe; die aus anderen Quellen direkt oder indirekt übernommenen Daten und Konzepte sind unter Angabe des Literaturzitats gekennzeichnet.

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Regensburg, 28.04.2009 Tobias Santl