Molecular Tools for Species Identification and Population Assessment of Marine Organisms

(Photo by M. Kochzius)

Tina A. Dohna

PhD Thesis, University of Bremen
Bremen, June 2015

Molecular Tools for Species Identification and Population Assessment of Marine Organisms

Dissertation submitted by

Tina A. Dohna

In partial fulfillment of the requirements for the degree of

Doctor of Natural Sciences (Dr. rer. nat.)

Faculty of Biology/Chemistry

University of Bremen

June, 2015

The present study was realized at the University of Bremen's Center for Environmental Research and Sustainable Technologies (UFT) in the Research Group - Biotechnology and Molecular Genetics, Faculty Biology/Chemistry. Funding was provided by the Federal Ministry of Education and Research (grant no. 03 F 0390B and 03 F 0472B) for the Project "Molecular Genetics as a Tool for the Management of Marine Ornamentals in Indonesia" as part of the larger project framework "Science for the Protection of Indonesian Coastal Ecosystems (SPICE)". Funding for open access publication of one of the manuscripts contained herein was generously provided by the University of Bremen.

TABLE OF CONTENTS

Acknowledgements 193

LIST OF FIGURES

LIST OF TABLES

Abbreviations

π	nucleotide diversity
µl	micro liter
µmol	micromol
Φ_{ST}	Overall genetic population structure
$\sum$ bs	cumulative bootstrap support
Abbr.	Abbreviations
AMOVA	Analysis of Molecular Variance
bp	base pair
5'COI 5'	end of the cytochrome oxidase subunit I
COI	cytochrome oxidase subunit I
CR	control region
CT	Coral Triangle
CTAB	Cetyl Trimethyl Ammonium Bromide
CTI	Coral Triangle Initiative
ddH_2O	double-distilled water
DNA	deoxyribonucleic acid
EBM	ecosystem based management
EtOH	Ethyl Alcohol
FDR	false discovery rate
F_{ST}	F-statistics
h	haplotype diversity
HEG	Homing Endonuclease Gene
H_e	expected heterozygosity
H_o	observed heterozygosity
HWE	Hardy–Weinberg Equilibrium
IBD	Isolation-by-Distance
IMA	Indo-Malay Archipelago
IPB	Indo-Pacific-Barrier
ITF	Indonesian Throughflow
ITS I	Internal Transcribed Spacer I
ITS II	Internal Transcribed Spacer II
K2P	Kimura two-parameter
k	number of clusters
ka	kilo annum -1000 years ago
kDa	kilo Daltons
ky	1000 years
LGM	last glacial maximum
m	meters
ma	million years ago
mean D_{est}	average overall genetic differentiation in the dataset
min	minutes
MP	Maximum Parsimony
MPCAH	Most Probable Common Ancestor Haplotype
m^3s^{-1}	cube meters per second
$ms^{-}1$	meters per second
Msat	microsatellite
MST	Minimum Spanning Tree
N_{haplo}	number of haplotypes
N_{ind}	number of individuals sampled
NJ	Neighbour-joining
ns	nucleotide substitution

P	probability
pairwise Φ_{ST}	pairwise population differentiation in the dataset
pairwise mean D_{est}	inter-population genetic differentiation in the dataset
PCR	Polymerase chain reaction
PLD	pelagic larval duration
rRNA	Ribosomal ribonucleic acid
s	seconds
SD	standard deviation
S.E.	standard error
SRP	signal recognition particle
SST	sea surface temperatures
T	temperature
y	year
ya	year ago

Summary

Sessile or site attached marine species rely on the dispersal of their pelagic larvae to ensure the exchange of reproductive individuals within and among subpopulations. The resultant and continued mixing of genetic identities constitutes their population connectivity and can ensure resilience against disturbance, disease or local extinctions. Studies focusing on population connectivity in centers of high biodiversity are particularly needed to protect and sustain these ecosystems in light of global climate change and increasing anthropogenic impacts from growing coastal populations and fisheries. Coral reef organisms, like anemonefishes and their host sea anemones, are ideal candidates to study the dynamics of larval dispersal, as adults are site attached and adult migration therefore does not factor in genetic mixing.

The overarching aim of this thesis is to develop, test and apply molecular markers in the study of different aspects of genetic and biological diversity in anemonefishes and their obligate symbiont sea anemone partners in the Indo-Malay Archipelago, adding to the body of scientific evidence needed to support biodiversity conservation in this "biodiversity hotspot". Specifically, the study furthers our understanding of connectivity in anemonefishes by presenting single species population genetic studies for, *Amphiprion perideraion* (**Chapter I**) and *A. sandaracinos* (**Chapter II**), where species specific structures are discussed in detail to highlight differences despite the highly similar life history and ecology of these fishes. This data is used as a basis for a multispecies approach to connectivity in anemonefishes by identifying and scaling regional barriers to geneflow among congeners (*Amphiprion perideraion*, *A. sandaracinos, A. clarkii* and *A. ocellaris*), making these results more accessible for application and implementation driven fields of research. By applying a comparative intergenomic (mitochondrial and nuclear markers) and an intrageneric (four species) approach, the mechanisms shaping genetic diversity in natural populations of anemonefishes are addressed and the variability in the system is explored.

The impact of host specialization (generalist vs. specialist) and the length of the pelagic larval phase are tentatively discussed in light of the overall genetic structure that could be detected for each species.

To heed the close association between anemonefishes and their sea anemone host, two mitochondrial and one nuclear marker are investigated as to their potential to delineate and identify species within the Actiniaria (**Chapter III**). Following a fourth research aim to study connectivity and diversity in host sea anemones, the attempted development of a set of polymorphic microsatellite loci is shown (**Chapter IV**).

Zusammenfassung

Sessile oder ortstreue marine Arten sind beim Austausch von reproduktiven Individuen innerhalb und zwischen Populationen auf die Zerstreuung ihrer pelagischen Larven angewiesen. Die daraus entstehende und fortlaufende Vermischung genetischer Identitäten wird als Konnektivität beschrieben und kann Populationen widerstandsfähiger gegen Störungen, Krankheit und lokales Aussterben machen. Studien, die sich mit der Konnektivität von Populationen in Zentren hoher Biodiversität befassen, werden besonders benötigt, um diese Ökosysteme zu schützen und zu erhalten im Licht von globalen Klimaveränderungen und steigenden anthropogenen Einwirkungen durch wachsende Küstenbevölkerung und Fischerei. Korallen Riff Organismen, wie Anemonenfische und ihre Seeanemonen Wirte, stellen ideale Kandidaten dar, um die Dynamiken der Larvenzerstreuung zu untersuchen, da die adulten Tiere an ihren Standort gebunden sind und sich kein genetischer Abdruck ihrer Migration ergibt.

Die allgemeine Zielsetzung dieser Arbeit besteht darin molekulare Marker zu entwickeln, zu testen und anzuwenden, um verschiedene Aspekte genetischer und biologischer Vielfalt in Anemonenfischen und ihren obligat symbiotischen Wirten im Indo-Malayischen Archipel zu studieren. Die Ergebnisse sollen zur wissenschaftlichen Beweislage beitragen, die für die Unterstützung des Artenschutzes in diesem 'Hotspot der Artenvielfalt' benötigt wird. Speziell treibt die Studie unser Verständnis von Konnektivität in Anemonenfischen voran, indem populationsgenetische Fallstudien für *Amphiprion perideraion* (**Chapter I**) und *A. sandaracinos* (**Chapter II**) vorgestellt und arteigene Strukturen im Detail diskutiert werden um Unterschiede, mit Blick auf die hohe Ähnlichkeit der Lebensgeschichte und Ökologie dieser Fische, hervorzuheben. Die Daten werden auch für einen artübergreifenden Ansatz zur Konnektivität in Anemonenfischen verwendet, indem regionale Barrieren zu Genfluß in Artverwandten (A*mphiprion perideraion*, *A. sandaracinos, A. clarkii* und *A. ocellaris*) identifiziert

und ihrem Ausmaß skaliert werden, um sie, unter anderem, Forschungsfeldern zugänglicher zu machen, die sich mit der Anwendung und Umsetzung solcher Ergebnisse befassen. Indem ein genomübergreifender (mitochondrialer und nukleare Marker) und artübergreifender (vier Arten) Ansatz gewählt wurde, können Mechanismen, die die Konnektivität in natürlichen Anemonenfischpopulationen steuern, besprochen werden und die Variabilität innerhalb dieses Artensystems untersucht werden. Der potenzielle Einfluss der Anzahl genutzter Wirtsanemonen (Generalist vs. Spezialist) und die Länge der pelagischen Larvenphase werden im Zusammenhang mit der Ausprägung genetischer Struktur unter den Arten besprochen.

Um die enge Verbindung zwischen Anemonenfisch und Wirt einzubeziehen, werden für die Wirtstiere genetische Marker auf ihre Anwendbarkeit in der Artenidentifizierung innerhalb der Actiniaria geprüft (Chapter III). Einem vierten Forschungsansatz folgend, der die Untersuchung von Konnektivität und Diversität in Wirtsanemonen zum Ziel hatte, wird der Versuch dargestellt polymorphe Microsatelliten für diese Fragestellung zu entwickeln (Chapter IV).

1.

Introduction

1.1. Study overview and aims

The terrestrial and marine flora and fauna of the Indo-Pacific Region are among the most diverse in the world, encompassing an epicenter of marine biodiversity, termed the 'Coral Triangle' (CT), covering most of the Indo-Malay Archipelago (Allen & Werner 2002, Carpenter & Springer 2005, Hoeksema 2007, Veron *et al.* 2009) (Fig. 1.1). Only quite recently was the Indo-Malay Archipelago identified as the true center of biodiversity, shifting the focus of conservationists from the Great Barrier Reef (Australia) to regions farther north (Veron 1995). High population densities in coastal communities in this region, poverty and an unregulated exploitation of reef resources are a continuing challenge in efforts to protect and preserve the coral reefs and other coastal ecosystems (Roberts *et al.* 2002, Nañola *et al.* 2011, Barber *et al.* 2014). The exceptional nature of the marine and terrestrial life found in this region has been the focus of much research following these basic questions and building on them:

1. What produced the exceptional species richness in this region?
2. How do we identify and quantify this diversity in order to detect change?
3. How can we preserve biodiversity in light of increasing pressure?

Molecular methods have been a principal tool in efforts to answer these questions. Direct observations of species and their offspring in the enormous expanse of the Indo-Pacific biome (or the sea in general) are difficult and can often not be realized at all. Molecular approaches based on sequence similarity in mitochondrial and nuclear genomes allow important inferences about the past and present processes shaping populations in the sea (Palumbi 1997, Benzie 1999, Hellberg 2009), which can be used to further focus research on emergent patterns, with these or other methods. Effective population sizes for marine species with large species ranges (tens of thousands of kilometers) are said to be on

the order of millions (Crandall *et al.* 2008). Studying the genetic identities of a subset of individuals from a metapopulations can reveal species histories (Timm *et al.* 2008), species identities (Knowlton 2000, Barber & Boyce 2006, Ward *et al.* 2009, Bucklin *et al.* 2011, Huelsken *et al.* 2013), population connectivity (Palumbi 2003, Hedgecock *et al.* 2007, Selkoe *et al.* 2008, Riginos & Liggins 2013), patterns of genetic diversity (Ward *et al.* 1994, Hughes *et al.* 2008) and adaptation, among other things.

The question of what caused the exceptionally high biodiversity in the IMA has been studied and discussed by others and will be summarized here to provide an overview. When contemporary genetic landscapes are studied, the imprint of historical events is often detected and can confound or overlay contemporary patterns. Most population genetic studies struggle to provide evidence that genetic discontinuities are a product of present day geneflow (or the lack thereof), rather than remnants of the evolutionary species history. For the interpretation of data addressing contemporary geneflow scenarios, a look into the recent past of the IMA is indispensable and is therefore included here in some depth.

This thesis presents research addressing 1) the molecular identification of sea anemones (Cnidaria, Hexacorallia, Actiniaria), 2) the genetic population structure detected in anemonefish in the IMA and 3) an example of how this data can be used to drive science based approaches to conservation of marine resources in the IMA by conducting an intrageneric (four *Amphiprion* sp.) synthesis of genetic landscapes. The organisms studied here are obligate symbionts, meaning that their survival rests on the mutually beneficial relationship between sea anemone and anemonefish. The high specialization and strong interdependence of reef associated organisms in general makes them extremely vulnerable to changes in reef health and structure. Both fish and sea anemones studied here are predominantly sessile species, moving only within a small range and achieving their population connectivity (the exchange of reproductive individuals) through pelagic larvae. To drive our understanding of how pelagic larvae connect marine populations across large

expanses of water, species with a sessile adult stage and pelagic larvae are ideal study subjects, as adult migration can be excluded in genetic mixing.

The fourth initial research objective was to study the population structures of sea anemones and their resident anemonefish concordantly, expecting to gain valuable insights to the role of breeding strategy (broadcast spawning in anemones vs. benthic brooding in anemonefish) and larval traits on population connectivity. An extensive microsatellite (short tandem repeats in the nuclear genome) library for three species of tropical sea anemones (*Heteractis crispa*, *Heteractis magnifica*, and *Entacmaea quadricolor*) was constructed and tested, but yielded not useable loci, so that this line of research could not be pursued further at this time. For completeness sake the procedure and the resulting microsatellite library is included here and will hopefully be of use for some yet undeveloped application and/or to other researchers pursuing similar lines of study.

1.2. The Indo-Malay Archipelago

1.2.1. Formation History of the IMA

A turbulent geographic and climatic history and complex contemporary geographic and oceanographic conditions are said to have created and maintained the exceptional marine biodiversity present in the Indo-Malay Archipelago (Roberts *et al.* 2002, Hoeksema 2007, Veron *et al.* 2009). Composed of 24,100 islands (including the Philippines), this archipel is situated within several large shallow sea basins separated by deep submarine trenches, which produces complex geographic and oceanographic dynamics. Exposure to extreme sea level variations during the ~50 glacial cycles of the Pleistocene shaped both terrestrial and marine biodiversity (Woodruff 2010). This region is recognized as the global center of marine biodiversity and has therefore a high priority in conservation efforts (Hoeksema 2007).

Sea level oscillations (up to 140m) during Pleistocene glacial cycles, starting about 3 Ma, led to the repeated exposure and re-submergence of large areas of marine habitat worldwide (Lambeck *et al.* 2002). In the IMA, the shallow Sunda and Sahul shelf areas were completely exposed during the last glacial maximum (LGM, approximately 17 kya) for at least the second time in the last 250 ky (Voris 2000). Receding water, captured in glacial formations elsewhere, left exposed marine habitat behind, forcing marine life to withdraw from coastal margins into deeper water and to the steep continental slopes (Potts 1983, Voris 2000). The waterline retreated at speeds exceeding 100 m /10 y on the continental shelves (Chappell & Thom 1977), eliminating resident coral reefs for the next few thousand years, until rising global temperatures reversed the process.

Sea water low stands not only eliminated large areas of marine habitat, but caused large scale ocean basin and marine habitat fragmentation by surfacing submarine geological features (Voris 2000). In addition to isolating smaller coastal inlets like Tomini Bay in the north-east of Sulawesi, Indonesia, this led to a wide land bridge between Australia and New Guinea and the fusion of all western IMA land structures (including Borneo) with the Eurasian mainland, cutting off the connection between the South China and Java Seas. The Sulu Sea was almost completely closed to water exchange with the adjacent Celebes and South China Seas, also severing the connection of the South China Sea to the central Archipelago. Emerging land structures during sea level low stands greatly reduced ocean basin connectivity in the IMA and minimized the strong current connecting the Indian and Pacific Oceans via the Indonesian Seas, now known as the Indonesian through flow (ITF).

Slowing of the dominant ocean currents within the IMA strongly limited connectivity and mixing of marine life inhabiting different basins or glacial refugia, thereby accelerating allopatric speciation of separated populations (McManus 1985). The ITF, carrying the majority of the 10 million m^3s^{-1} of water connecting the Pacific and Indian Oceans (Gordon *et al.* 2003), is instrumental for region-wide marine connectivity and mixing by acting as a

water mass conveyer belt from the Celebes Sea, through the Makassar Strait, into the Flores Sea and finally the Indian Ocean. Fusion of most of the lesser Sunda Islands and a large reduction in the width of the Timor Passage at sea level low stands decelerated the high speed ITF (contemporary flow estimates: 1 ms^{-1}), and water transport through the Lifamatola Passage into the Banda Sea (Wyrtki 1961). This not only decreased replacement rates and water transport within the Banda and Flores Seas, but also enhanced the impact of freshwater runoff from the emerged shelf areas on marine life, increasing habitat heterogeneity and decreasing suitable marine habitat for coral reefs (Potts 1983, reviewed in Hoeksema 2007).

The gradual reestablishment of water connections within the IMA and contemporary current patterns have not been able to erase the impact of Indian and Pacific Ocean vicariance on regional divergence seen in many species (e.g. Chenoweth *et al.* 1998, Barber *et al.* 2002, Lourie *et al.* 2005, DeBoer *et al.* 2008, Knittweis *et al.* 2008, Timm & Kochzius 2008, Gaither *et al.* 2011). Though sea levels rose continuously after the LGM, recovery rates of marine habitat by flooding where highest between 15 and 10 ka (Hanebuth *et al.* 2000), with a surge of range expansions onto the Sunda shelf about 14 ka (Sathiamurthy & Voris 2006). The genetic signature of this range expansion has been detected in many studies of contemporary population structure (Palumbi 1996, 1997, Benzie 1998, 1999a, b, 2000, Chenowith *et al.* 1998, Barber *et al.* 2000, McCartney *et al.* 2000, Williams 2000, Perrin & Borsa 2001, Chenoweth & Hughes 2003, Uthicke & Benzie 2003, Lind *et al.* 2007). Generally speaking, repeated and prolonged habitat fragmentation, ocean basin isolation and reconnection are believed to have contributed to the overall biodiversity that is found here today.

1.2.2. The Indo-Malay Region: "An Evolutionary Cauldron"

Quite a large number of non-mutually exclusive hypotheses have been formulated to account for the exceptionally high biodiversity in the IMA. Among them are: **1)** centre of origin (McManus 1985, Wilson & Rosen 1998), **2)** centre of accumulation (Ladd 1960) and **3)** region of overlap (Woodland 1983) and various derivatives of these approaches. The "centre of origin" hypothesis postulates that the high biodiversity was born out of the intrinsically complex nature of geological formations constituting the CT and its history of repeated drastic geological and oceanographic change during eustatic sea level oscillations. Others have further argued that selection pressure from the environmental heterogeneity present in the CT drove speciation processes and is responsible for the species richness found there (Briggs 2005, Rocha & Bowen 2008). The "center of accumulation" hypothesis predicts that speciation took place in isolated locations peripheral to the CT and that wind and current patterns ensured the accumulation of novel species in its central area (Ladd 1960, Jokiel & Martinelli 1992). Like the "center of accumulation" hypothesis, the "region of overlap" hypothesis agues for the accumulation of novel species from peripheral locations in the center of the CT. However, this theory directly implicates the Indo-Pacific- Barrier (IPB) as the primary factor responsible for the high species diversity in the CT.

Despite the large body of evidence that has been generated for each of these hypotheses no clear consensus has been reached (Connolly *et al.* 2003, Mora *et al.* 2003, Halas & Winterbottom 2009). This has led to suggestions that the simultaneous and combined effect of all three processes on different scales may most accurately characterize the biotic formation in the CT (Wallace 1997, Randall 1998, Allen 2003; Barber & Bellwood 2005). It remains undisputed that the existence of a globally unsurpassed marine biodiversity in the CT and the Indo-Malay-Archipelago at large is due to the geological history and contemporary geological and oceanographic complexity of the region, still driving speciation today.

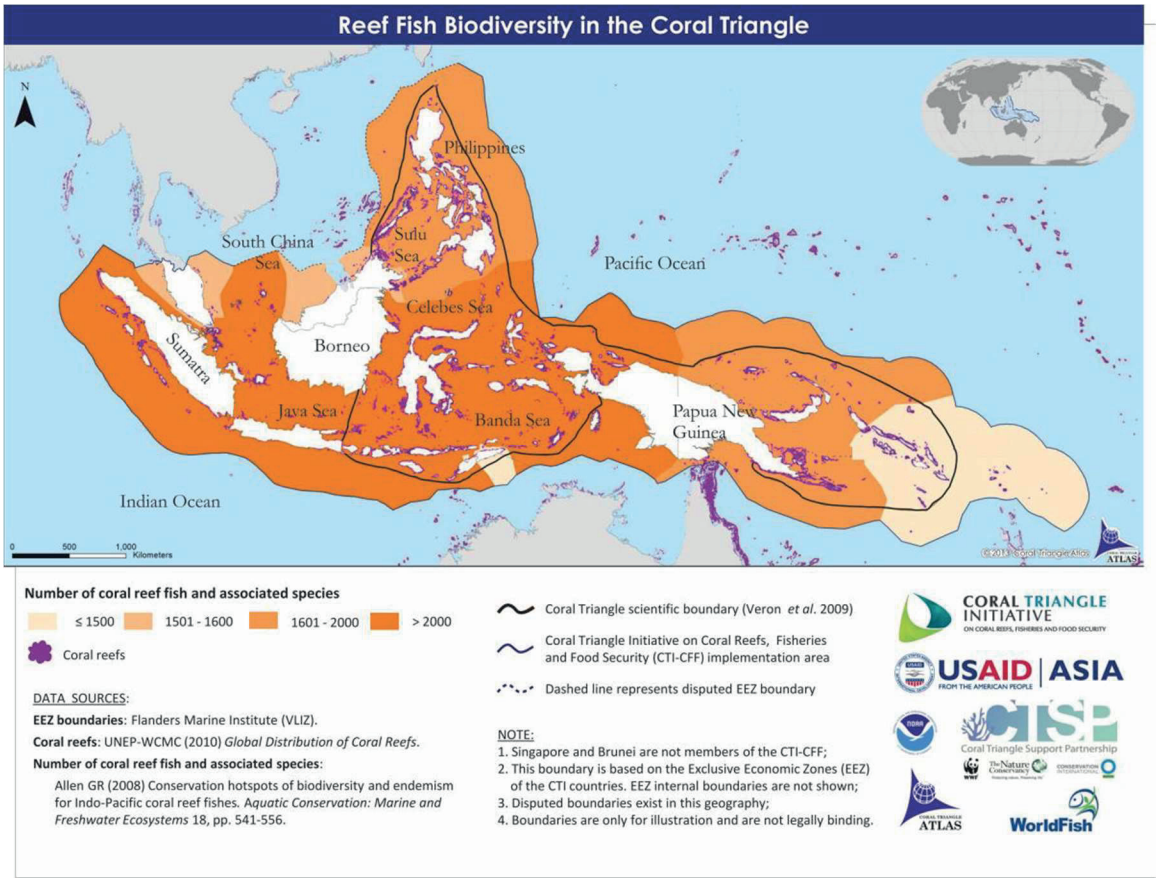


Figure 1.1 Map depicting the Indo-Malay Archipelago, including the Philippines. The scientific boundaries of the Coral Triangle (Veron *et al.* 2009) are drawn in black. Color shadings correspond to reef fish biodiversity (see legend) in the region (after Allen 2008). The author takes no credit for any part of this map, which was provided by the Coral Triangle Initiative with the corresponding figure legend. Merely regional labels were redrawn to fit the format of this document. The overall boundary of the color coded region corresponds to the Economic Exclusive Zones (EEZ) of the participating countries of the Coral Triangle Initiative.

1.3. Coral Reefs and Biodiversity

1.3.1. The Coral Reef Habitat

The IMA, occupying only 10% of the equatorial circumference has the highest concentration of reefs and the highest coral diversity found worldwide (Veron *et al.* 2009). The CT, which covers most of the central and eastern IMA (Fig. 1.1), hosts 76% of the

total global zooxanthellate coral species (corals hosting symbiotic algae), earning it highest global conservation priority. The contemporary coral reefs of the IMA are built predominantly by scleractinian corals, which have seen a global decrease through mass bleaching (loss of symbiont algae and pigments that can cause reef demise), triggered by elevated sea surface temperatures (SST)(Brown 1997). More than one third of global coral species are already considered threatened (Carpenter *et al.* 2008) and coral reefs are regarded as the most highly impacted marine ecosystems (Halpern *et al.* 2008) with an unprecedented decline of coral reefs worldwide (Gardner *et al.* 2003, Pandolfi *et al.* 2005, Bruno & Selig 2007). Climate change predictions describe dire times for coral reefs (Hoegh-Guldberg 1999, Hughes *et al.* 2003, Hoegh-Guldberg *et al.* 2007) resulting in a dramatic impact on reef-associated organisms (Jones *et al.* 2004, Garpe *et al.* 2006) and services, many of which are also important to coastal community subsistence. A mere 1 to 2°C increase of mean SST above the long term average over an extended period (weeks) can exterminate entire reef systems (Hoegh-Guldberg 1999). The calcareous remnants of "dead" reefs can retain their structural integrity for long periods, but no longer provide the biological function needed to maintain its reef dependent communities, of which the large majority will disappear with the demise of functional coral colonies.

Many coral species can subsist without a reef structure in adverse environments (as single polyps or colonies; Veron *et al.* 2011), but most reef-dwelling organisms are highly dependent on reef services and habitat. The immense specialization and niche compartmentalization that explains the coexistence of such an immense number of species in a relatively small area (up to 280 species ha^{-1}, Veron *et al.* 2009) makes these organisms especially vulnerable to changes in the complexity and diversity of the reef system. Many species engage in intricate obligate symbiotic relationships with other organisms, which can produce a cascade of extinction once one of the partners cannot subsist under changed conditions. The symbiotic relationship between anemonefish (Family: Pomacentridae,

Subfamily: Amphiprioninae), their host sea anemones (Phylum: Cnidaria, Order: Actiniaria) and algal symbionts (*Symbodinium* sp.) is a very good example of this type of specialized interdependence, which is found in many reef organisms (Fautin & Allen 1997, Paulay 1997). The anemonefish cannot survive or recruit without a suitable anemone host, which in turn may fall victim to butterflyfishes (Chaetodondidae) predation without the protection of resident anemonefish and/or may succumb to bleaching when algal symbionts are expelled in reaction to thermal, ultra-violet or toxic stress (Saenz-Agudelo *et al.* 2011). The vulnerability of different anemone fish populations to changes in host availability is also linked to their degree of specialization in host acceptance, a result of competition among those species with niche overlap. This fragile linkage between different reef inhabitants is very common in reef systems. It often involves the corals themselves (e.g. Pigmy seahorses, butterfly fish feeding on coral polyps, specialized camouflage, invertebrates inhabiting barren coral structures) and underlines the dependence of these species and species groups on the existence of a reef structure without which they could not exist in the otherwise naturally barren tropical submarine realm.

1.3.2. Threats to Coral Reefs and Reef Organisms

Global and regional developments imperil the shallow water coral reefs of the IMA. Coastal degradation, pollution, overexploitation, and climate change all pose serious threats that require prompt action to avert irreversible damage. This region consists primarily of island states, where 350 million people live within 50 km of the coast, relying on ocean resources for their subsistence, transport and trade (Burke *et al.* 2002, 2012). Economic and population growth is increasing the burdens on reef systems throughout the region (Chou 1997). While regional developments can often be curtailed and local residents empowered, global developments such as climate change, market pressures and pollution require the attention of a global audience.

The list of threats to coral reefs is long and ever increasing (Knowlton 2001, Hoegh-Guldberg 2004). Most problematic is the impact of the burgeoning rural population which not only directly exploits resources (unchecked) but brings pollution and an increased sediment and nutrient load in river discharge, known to negatively affect corals reefs (Pandolfi *et al.* 2003, Bruno & Selig 2007). High nutrient input (eutrophication) via river plumes and agricultural runoff give algae, sponges and other filter-feeding organisms an advantage over the slow coral growth, a problem compounded by increased fishing pressure on herbivores (Kinsey 1988).

The global destruction of reef associated ecosystems like mangrove stands and sea grass beds has also been implicated in reef health and diversity (Shepherd *et al.* 1989, Spalding 1998, Nagelkerken *et al.* 2002). Mangrove logging for mosquito control, aquaculture space, firewood, and building material removes an important buffer zone for nutrient and sediment influx to coastal coral reefs, in addition to destroying the nursery habitat of many species recruiting to coral reefs at some stage of their lives (Nagelkerken *et al.* 2002, 2008). Research has shown that the absence mangroves and sea grass beds in the vicinity of coral reefs can lead to a significant recruitment reduction of species using these ecosystems during development (Nagelkerken *et al.* 2002).

The ornamental fishery is a global multibillion dollar business, exerting strong fishing pressure on sought after species, many of them endemics or extreme habitat specialists (e.g. Hawkins *et al.* 2000, Vagelli & Erdmann 2002, Shuman *et al.* 2005, Maduppa *et al.* 2014). Indonesia controls about 7.5% of the global ornamental fishery market, with marine ornamental exports estimated at US$ 11.66 million (in 2009) to Asian importers in Singapore, Malaysia, China and Hong Kong and in shipments to Europe and the United States (Alfian 2010). According to the Indonesian Ministry of Trade's National Agency for Export Development (NAFED), Indonesia is hoping to expand trade relations to the Middle East in order to secure a larger market share (Alfian 2010). Only 5% of exported marine fishes

stem from fish cultures, with catches from the wild making up the main bulk of the total export volume of 911 Tons (T) in 2009 (Alfian 2010). Where investigated, mortality rates are extremely high (24-51% mortality at an Indonesian export facility, Schmidt & Kunzman 2005; 30-40% mortality at a Philippine facility, Vallejo 1997), suggesting that the actual volume of wild caught specimens is much larger, even if they do not survive or are too damaged (injured) to be exported. Fish culture has been successfully implemented and tested throughout the Coral Triangle, whereby it shows potential to supplement and diversify family incomes, which, nevertheless, will be generated to the largest degree through traditional fisheries and wild caught ornamentals (Pomeroy & Balboa 2004, Ferse *et al.* 2012a, b, Williams *et al.* 2014). To meet market demands, Indonesian exporters are targeting more remote collection sites in West Papua and in the Moluccas, expanding fishing pressure on previously unexploited stocks (Williams *et al.* 2014). While the food fish sector by far outweighs the marine ornamental trade in catch volume in Indonesia and other Asian nations (Erdmann & Pet-Soede 1997, Mous *et al.* 2000, Scales *et al.* 2007, Radjawali 2012, Williams *et al.* 2014), the latter tends to target rare endemics and extreme habitat specialists, which are most vulnerable to exploitation, being limited in their distribution and habitat availability (Hawkins *et al.* 2000).

While blast fishing methods are used primarily by the food fish fishery, the damage inflicted by the detonation devices affects all reef and reef associated organisms. Blast fishing is a common method in Asian waters since WWII, whereby fish, stunned by an underwater explosion, drift to the surface and are there collected by the fishermen (Galvez *et al.* 1989, Djohani 1995, Pet-Soede & Erdmann 1998). This practice creates dead coral rubble fields that do not recover naturally, as scleractinian coral recruits (reef builders) cannot settle on the continuously revolving remnant coral fragments moved by waves and currents (Fox *et al.* 2003, Fox & Caldwell 2006) and are additionally blocked from settling by soft coral and algal overgrowth (Fox *et al.* 2003). Personal observations by the thesis author in the

Spermonde Archipelago, South Sulawesi, Indonesia, have seen a switch to explosive devices that detonate above the reef structure, in such limiting the amount of structural damage to the reef by blast-fishermen. Although long outlawed, blast fishing is still a common fishing method. In personal interviews conducted by the thesis author, several Spermonde fishermen reported paying off Coast Guard officials prior to departing on blast fishing expeditions. "Fines" calculated by the officials were reported to be scaled according to the size of the fleet and number of fishermen participating. Further, fishermen argued that fines were nevertheless low enough to make blast fishing expeditions highly profitable and, due to the preemptively paid "fines", without any danger of prosecution.

1.4. Molecular Methods

1.4.1. Molecular Methods to address species identification and population connectivity

Molecular methods have been increasingly used to study marine life because direct observations on large scales and of big populations are often difficult or not feasible at all. Their application has led to an increased understanding of biogeographic species boundaries, intraspecific population divergence and population connectivity (Palumbi 1994, 1996, Knowlton 2000), in addition it helped in discovering new and cryptic species (marine metazoans reviewed in Bucklin *et al.* 2011). The underlying concept is that populations will diverge (genetically) over time if there is no or little reproductive contact (geneflow) between them, while increased geneflow diminishes population differentiation (Slatkin 1987). On an evolutionary timescale, this process can lead to the formation of highly divergent genetic lineages or new species, either in allopatry (physical isolation of lineages) or in sympatry (ecological niche isolation of lineages) (Campbell & Reese 2002). The latter is thought to occur only rarely and in highly complex environments. In phylogeography and population

genetic analysis the focus is on finding and analyzing genetic markers that reflect the differenciation among populations of the same species (high intraspecific variation), while the aim of barcoding is to find and use genetic markers with few intraspecific and many interspecific (between species) differences. In the molecular research approach selected fragments of the nuclear or mitochondrial genome are inspected for base pair differences in the DNA sequences. Marker choice and analytical methods are chosen based on the research question, spatial scale and resource availability.

1.4.2. Genetic Markers

Genetic markers derive either from the mitochondrial or the nuclear genome and vary in their field of application, with differences found among species and groups of organisms (e.g. plants/animals) (Wan *et al.* 2004). The mitochondrial genome (mtDNA) is exclusively maternally inherited and can therefore be assumed to be present as a single copy, with few exceptions found to date and limited to bivalves (Liu *et al.* 1996, Passamonti & Scali 2001, Curole & Kocher 2002, Serb & Lydeard 2003, Filipowicz *et al.* 2008, Theologidis *et al.* 2008). This circumvents the need for cloning, otherwise required to investigate discrepancies between the maternal and paternal copy of a gene. Nuclear copies of mitochondrial genes (Numt) have also been found, but can be identified as pseudogenes due to differences in the nuclear and mitochondrial genetic code (Zhang & Hewitt 2003). Markers can be coding or non-coding, meaning that the base sequence either translates into a functional amino acid product (coding) or contains no such information (non-coding). Markers used in population genetic analysis and species identifications are selected based on their variability, ease of amplification, neutrality (not under selection), and interspecific coverage (comparability across species). Non-coding regions are generally more variable because mutations are usually of little functional consequence, not leading to errors in transcription.

1.4.3. Barcoding

Barcoding based on species-specific DNA sequence data (barcodes) is an important tool to support efforts at determining and preserving biodiversity and has therefore been used for the assessment of many different organism groups and ecosystems. Of the estimated 11 million species on earth, 1.9 million have been described so far. Many may never be discovered, owing to the highest-ever recorded human-induced species extinction rate (Pimm *et al.* 1995, Chapman 2009). Accompanying the high interest in barcoding studies and an increase in the number of studies focusing on biodiversity since the 1990's, the interest of young researchers in traditional taxonomy has slumped, although some taxonomic knowledge continues to be essential for any biological study (Radulovici *et al.* 2010). Marine biodiversity has not received as much attention as its terrestrial counterpart, but the value of DNA barcoding is increasingly appreciated in marine environments where cryptic speciation, phenotypic plasticity, and complex life cycles are hampering the ability to study and interpret biological communities (Gómez *et al.* 2007, Vrijenhoek 2009, McFadden *et al.* 2011).

A commonly suggested threshold for species detection in barcoding studies is 10X the mean pairwise intra-specific genetic distance (Hebert *et al.* 2003b). To preserve biodiversity, it is essential to know this "variety of life" and be familiar with ways to quantify it. In the marine realm barcoding is particularly valuable because the accessibility of many environments is limited and ways to identify species by taking small (non-lethal) samples and without necessitating the removal of organisms, are an immense resource. Species-barcoding may help to identify unknown specimens (e.g. Hebert *et al.* 2003a, b, 2004a, Hajibabaei *et al.* 2006, Gómez *et al.* 2007) and confirm species identities (Herbert *et al.* 2004b, Moritz & Cicero 2004, Clare *et al.* 2007). Therefore, it fills an important taxonomic gap and has led to the discovery of new and cryptic species, apart from being an immense contribution to ecological

studies dealing with species which are hard to identify or distinguish.

The mitochondrial cytochrome oxidase subunit I (COI) is the most frequently used gene fragment for barcoding (Hebert *et al.* 2003a), usually exhibiting low intraspecific (< 3%) and higher interspecific (10-25%) sequence divergence (Hebert *et al.* 2003b, Stoeckle 2003). If there is no overlap in the degree of sequence variation found at intra- and interspecific taxonomic levels, one speaks of a "barcoding gap", which allows unknown sequences to align with conspecifics in a global database. Barcoding with COI has been successful in a very large number of taxa (Bucklin *et al.* 2011) but remains problematic for basal diploblasts (e.g. sponges, corals, and sea anemones) (Shearer *et al.* 2002, Schröder *et al.* 2003, Wörheide 2006, Shearer & Coffroth 2008). Substitution rates in the mitochondrial genome of cnidarians have been shown to be much slower than those of the nuclear DNA (Shearer *et al.* 2002), contrary to patterns seen in higher metazoans. Identical sequences among congenerics and only distantly related anthozoan (Cnidaria, Anthozoa) taxa are not uncommon, but the mechanisms controlling sequence evolution have not been fully understood. Two alternative evolutionary pathways have been proposed to explain the slow sequence evolution in certain organisms. One hypothesis suggests a slow evolution rate in the mitochondrial genome of early metazoans, followed by a secondary acceleration of sequence evolution in the Bilateria (Shearer *et al.* 2002). In contrast, the slowdown in the Anthozoa could also be a secondarily acquired feature, with the fast sequence evolution being the basic condition in metazoans. Most research on anthozoan sequence evolution in the COI barcoding fragment has focused on scleractinian corals (reef building corals) (e.g. Shearer *et al.* 2002, Shearer & Coffroth 2006, 2008), extrapolating results for the whole of the class. Sea anemones (Cnidaria, Anthozoa, Hexacorallia, Actiniaria) have not been studied in this context, despite the large number of known species.

1.4.4. Alternatives and Additions to Molecular Approaches

Molecular approaches are by no means the only approach taken for species delineation and for the study of population dynamics. However, molecular evidence can often be used to confirm ecological, taxonomic, and historic observations. Strong discordance between molecular results and data generated by other methods (e.g. modeling, direct observation, taxonomic identification, mark/recapture experiments), may point at possible fundamental misunderstandings of an explored phenomenon and/or require revision of common conceptions (e.g. Begga & Waldman 1999, Berumen *et al.* 2010, Bucklin *et al.* 2011, Kool *et al.* 2011).

Examples of methodological approaches relevant to the research presented in this thesis include the direct tracking of larvae in situ or through mark/recapture experiments (Jones *et al.* 2005, Leis *et al.* 2006), including otolith analysis (calcium carbonate accretion in the inner ear) using microchemistry (Swearer *et al.* 2003), though the validity of this method has been questioned recently (*Amphiprion percula*, Berumen *et al.* 2010). Another promising method of marking larval otoliths is through maternal transmission of injected stable isotope signatures on larval otoliths (*Amphiprion melanopus*, Thorrold *et al.* 2006), though detrimental effects on larval growth and survival have not been conclusively studied (Starrs *et al.* 2014). Biophysical dispersal models for the IMA have also been developed (Kool *et al.* 2011, Treml *et al.* 2012, 2015), producing simulated patterns of regional diversity, population connectivity and isolation. These models are a very valuable resource, because the poorly understood temporal scale of population genetic patterns can be interpreted against this background. They focus exclusively on contemporary population connectivity, most relevant when population genetic data is to be used for spatial planning of marine resource management and protection.

REFERENCES

Alfian. 2010. Indonesian Ornamental Fish: Redefining the global status. In H.I. Kresnarini, T. Widayanti, and Z. Bachtar (eds.). Export News Indonesia, pp. 1-10. National Agency for Export Development (NAFED), The Ministry of Trade, Republic of Indonesia.

Allen, G.R., and M. Adrim. 2003. Coral reef fishes of Indonesia. Zool. Studies 42:1-72.

Allen, G.R., and T.B. Werner. 2002. Coral reef fish assessment in the "coral triangle" of southeastern Asia. Environ. Biol. Fish. 65:209-214.

Barber, P.H., M.C.A. Ablan-Lagman, Ambariyanto, R.G.S. Berlinck, D. Cahyani, E.D. Crandall, *et al.* 2014. Advancing biodiversity research in developing countries: the need for changing paradigms. Bull. Mar. Sci. 90:187–210.

Barber, P.H., and D.R. Bellwood. 2005. Biodiversity hotspots: evolutionary origins of biodiversity in wrasses (Halichoeres: Labridae) in the Indo-Pacific and new world tropics. Phylogenet. Evol. 35:235-253.

Barber, P.H., and Boyce, S.L. 2006. Estimating diversity of Indo-Pacific coral reef stomatopods through DNA barcoding of stomatopod larvae. Proc. R. Soc. B 273: 2053-2061.

Barber, P.H., S.H. Cheng, M.V. Erdmann, K. Tengardjaja, and Ambariyanto. 2011. Evolution and conservation of marine biodiversity in the Coral Triangle: insights from stomatopod Crustacea. Crustacean Iss.19:129-156.

Barber, P.H., S.R. Palumbi, M.V. Erdmann, and M.K. Moosa. 2000. A marine Wallace's line? Nature 406:692-693.

Barber, P.H., S.R. Palumbi, M.V. Erdmann, and M.K. Moosa. 2002. Sharp genetic breaks among populations of *Haptosquilla pulchella* (Stomatopoda) indicate limits to larval transport: patterns, causes, and consequences. Mol. Ecol. 11:659-674.

Begga, G.A., and J.R. Waldman. 1999. A holistic approach to fish stock identification. Fish. Res. 43:35-44.

Benzie, J. 1999a. Genetic structure of coral reef organisms: ghosts of dispersal past. Am. Zool. 39:131-145.

Benzie, J.A.H. 1999b. Major genetic differences between crown-of-thorns starfish (*Acanthaster planci*) populations from the Indian and Pacific Oceans. Evolution 53:1782-1795

Benzie, J.A.H. 1998. Genetic structure of marine organisms and SE Asian biogeography. In Hall, R. and J.D. Holloway (eds). Biogeography and Geological Evolution of SE Asia, pp.197-209. Backhuys Publishers, Leiden.

Benzie, J.A.H. 2000. The detection of spatial variation in widespread marine species: methods and bias in the analysis of crown-of-thorns starfish population structure. Hydrobiologia 420:1-14.

Berumen, M.L., H.J. Walsh, N. Raventos, S. Planes, G.P. Jones, V. Starczak, and S.R. Thorrold. 2010. Otolith geochemistry does not reflect dispersal history of clownfish larvae. Coral Reefs 29:883.

Briggs, J.C. 2005. The marine East Indies: diversity and speciation. J. Biogeogr. 32:1517-1522.

Brown, B. E. 1997. Coral bleaching: causes and consequences. Coral Reefs 16:129-138.

Bruno, J.F., and E.R. Selig. 2007. Regional decline of coral cover in the Indo-Pacific: timing, extent, and subregional comparisons. PLoS One 2:e711.

Bucklin, A., D. Steinke, and L. Blanco-Bercial. 2011. DNA barcoding of marine metazoa. Ann. Rev. Mar. Sci. 3:471-508.

Burke, L., K. Reytar, M.D. Spalding, and A. Perry. 2012. Reefs at risk revisited in the Coral Triangle. World Resources Institute, Washington, DC.

Burke, L., E. Selig, and M. Spalding. 2002. Reefs at risk in Southeast Asia. World Resources Institute, Washington, DC.

Campbell, N., and J. Reese. 2002. Biology (6th ed.). Benjamin Cummings, San Francisco.

Carpenter, K.E., and V. Springer. 2005. The center of the center of marine shore fish biodiversity: the Philippine Islands. Environ. Biol. Fish. 72:467-480.

Carpenter, K.E., M. Abrar, G. Aeby, R.B. Aronson, S. Banks, A. Bruckner, *et al.* 2008. One-third of reef-building corals face elevated extinction risk from climate change and local impacts. Science 321:560-563.

Chapman, A.D. 2009. Numbers of living species in Australia and the World (2nd ed.). Australian Biodiversity Information Services, Toowoomba, Australia.

Chappell, J. and B.G. Thom. 1977. Sea levels and coasts. In J. Allen, J. Golson, and R. Jones (eds.). Sunda and Sahul: Prehistoric Studies in Southeast Asia, Melanesia and Australia, pp. 275–291. Academic Press, London.

Chenoweth, S.F., and J.M. Hughes. 2003. Oceanic interchange and nonequilibrium population structure in the estuarine dependent Indo-Pacific tasselfish, *Polynemus sheridani*. Mol. Ecol. 12:2387-2397.

Chenoweth, S.F., J.M. Hughes, C.P. Keenan, and S. Lavery. 1998. When oceans meet: a teleost shows secondary integradation at an Indian-Pacific interface. Proc. R. Soc. Lond. B 265:415-420.

Chou, L. M. 1997. The status of Southeast Asian coral reefs. Proceedings of the Eighth International Coral Reef Symposium 1:317-322.

Connolly, S.R., D.R. Bellwood, and T.P. Hughes. 2003. Indo-Pacific biodiversity of coral reefs: deviations from a mid-domain model. Ecology 84:2178-2190.

Crandall, E.D., M.A. Frey, R.K. Grosberg, and P.H. Barber. 2008. Contrasting demographic history and phylogeographical patterns in two Indo-Pacific gastropods. Mol. Ecol. 17:611-626.

Curole, J.P., and Kocher T.D. 2002. Ancient sex-specific extension of the cytochrome c oxidase II gene in bivalves and the fidelity of doubly-uniparental inheritance. Mol. Biol. Evol. 19:1323-1328.

DeBoer, T.S., M.D. Subia., M.V. Erdmann, K. Kovitvongsa, and P.H. Barber. 2008. Phylogeography and limited genetic connectivity in the endangered boring giant clam across the Coral Triangle. Conserv. Biol. 22:1255-1266.

Djohani, R. 1995. The combat of dynamite and cyanide fishing in Indonesia, p. 47. The Nature Conservancy, Jakarta.

Erdmann M.V., and L. Pet-Soede. 1997. How fresh is too fresh? The live reef food fish trade in Eastern Indonesia. SPC Live Reef Fish Info Bull. 3:41-43.

Fautin, D.G., and G.R. Allen. Revised edition 1997. Anemone fishes and their host sea anemones. Western-Australian Museum, Perth, Australia.

Ferse S.C.A., M. Glaser, M. Neil, and K.S. Máñez. 2012a. To cope or to sustain? Eroding long-term sustainability in an Indonesian coral reef fishery. Reg. Environ. Change 14:2053-2065.

Ferse S.C.A., L. Knittweis, G. Krause, A. Maddusil, and M. Glaser. 2012b. Livelihoods of ornamental coral fishermen in south Sulawesi/Indonesia: implications for management. Coast. Manag. 40:525.555.

Filipowicz, M., A. Burzyń ski, B. Smietanka , and R. Wenne. 2008. Recombination in mitochondrial DNA of European mussels *Mytilus*. J. Mol. Evol. 67:377-388.

Fox, H.E., and Caldwell R.L. 2006. Recovery from blast fishing on coral reefs: a tale of two scales. Ecol. Appl. 16:1631-1635.

Fox, H.E., J.S. Pet, R. Dahuri, and R.L. Caldwell. 2003. Recovery in rubble fields: long-term impacts of blast fishing. Mar. Pollut. Bull. 46:1024-1031.

Gaither, M.R., B.W. Bowen, T.-R. Bordenave, L.A. Rocha, S.J. Newman, J.A. Gomez, *et al.* 2011. Phylogeography of the reef fish *Cephalopholis argus* (Epinephelidae) indicates Pleistocene isolation across the Indo-Pacific Barrier with contemporary overlap in The Coral Triangle. BMC Evol. Biol. 11:189.

Galvez, R., T.C. Hingco, C. Bautistsa, and M.T. Tungpalar. 1989. Sociocultural dynamics of blast fishing and sodium cyanide fishing in two fishing villages in the Lingayen Gulf area. In: G. Silvestre, E. Miclat, and T.-E.Chua, (eds.). Towards sustainable development of the coastal resources of Lingayen Gulf, Philippines, pp. 43-62. International Centre for Living Aquatic Resources and Management, Manila.

Gardner, T.A., I.M. Cote, J.A. Gill, A. Grant, and A.R. Watkinson. 2003. Long-term region-wide declines in Caribbean corals. Science 301:958-960.

Garpe, K.C., S.A.S. Yahya, U. Lindahl, and M.C. Ohman. 2006. Long-term effects of the 1998 coral bleaching event on reef fish assemblages. Mar. Ecol. Prog. Ser. 315:237-247.

Gómez, A., P.J. Wright, D.H. Lunt, J.M. Cancino, G.R. Carvalho, and R.N. Hughes. 2007. Mating trials validate the use of DNA barcoding to reveal cryptic speciation of a marine bryozoan taxon. Proc. R. Soc. Lond. B 274:199-207.

Gordon, A.L., R.D. Susanto, and K. Vranes. 2003. Cool Indonesian through flow as a consequence of restricted surface layer flow. Nature 425:21-25.

Hajibabaei, M., D.H. Janzen, J.M. Burns, W. Hallwachs, and P.D.N. Hebert. 2006. DNA barcodes distinguish species of tropical Lepidoptera. Proc. Natl. Acad. Sci. USA 103:968-971.

Halas, D., and R. Winterbottom. 2009. A phylogenetic test of multiple proposals for the origins of the East Indies coral reef biota. J. Biogeogr. 36:1847-1860.

Hanebuth, T., K. Stattegger, and P.M. Grootes. 2000. Rapid flooding of the Sunda Shelf: a late-glacial sea-level record. Science 288:1033-1035.

Halpern, B.S., S. Walbridge, K.A. Selkoe, C.V. Kappel, F. Micheli, C. D'Agrosa, *et al.* 2008. A global map of human impact on marine ecosystems. Science 319:948-952.

Hawkins, J.P., C.M. Roberts, and V. Clark. 2000. The threatened status of restricted-range coral reef fish species. Anim. Conserv. 3:81-88.

Hebert, P.D.N., A. Cywinska, S.L. Ball, and J.R. deWaard. 2003a. Biological identifications through DNA barcodes. Proc. R. Soc. Lond. B 270:313-321.

Hebert P.D.N., E.H. Penton, J.M. Burns, D.H. Janzen, and W. Hallwachs. 2004a. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proc. Natl. Acad. Sci. USA 101:14812-17.

Hebert P.D.N., S. Ratnasingham, and J.R. deWaard. 2003b. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proc. R. Soc. Lond. B 270:96-99.

Hebert P.D.N., M.Y. Stoeckle, T.S. Zemlak, and C.M. Francis. 2004b. Identification of birds through DNA barcodes. PLoS Biol. 2:e312.

Hedgecock, D., P.H. Barber, and S. Edmands. 2007. Genetic approaches to measuring connectivity. Oceanography 20:70-79.

Hellberg, M.E. 2009. Gene Flow and Isolation among Populations of Marine Animals. Annu. Rev. Ecol. Evol. S. 40:291-310.

Hoegh-Guldberg, O. 1999. Climate change, coral bleaching and the future of the world's coral reefs. Mar. Freshwater Res. 50:839-866.

Hoegh-Guldberg, O. 2004. Coral reefs in a century of rapid environmental change. Symbiosis 37:1-31.

Hoegh-Guldberg, O., P.J. Mumby, A.J. Hooten, R.S. Steneck, P. Greenfield, E. Gomez, *et al.* 2007. Coral reefs under rapid climate change and ocean acidification. Science 318:1737-1742.

Hoeksema, B.W. 2007. Delineation of the Indo-Malayan centre of maximum marine biodiversity: the Coral Triangle. In: W. Renema (ed.). Biogeography, time and place: distributions, barriers and islands, pp. 117–178. Springer Netherlands, Dordrecht.

Huelsken, T., J. Keyse, L. Liggins, S. Penny, E.A. Treml, and C. Riginos. 2013. A Novel Widespread Cryptic Species and Phylogeographic Patterns within Several Giant Clam Species (Cardiidae: *Tridacna*) from the Indo-Pacific Ocean. PLoS ONE 8: e80858. doi:10.1371/journal.pone.008085

Hughes, A.R., B.D. Inouye, M.T. Johnson, N. Underwood, and M. Vellend. 2008. Ecological consequences of genetic diversity. Ecol. Lett. 11:609-623.

Hughes, T.P., A.H. Baird, D.R. Bellwood, M. Card, S.R. Connolly, C. Folke, *et al.* 2003. Climate change, human impacts and the resilience of coral reefs. Science 301:929-933.

Jokiel, P., and F.J. Martinelli. 1992. The vortex model of coral reef biogeography. J. Biogeogr. 19:449-458.

Jones, G.P., M.J. McCormick, M. Srinivasan, and J.V. Eagle. 2004. Coral decline threatens fish biodiversity in marine reserves. Proc. Natl. Acad. Sci. USA 101:8251-8253.

Jones, G.P., S. Planes, and S.R. Thorrold. 2005. Coral reef fish larvae settle close to home. Curr. Biol. 15:1314–1318.

Kinsey, D.W. 1988. Responses of coral reef systems to elevated nutrients. In: C.L. Baldwin (ed.). Nutrients in the Great Barrier Reef region, pp. 55-65. Workshop Series No. 10. GBRMPA, Townsvillle.

Knittweis, L., W.E. Kraemer, J. Timm, and M. Kochzius. 2008. Genetic structure of *Heliofungia actiniformis* (Scleractinia: Fungiidae) populations in the Indo-Malay Archipelago: implications for live coral trade and management efforts. Conserv. Genet. 10:241-249.

Knowlton, N. 2000. Molecular genetic analyses of species boundaries in the sea. Hydrobiologia 420:73-90.

Knowlton, N. 2001. The future of coral reefs. Proc. Natl. Acad. Sci. USA 98:5419-5425.

Kool, J.T., C.B. Paris, P.H. Barber, and R.K. Cowen. 2011. Connectivity and the development of population genetic structure in Indo-West Pacific coral reef communities. Glob. Ecol. Biogeogr. 20:695-706.

Lambeck, K., T.M. Esat, and E.K. Potter. 2002. Links between climate and sea levels for the past three million years. Nature 419:199-206.

Ladd, H.S. 1960. Origin of the Pacific Island molluscan fauna. Am. J. Sci. 258:137-150.

Leis, J.M., A.C. Hay, and T. Trnski. 2006. *In situ* behavioural ontogeny in larvae of three temperate, marine fishes. Mar. Biol. 148, 655-669.

Liu, H.P., J.B. Mitton, and S.K.Wu. 1996. Paternal mitochondrial DNA differentiation far exceeds maternal mitochondrial DNA and allozyme differentiation in the fresh-water mussel, *Anodonta grandis grandis*. Evolution 50:952-957.

Lind, C.E., B.S. Evans, J.J.U. Taylor, and D.R. Jerry. 2007. Population genetics of a marine bivalve, *Pinctada maxima*, throughout the Indo-Australian Archipelago shows differentiation and decreased diversity at range limits. Mol. Ecol. 16:5193-5203.

Lourie, S., D.M. Green, and C.J. Vincent. 2005. Dispersal, habitat differences, and comparative phylogeography of Southeast Asian seahorses (Syngnathidae: *Hippocampus*). Mol. Ecol. 14:1073-1094.

Maduppa, H.H., K. von Juterzenka, M. Syakir, and M. Kochzius. 2014. Socio-economy of marine ornamental fishery and its impact on the population structure of the clown anemone fish, *Amphiprion ocellaris*, and its host anemones in Spermonde Archipelago, Indonesia. Ocean Coast. Manage. 100:41-50.

McCartney, M.A., G. Keller, and H.A. Lessios. 2000. Dispersal barriers in tropical oceans and speciation in Atlantic and eastern Pacific sea urchins of the genus *Echinometra*. Mol. Ecol. 9:1391-1400.

McFadden, C. S., Y. Benayahu, E. Pante, J.N. Thoma, P.A. Nevarez, and S.C. France. 2011. Limitations of mitochondrial gene barcoding in Octocorallia. Mol. Ecol. Resour. 11:19-31.

McManus, J.W. 1985. Marine speciation, tectonics, and sea-level changes in Southeast Asia. In: Proceedings of the Fifth International Coral Reef Congress, pp. 133-138. Antenne Museum-EPHE, Moorea, Tahiti.

Mora, C., P.F. Sale, J.P. Kritzer, and S.A. Ludsin. 2003. Patterns and processes in reef fish diversity. Nature 421:933-936.

Moritz C, Cicero C. 2004. DNA barcoding: promise and pitfalls. PLoS Biol. 2:1529-1531.

Mous, P.J., L. Pet-Soede, M. Erdmann, H.S.J. Cesar, Y. Sadovy, and J.S. Pet. 2000. Cyanide fishing on Indonesian coral reefs for the live food fish market-What is the problem? SPC Live Reef Fish Info Bull. 7:20-27.

Nagelkerken, I., S.J.M. Blaber, S. Bouillon, P. Green, M. Haywood, L.G. Kirton, *et al.* 2008. The habitat function of mangroves for terrestrial and marine fauna: A review. Aquat. Bot. 89:155-185.

Nagelkerken, I., C.M. Roberts, G. van der Velde, M. Dorenbosch, M.C. van Riel, E. Cocheret de la Morinière, and P.H. Nienhuis. 2002. How important are mangroves and seagrass beds for coral-reef fish? The nursery hypothesis tested on an island scale. Mar. Ecol. Prog. Ser. 244:299-305.

Nañola, C.L.J., P.M. Aliño, and K.E. Carpenter. 2011. Exploitation-related reef fish species richness depletion in the epicenter of marine biodiversity. Environ. Biol. Fish. 90:405-420.

Palumbi, S.R. 1994. Genetic divergence, reproductive isolation and speciation in the sea. Annu. Rev. Ecol. Syst. 25:547-572.

Palumbi, S.R. 1996. What can molecular genetics contribute to marine biogeography? An urchin's tale. J. Exp. Mar. Biol. Ecol. 203:75-92.

Palumbi, S.R. 1997. Molecular biogeography of the Pacific. Coral Reefs 16:47-52.

Palumbi, S.R. 2003. Population genetics, demographic connectivity, and the design of marine reserves. Ecol. Appl. 13:146-158.

Pandolfi, J.M., R.H. Bradbury, E. Sala, T.P. Hughes, K.A. Bjorndal, R.G. Cook, *et al.* 2003. Global Trajectories of the Long-Term Decline of Coral Reef Ecosystems. Science 301: 955-958.

Pandolfi, J.M., J.B.C. Jackson, N. Baron, R.H. Bradbury, H.M. Guzman, T.P. Hughes, *et al.* 2005. Ecology - are US coral reefs on the slippery slope to slime? Science 307:1725-1726.

Passamonti, M., and V. Scali. 2001. Gender-associated mitochondrial DNA heteroplasmy in the venerid clam *Tapes philippinarum* (Mollusca, Bivalvia) Curr. Genet. 39:117-124.

Paulay, G. 1997. Diversity and distribution of reef organisms. In: C.E. Birkeland (ed.). Life and Death of Coral Reefs, pp. 298-353. Chapman & Hall, NY.

Perrin, C., and P. Borsa. 2001. Mitochondrial DNA analysis of the geographic structure of Indian scad mackerel in the Indo-Malay archipelago. J. Fish Biol. 59:1421-1426.

Pimm, S.L., G.J. Russel, J.L. Gittleman, and T.M. Brooks. 1995. The future of biodiversity. Science 269:347-350.

Pomeroy, R.S., and C. Balboa. 2004. The financial feasibility of small-scale marine ornamental aquaculture in the Philippines. Asian Fish. Sci. 17:365-376.

Potts, D.C. 1983. Evolutionary disequilibrium among Indo-Pacific corals. Bull. Mar. Sci. 33: 619-632.

Radjawali I. 2012. Examining local conservation and development: live reef food fishing in Spermonde Archipelago, Indonesia. J. Integr. Coast. Zone Manag. 12:545-557.

Radulovici, A.E., P. Archambault, and F. Dufresne. 2010. DNA Barcodes for Marine Biodiversity: Moving fast forward? Diversity 2:450-472.

Randall, J.E. 1998. Zoogeography of shorefishes of the Indo-Pacific region. Zool. Studies 37:227-268.

Riginos, C., and L. Liggins. 2013. Seascape genetics: populations, individuals, and genes marooned and adrift. Geogr. Compass. 7:197-216.

Roberts, C.M., C.J. McClean, J.E.N. Veron, J.P. Hawkins, G.R. Allen, D.E. McAllister, *et al.* 2002. Marine biodiversity hotspots and conservation priorities for tropical reefs. Science 295:1280-1284.

Rocha, L.A., and B.W. Bowen. 2008. Speciation in coral reef fishes. J. Fish. Biol. 72:1101-1121.

Saenz-Agudelo, P., G.P. Jones, S.R. Thorrold, and S. Planes. 2011. Detrimental effects of host anemone bleaching on anemonefish populations. Coral Reefs 30:497-506.

Sathiamurthy, E., and H.K. Voris. 2006. Maps of Holocene sea level transgression and submerged lakes on the Sunda Shelf. Nat. His. J. Chulalongkorn University. Suppl 2:1-43.

Scales, H., A. Balmford, and A.Manica. 2007. Impacts of the live reef fish trade on populations of coral reef fish off northern Borneo. Proc. R. Soc. B. 274:989-994.

Selkoe, K.A., C.M. Henzler, and S.D. Gaines. 2008. Seascape genetics and the spatial ecology of marine populations. Fish Fish. 9:363-377.

Serb, J.M., and C. Lydeard. 2003. Complete mtDNA sequence of the North American freshwater mussel, *Lampsilis ornata* (Unionidae): an examination of the evolution and phylogenetic utility of mitochondrial genome organization in Bivalvia (Mollusca). Mol. Biol. Evol. 20:1854-1866.

Shearer, T.L., and M.A. Coffroth. 2006. Coral recruitment patterns at the Flower Garden Banks and the Florida Keys using genetic methods to identify recruits. Mar. Ecol. Prog. Ser. 306:133-142.

Shearer, T.L., and M.A. Coffroth. 2008. Barcoding corals: limited by interspecific divergence, not intraspecific variation. Mol. Ecol. Res. 8:247-255.

Shearer, T.L., M.J.H. Van Oppen, S.L. Romano, and G. Wörheide. 2002. Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). Mol. Ecol. 11:2475-2487.

Shepherd, S.A., A.J. McComb, D.A. Bulthuis, V. Neverauskas, D.A. Steffensen, and R. West. 1989. Decline of seagrasses. In: A. W. D. Larkum, A. J. McComb and S. A. Shepherd (eds.). Biology of seagrasses, pp. 346-393. Elsevier, Amsterdam.

Shuman, C., Hodgson, G., Ambrose, R., 2005. Population impacts of collecting sea anemones and anemone fish for the marine aquarium trade in the Philippines. Coral Reefs 24:564-573

Slatkin, M. 1987. Gene flow and the geographic structure of natural-populations. Science 236:787-792.

Spalding, M.D. 1998. Patterns of biodiversity in coral reefs and mangroves: global and local scales. PhD thesis, University of Cambridge

Starrs, D., J.T. Davis, J. Schlaefer, B.C. Ebner, S.M. Eggins, and C.J. Fulton. 2014. Maternally transmitted isotopes and their effects on larval fish: a validation of dual isotopic marks within a meta-analysis context. Can. J. Fish. Aquat. Sci. 71:387-397.

Stoeckle, M. 2003. Taxonomy, DNA and the barcode of life. Bioscience 53:2-3.

Swearer, S.E., G.E. Forrester, M.A. Steele, A.J. Brooks, and D.W. Lea. 2003. Spatio-temporal and interspecific variation in otolith trace-elemental fingerprints in a temperate estuarine fish assemblage. Estuar. Coast. Shelf S. 56:1111-1123.

Theologidis, I., S. Fodelianakis, M.B. Gaspar, and E. Zouros. 2008. Doubly uniparental inheritance (DUI) of mitochondrial DNA in *Donax trunculus* (Bivalvia: Donacidae) and the problem of its sporadic detection in Bivalvia. Evolution 62:959-970.

Thorrold, S.R., G.P. Jones, S. Planes, and J.A. Hare. 2006. Transgenerational marking of embryonic otoliths in marine fishes using barium stable isotopes. Can. J. Fish. Aquat. Sci. 63:1193-1197.

Timm, J., M. Figiel, and M. Kochzius. 2008. Contrasting patterns in species boundaries and evolution of anemonefishes (Amphiprioninae, Pomacentridae) in the centre of marine biodiversity. Mol. Phylogenet. Evol. 49:268-276.

Timm, J., and M. Kochzius. 2008. Geological history and oceanography of the Indo- Malay Archipelago shape the genetic population structure in the false clown anemonefish (*Amphiprion ocellaris*). Mol. Ecol. 17:3999-4014.

Treml, E.A., J.J. Roberts, Y. Chao, P.N. Halpin, H.P. Possingham, and C. Riginos. 2012. Reproductive output and duration of the pelagic larval stage determine seascape-wide connectivity of marine populations. Integr. Comp. Biol. 52:525-537.

Treml, E.A., J.J. Roberts, P.N. Halpin, H.P. Possingham and C. Riginos. 2015. The emergent geography of biophysical dispersal barriers across the Indo-West Pacific. Divers. Distrib. 21:465-476.

Uthicke, S., and J.A. Benzie. 2003. Gene flow and population history in high dispersal marine invertebrates: mitochondrial DNA analysis of *Holothuria nobilis* (Echinodermata: Holothuroidea) populations from the Indo-Pacific. Mol. Ecol. 12:2635-2648.

Vagelli, A. and M. Erdmann. 2002. First comprehensive ecological survey of the Banggai cardinalfish, *Pterapogon kauderni*. Env. Biol. Fish. 63:1-8.

Veron, J.E.N. 1995. Corals in space and time: the biogeography and evolution of the Scleractinia. Cornell University Press, Ithaca, New York

Veron, J.E.N., L.M. DeVantier, E. Turak, A.L. Green, S. Kininmoth, M. Stafford-Smith, *et al.* 2009. Delineating the Coral Triangle. Galaxea 11:91-100.

Veron, J.E.N., E. Turak, L.M. DeVantier, M.G. Safford-Smith, and S. Kininmonth. 2011. Coral Geographic. www.coralgeographic.com, version 1.11 Aust. Inst. Mar. Sci.

Voris, H.K. 2000. Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. J. Biogeogr 27:1153-1167.

Vrijenhoek, R.C. 2009. Cryptic species, phenotypic plasticity, and complex life histories: Assessing deep-sea faunal diversity with molecular markers. Deep-Sea Res. II 56:1713-1723.

Wallace, C.C. 1997. The Indo-Pacific center of coral diversity re-examined at species level. In: H. A. Lessios and I. G. Macintyre (eds.). Proceedings of the 8th International Coral Reef Symposium, pp. 365-370. Smithsonian Tropical Research Institute, Balboa (Panama).

Wan, Q.-H., H. Wu, T. Fujihara, and S.-G. Fang. 2004. Which genetic marker for which conservation genetics issue? Electrophoresis 25:2165-2176.

Ward, R.D., R. Hanner, and P.D. Hebert. 2009. The campaign to DNA barcode all fishes, FISH-BOL. J. Fish Biol. 74:329-356.

Ward, R.D., M. Woodwark, and D.O.F. Skibinski. 1994. A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. J. Fish Biol. 44:213-232.

Williams, S.L., N. Janetski, J. Abbott, S. Blankenhorn, B. Cheng, R.E. Crafton, *et al.* 2014. Ornamental Marine Species Culture in the Coral Triangle: Seahorse Demonstration Project in the Spermonde Islands, Sulawesi, Indonesia. Environ. Manage. 54:1342-1355.

Williams, S.T. 2000. Species boundaries in the starfish genus *Linckia*. Mar. Biol. 136:137-148.

Wilson, M.E.J. and B.R. Rosen. 1998. Implications of paucity of corals in the Paleogene of SE Asia: plate tectonics or centre of origin? In: R. Hall and J.D. Holloway (eds.). Biogeography and geological evolution of SE Asia, pp. 165-195. Backhuys Publishers, Leiden.

Wörheide, G. 2006. Low variation in partial cytochrome oxidase subunit I (COI) mitochondrial sequences in the coralline demosponge Astrosclera willeyana across the Indo-Pacific. Mar. Biol. 148:907-912

Woodland, D.J. 1983. Zoogeography of the Siganidae (Pisces): an interpretation of distribution and richness patterns. Bull. Mar. Sci. 33:713-717.

Woodruff, D.S. 2010. Biogeography and conservation in South-East Asia: how 2.7 million years of repeated environmental fluctuations affect today's patterns and the future of the remaining refugial-phase biodiversity. Biodivers. Conserv. 19:919-941.

Wyrtki, K. 1961. Physical oceanography of the Southeast Asian waters. NAGA Report, vol. 2. University of California, San Diego, Scripps Institute of Oceanography, San Diego.

Zhang, D.-X., and G.M. Hewitt. 2003. Nuclear DNA analyses in genetic studies of populations: practice, problems and prospect. Mol. Ecol. 12:563-584

2.

Thesis Aims

2. Thesis Aims

The main objectives of this thesis were to:

1) Investigate the genetic population structures of *Amphiprion perideraion* and *Amphiprion sandaracinos* across a large portion of their range. Results were expected to contribute to our understanding of factors affecting diversity and population connectivity of sessile marine species.

2) Identify common geneflow barriers among all four anemonefish species studied so far, *A. sandaracinos* (Dohna *et al.* in preparation), *A. perideraion* (Dohna *et al.* 2015), *Amphiprion ocellaris* (Timm & Kochzius 2008; Timm *et al.* 2012) and *Amphiprion clarkii* (Rodriguez Moreno, unpublished data), by applying an integrative approach. This type of approach is urgently needed to consolidate multispecies patterns to a format that can be directly applied to spatial management of marine resources in the Indo-Malay Archipelago and elsewhere. The research was expected to identify regional diversity gradients and common genetic barriers, which are important factors to consider for the protection of (genetic) biodiversity and population persistence under pressure from fisheries and habitat reduction.

3) Develop a set of microsatellite loci with which to study diversity and population connectivity in three species of tropical sea anemones. This approach was expected to provide direct evidence for the role of spawning strategy on genetic structure by comparing the anemonefish (benthic brooders) and symbiont sea anemones (broadcast spawners) genetic patterns derived from the analysis of identical sampling sites. This is a very fundamental question in the field. Unfortunately, this objective could not be realized, because no suitable loci were found within the frame of the study.

4) Test different molecular markers of high potential for genetic fingerprinting (barcoding) of sea anemones, if the conventional barcoding marker (5' fragment of the Cytochrome Oxidase Subunit I) would fail to delineate species. Because barcoding has been problematic in some members of the Hexacorallia (main focus on scleractinian corals and zoantharians), negative results have been extrapolated for the group as a whole, without direct evidence.

3.

Publication Outline

3. Publication Outline

Publication 1

Title: Limited connectivity and a phylogeographic break characterize populations of the pink anemonefish, *Amphiprion perideraion*, in the Indo-Malay Archipelago: inferences from a mitochondrial and microsatellite loci

Authors: Tina Dohna, Janne Timm, Lemia Hamid and Marc Kochzius

Journal: Ecology and Evolution, Volume 5, Issue 8, Pages: 1717–1733, April 2015

The idea for this study was developed by Tina Dohna, Janne Timm and Marc Kochzius. Sampling in the field was done by Janne Timm, Marc Kochzius and Agus Nuryanto. Mitochondrial sequences were produced by Lemia Hamid as part of her Masters Thesis and Christian Seidel as part of a HiWi contract. Microsatellites were amplified and scored by Tina Dohna. All population genetic analyses of both datasets were carried out by Tina Dohna, as Lemia Hamid's sequence alignment erroneously included some sequences of another anemonefish. The manuscript was written by Tina Dohna, with revisions by Marc Kochzius, Janne Timm and several anonymous reviewers.

Publication 2

Title: Striving for fusion: Phylogeography of the orange anemonefish, *Amphiprion sandaracinos*, as a basis for a synergized genetic landscape of four congeners (*Amphiprion* spp.)

Authors: Tina Dohna, Marc Kochzius, Maria Liebsch, Melina Rodríguez Moreno and Janne Timm

Journal: anticipated submission to Diversity and Distributions

The idea for this study was developed by Tina Dohna, Janne Timm and Marc Kochzius. Sampling in the field was done by Marc Kochzius, Janne Timm and Agus Nuryanto. Mitochondrial sequences of *A. sandaracinos* were produced by Maria Liebsch as part of her Master Thesis. Microsatellites for *A. sandaracinos* were amplified and scored by Tina Dohna. Melina Rodriguez tested and amplified microsatellite loci for *Amphiprion clarkii* as part of her diploma thesis, while mitochondrial sequences were produced by Janne Timm. Overall and pairwise population differentiation index for *Amphiprion ocellaris* was derived from a publication by Janne Timm. Population genetic analysis of *A. sandaracinos* and statistical

analyses with all four species were carried out by Tina Dohna. The manuscript was written by Tina Dohna with revisions by Janne Timm and Marc Kochzius.

Publication 3

Title: Obstacles to molecular species identification in sea anemones (Hexacorallia: Actiniaria) with COI, a COI intron, and ITS II

Authors: Tina Dohna and Marc Kochzius

Journal: Marine Biodiversity: DOI 10.1007/s12526-015-0329-5, 2015

The idea to this study was developed by Tina Dohna and Marc Kochzius. All sampling was done by Janne Timm, Marc Kochzius and Agus Nuryanto. The laboratory analysis was carried out by Tina Dohna at the UFT Bremen. The computer analysis was also carried out by Tina Dohna. The manuscript was written by Tina Dohna with revisions and improvements by Marc Kochzius, Janne Timm and two anonymous reviewers.

4.

Principles of Applied Methods

4. Principles of Applied Methods

4.1. Population genetic analysis (Chapters I and II)

4.1.1. Sequence Markers

Sequence markers, such as the mitochondrial Control Region (CR), used in Chapters I and II derive their power from sequence (dis)similarity at all nucleotide positions within the compared sequences. Mitochondrial markers have been extensively used in population genetics, given the relative ease of amplification (in most species no cloning is necessary) and the higher variability of mitochondrial DNA as compared to the nuclear genome. The mitochondrial CR is non-coding and has been shown to be highly variable in fish and other vertebrates. Therefore, this locus was chosen for analyzing the population genetic structure of the fish species studied in Chapters I and II.

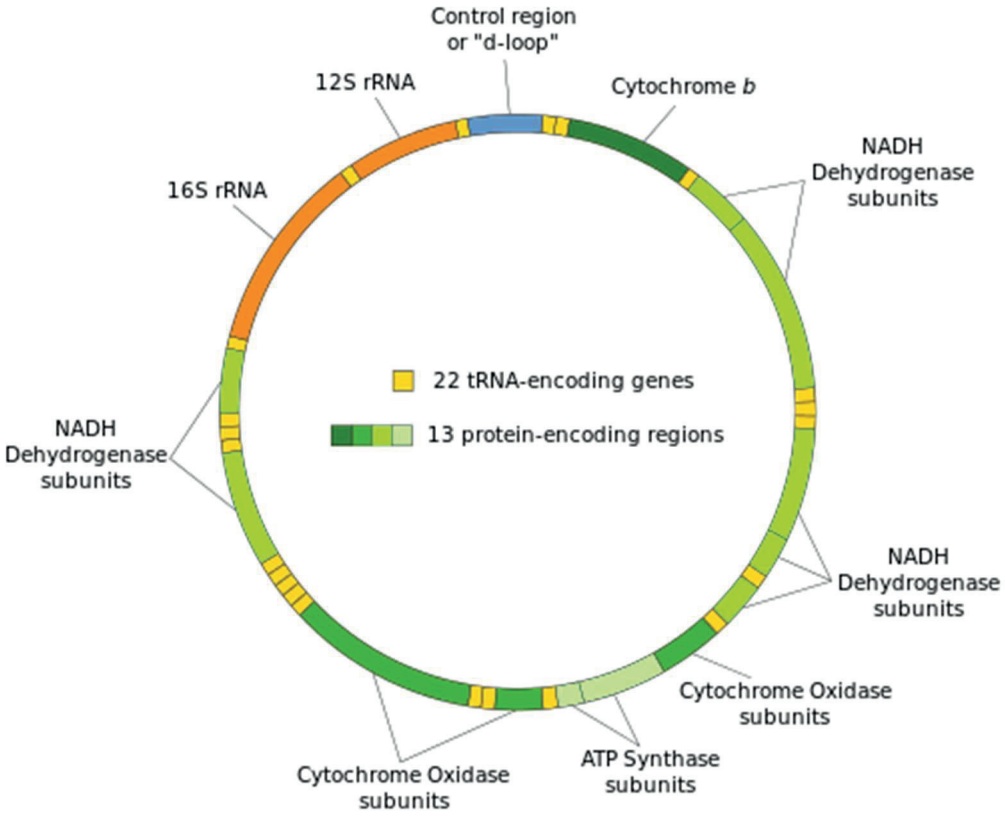


Figure 4.1 General organization of a vertebrate mitochondrial genome, including the putative Control Region or displacement loop (d-loop) initiating replication of the Heavy strand of the genome and used as a sequence marker in this study (graphic retrieved from http://commons.wikimedia.org/wiki/File:Mitochondrial_DNA_de.svg on 20.5.2015).

Consensus sequences of CR haplotypes were produced by editing forward and reverse sequence strands in Seqman (ver. 4.05 DNAStar) and were further aligned with Clustal W (Thompson *et al.* 1994) as implemented in BioEdit (ver. 7.0.0.1, Hall 1999). Sequences were trimmed to the shortest common sequence length in the alignment prior to further analyses to allow base-by-base comparisons.

4.1.2. Neutrality testing

An underlying assumption of population genetic analysis is that molecular markers are neutral, meaning that they are not subject to natural selection. Therefore, the neutrality of the mitochondrial CR marker was determined on the basis of Fu's F_S (Fu 1997), Tajima's

D (Tajima 1989, 1993), and Chakraborty's test of amalgamation (Ewens 1972, Chakraborty 1990), in DnaSP (ver.5.0, Librado & Rozas 2009). Tajima's D uses two mutation indices to distinguish between the genetic signature of population expansion, bottlenecks and selection. Fu's F_S is very sensitive to population expansions and selection. Recent demographic expansion leads to an excess of low frequency haplotypes in the dataset, producing significant negative values for F_S. Chakraborty's test of amalgamation tests the selective neutrality of the marker and population homogeneity. A significant test statistic can indicate the amalgamation of previously separated populations if the selective neutrality of the marker has been established with other methods.

4.1.3. Genetic diversity and population structure

Nucleotide and haplotype diversities for all populations were calculated according to Nei (1987). Nucleotide diversity (π) describes the average number of nucleotide differences per site between two randomly chosen sequences from a population and is a measure of the diversity found within the sampled group (e.g. population). The haplotype diversity (*h*) is also a measure of diversity, describing the uniqueness of haplotypes within the sample. Overall genetic population structure in the dataset (Φ_{ST}) and pairwise population differentiation (pairwise Φ_{ST}) were ascertained with an Analysis of Molecular Variance (AMOVA). The Φ_{ST} statistic ranges between 0.00 (no genetic difference between subpopulations, high geneflow) to 1.00 (complete genetic differentiation, no geneflow) and is an overall proxy for the differentiation among subpopulation within a metapopulation sample due to genetic drift. Pairwise Φ_{ST}, differentiation between population pairs, were computed and the corresponding p-values were adjusted to control for the False Discovery Rate (FDR) according to Benjamini & Hochberg (1995). The downward adjustment of the significance threshold in multiple testing is performed to compensate for Type 1 error, which leads

to erroneous rejection of the null hypothesis. The proportion of false positive test outcomes increases proportionately with the number of hypotheses tested.

Hierarchical AMOVA was applied to test the amount of variation in the dataset that could be explained by differences between groups, when populations were assigned to different smaller or larger regional groups. Groups for testing were chosen to represent regional assemblages and/or to reflect gene flow barriers detected in pairwise population comparisons. Interpretation of significant pairwise population differences adds scale to the extent of local or regional population differentiation, otherwise masked by the rather broad groupings achieved in a hierarchical AMOVA.

All haplotypes in the datasets were included in the construction of a Minimum Spanning Tree (MST) (Kruskal 1956, Prim 1957), inferring the most probable scenario of haplotype descent based on Euclidean squared distances between all haplotype pairs. Clades are identified by grouping closely associated haplotypes in the MST, which are characterized by less mutational steps between them, than to other haplotypes in the MST. Terminal single outlier haplotypes (highly divergent alleles within clades) were not declared clades, because the large number of unsampled intermittent mutational steps leading to these alleles challenge their position in the MST and may indicate unsampled clades, which would provide closer associations. The relative frequency of clades at each location was visualized with pie charts imposed onto a map of the sampling area so that the geographical spread of clades could be visualized and interpreted. The geographical restriction of divergent clades to specific regions or sampling sites was interpreted as evidence for genetic drift in these populations and a restriction of geneflow to and from other subpopulations. Population samples containing members of many clades were interpreted as resulting from higher geneflow scenarios and greater connectivity to other regions sharing members of these clades.

4.1.4. Microsatellites

Frequency markers, such as microsatellites (Msat), are used to determine allele frequencies in populations, potentially showing if gene flow is present. For example, if two or more populations share alleles that are otherwise rather rare, gene flow is assumed to be connecting these populations. Microsatellites are co-dominant markers consisting of short tandem repeat motifs two to six nucleotides in length and common in all eukaryotic genomes (Tautz 1989). Alleles vary in the number of repetitions of the motif, so that length variation is ideally always exactly some multiple of the motif length. The high variation found in some microsatellites can allow for paternity testing (Jones *et al.* 2005) and sibling studies (Selkoe *et al.* 2006). Flanking regions of microsatellites are often conserved within a species, but cannot be amplified in other related species. Variability at the same locus may also be drastically different (highly polymorph vs. monomorph) in closely related species, so that each locus needs to be tested for its suitability for a given species. All microsatellite loci applied to population genetic analysis in Chapters I and II were tested for their suitability.

4.1.5. Evaluating microsatellite loci

The suitability of the microsatellite loci for population genetic analysis in *A. perideraion* and *A. sandaracinos* was evaluated prior to inclusion, since none of the loci had previously been isolated and tested for these species. The expected and observed heterozygosities of loci in each population and overall were resolved in Arlequin, testing for significant deviations from Hardy-Weinberg equilibrium in the distribution of alleles. A likelihood-ratio test was used to detect linkage disequilibrium between pairs of loci (Excoffier & Slatkin 1998). Truly linked loci were expected to display linkage across most of the tested populations. Loci were assessed to check for null alleles and large allele dropouts. Null alleles do not amplify due to mutations in the primer binding sites, falsely

indicating homozygous individuals. Null alleles simulate a heterozygote deficit and artificially inflate the genetic structure detected. These loci usually show large differences between the expected and observed heterozygosity. Loci that indicated null alleles but had no large differences between expected and observed heterozygosity where removed from the dataset and the overall genetic structure was recalculated to assess their impact on this value.

4.1.6. Genetic diversity and population structure - Microsatellites

The program FSTAT (ver. 2.9; Goudet 1995) was used to determine the mean gene diversity and allelic richness in each population. These indices correspond conceptually to nucleotide and haplotype diversity in the mitochondrial genome, but are based on allele frequency data, instead of sequence information.

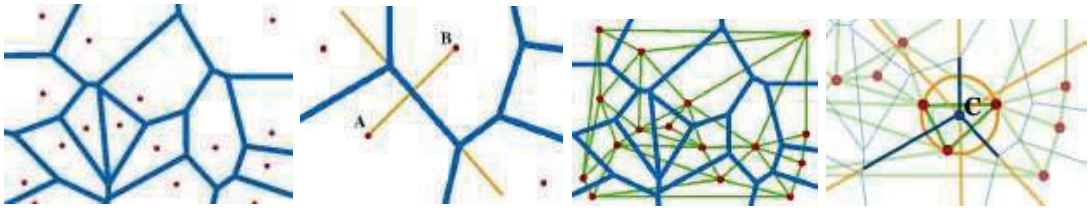
A derivative of the differentiation index D (Jost 2008) was calculated to detect average overall (mean D_{est}) and inter-population (pairwise mean D_{est}) genetic differentiation in the dataset (Gerlach *et al.* 2010). The inability of F_{ST} to accurately reflect population differentiation when diversity within populations is high (as with polymorphic microsatellites) has been repeatedly discussed and confirmed (reviewed in Meirmans & Hedrick 2011). F_{ST} is expected to detect significant structure when present, but fails to rank gene flow scenarios correctly (Gerlach *et al.* 2010). This has led to new indices, such as the derivative of Jost's D, the D_{est} index, employed here. To determine the correlation between pairwise population F_{ST} values and corresponding D_{est} values a Mantel's test was conducted.

A hierarchical AMOVA was run for several different scenarios of population groupings with the microsatellite dataset. Inferences drawn from pairwise distance calculations were used for grouping decisions. The population structure within and among *A. perideraion* populations was further investigated with a model-based clustering method

implemented in STRUCTURE (Pritchard *et al.* 2000). The model applies a Bayesian likelihood approach to estimate the probability of correctly dividing all genotypes in the dataset among *k* number of clusters. All individuals in the *A. perideraion* dataset were additionally labeled according to sampling location, so that the LOCPRIOR admixture model could be applied (Hubisz *et al.* 2009). No information about the geographic distance between sampling locations was included. If population structure is detected by other means (mean D_{est} and F_{ST} in this case), but the structure is too weak for a clear assignment of individuals to clusters, then this option allows the *a priori* use of the sampling location information, if it is informative about ancestry. If informative, clustering solutions that correlate with sampling location are preferentially chosen by the program. This approach is not expected to produce artificial structure where none is present, as the model ignores the *a priori* information if no correlation with ancestry of individuals is detected. The final number of clusters (*k*) for the assignment of samples was chosen based on the highest median estimated ln probability found among all tested values of *k*. Individual samples were assigned to clusters based on the estimated membership (mean value of *q*). The proportion of samples assigned to different clusters at each sampling location was visualized by means of pie chart diagrams, superimposed on a map of the sampling area. An artificial *q*-value threshold difference ($\geq$ 0.25) was enforced for a clear assignment of samples to one of the proposed groups. When this value could not be reached, samples were treated as potential descendants of mixed ancestry and marked as such in the corresponding pie charts.

4.1.7. Genetic Barriers

To identify and evaluate genetic barriers among four species of anemonefish, pairwise genetic distances were used to develop a landscape reflecting common geneflow barriers and regions of connectivity in the IMA (Chapter II).



A B C D

Figure 4.2 A-D depicts the development of a geometric landscape by the program BARRIER (ver. 2.2) from location data to identify genetic barriers among locations within this landscape. Red dots signify the individual sampling locations which are entered with their respective X/Y coordinates. In **A)** sampling points our enclosed by Voronoï tessellation (in blue), identifying their direct neighborhood shown in **B)** where the two points connected by the yellow line are neighbors, because they share a polygon edge. In the Delaunay triangulation (in green) is added, triangulating all points based on the **D)** circumcircle property, which determines that segments of the Voronoï tessellation crossing the edges of the same Delaunay triangle will meet at a point which is the center of that triangle (blue point, which is also the sampling point) (Figure adapted from Manni & Guérard 2004).

Using the program BARRIER (ver. 2.2, Manni *et al.* 2004), each sampling location is enclosed by a polygon (Voronoï tessellation), adhering to the geometry depicted and described in Fig. 4.2 A-D. The applied method produces a two dimensional surface of the sampling area, connecting neighboring sampling sites via shared polygonal segments (Fig 4.2 A). Each shared segment is associated with the pairwise population difference calculated prior and by other means (see previous sections). With the use of "virtual points" the sampling area is demarcated and large land structures can be included to prevent neighborhoods from forming between locations actually separated by large barriers, such as islands (Fig. 4.3 A&B). The model sends a flow along the polygon edges across the polygonal landscape, directing the flow along segments that constitute a higher rate of change in the input variable (in this case genetic distance) moving from on polygon segment to the next.

In the first step, barriers were identified and ranked according to their order of appearance, with those appearing first assumed to be of higher impact. In a second step, the pairwise genetic distance matrix was bootstrapped and run across the map again, lending bootstrapped support for individual barriers. This procedure was carried out individually for

all four species with both mitochondrial and microsatellite (where available) data. The resultant maps where used 1) to identify barriers (polygon edges) shared by several species and 2) to rank these shared barriers by their cumulative bootstrap support, achieved by adding bootstrap values from the individual bootstrapped maps. The cumulative bootstrap values are thereby inflated by 1) this being a high ranking barrier in individual species, 2) mito-nuclear concordance (both CR and Msat data support the barrier) and 3) several species sharing this barrier. Bootstrap values are merely used to add scale to the detected barriers, since the significance of the genetic differences among locations was established by other methods (see previous sections).

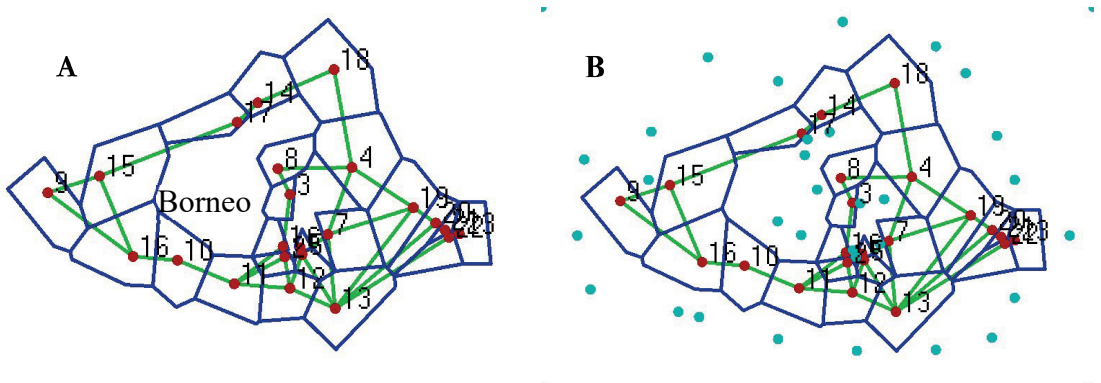


Figure 4.3 The Voronoï tessellation (blue) and Delaunay triangulation (green) for *Amphiprion ocellaris* sampling locations (red dots) is shown in **A** of this figure, while **B** illustrates the use of virtual points (light blue) to determine the boundaries of the sampling region and to include large land structures, such as the Islands of Borneo (labeled) and Sulawesi (smaller space to the right of Borneo) here. This is done to prevent neighborhoods forming between sampling locations separated by large structures in the real world.

4.2. Molecular species identification in the Actiniaria (Chapter III)

4.2.1. Barcoding

Barcoding is based on the simple premise that if a suitable gene fragment is analyzed, the sequence similarity among individuals of a species will be higher than that between species. When a threshold value of sequence divergence is crossed (recommendations vary between taxa, 2-10%), the conclusion is, that there is species delineation. The difference

between intraspecific variation and interspecific divergence should be large enough for the range of the two values not to overlap. This is called the barcoding gap. If a barcoding gap is absent, species delineation cannot be achieved by genetic barcodes alone.

4.2.2. Sequence alignments

To prepare sequences for analysis, forward and reverse sequence strands were aligned and edited using the SeqMan program (ver. 4.0.5, DNASTAR) and a multiple alignment was constructed using ClustalW (Thompson *et al.* 1994) as implemented in the software BioEdit (ver. 7.0.9.0) (Hall 1999) from COI and COI intron sequences each. When aligning ITS sequences, large sequence gaps are common, due to the high variability at this locus. The ClustalW algorithm, which was used to align COI and the COI intron, does not deal well with large gaps in alignments and has the tendency to "shred" sequences. ITS II sequences were therefore aligned using MAFFT 7 (online version; Katoh & Standley 2013) and poorly aligned positions and highly divergent regions were removed with GBlocks 0.91b (Castresana 2000, Talavera & Castresana 2007).

4.2.3. Calculations of intra- and interspecific divergence

Intra- and interspecific sequence divergences were calculated using the Kimura two-parameter (K2P) model of base substitution (Kimura 1980) for COI (coding sequence), and simple pairwise differences for the COI Intron dataset (non-coding sequence) to account for substitution rate difference in coding vs. non-coding DNA fragments. The K2P model is a nucleotide-by-nucleotide distance method, which assumes that the four nucleotides are present in equal frequencies, while transition and transversion rate differences are accounted for. Transitions are base substitutions (mutations) where an interchange of a purine for a

purine or a pyrimidine for pyrimidine takes place. Transversion constitute a purine to pyrimidine mutation, or vice versa. Despite there being twice as many possible transversions to transitions, the ladder occurs in much higher frequencies and is more likely to persist, bearing less potential for conformational irregularities ("wobbles") and amino acid changes. Maximum Parsimony (MP) and Neighbor-joining (NJ) trees, including bootstrap analysis, were performed using MEGA 4 (Kumar *et al.* 2004), as were the calculations of intra- and interspecific genetic divergence (K2P genetic distances). The underlying principle of MP is to construct a tree using the least number of evolutionary steps to infer the topology (Felsenstein 1983). The tree topology of the phylogenetic reconstruction is built on the assumption that shared characters reflect a shared history with a higher probability than an evolution of these characters in parallel (Wägele 2000). As a pure distance-based method, NJ constructs the tree in hierarchical order based on a distance matrix of the sequences, arranging them such, that the sum of all branch lengths is minimized (Saitou & Nei 1987). The Maximum Likelihood (ML) algorithm was used to construct an ITS II tree in PhyML (online version, Guindon *et al.* 2010) and to calculate intra- and interspecific genetic distances for this locus. The ML method generates a multitude of trees based on different models and then searches for the tree with the highest probability of correctly depicting the evolutionary history and phylogenetic relationships of the submitted sequences. Distance calculations were performed in order determine if a barcoding gap was present or not (no overlap between intra- and interspecific genetic distances), while trees were constructed to visualize which families or genera could not be delineated.

REFERENCES

Castresana, J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol. Biol. Evol. 17:540-552.

Chakraborty, R. 1990. Mitochondrial DNA polymorphism reveals hidden heterogeneity within some Asian populations. Am. J. Hum. Genet. 47:87-94.

Ewens, W.J. 1972. The sampling theory of selectively neutral alleles. Theor. Popul. Biol. 3:87-112.

Excoffier, L., and M. Slatkin. 1998. Incorporating genotypes of relatives into a test of linkage disequilibrium. Am. J. Hum. Genet. 62:171-180.

Felsenstein, J. 1983. Parsimony in Systematics: Biological and Statistical Issues. Annu. Rev. Ecol. Sys. 14:313-333.

Fu, Y.-X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915-925.

Gerlach, G., A. Jueterbock, P. Kraemer, J. Deppermann, and P. Harmand. 2010. Calculations of population differentiation based on G(ST) and D: forget G(ST) but not all of statistics! Mol. Ecol. 19:3845-3852.

Goudet, J. 1995. Fstat version 1.2: a computer program to calculate F-statistics. J. Hered. 86:485-486.

Guindon, S., J.F. Dufayard, V. Lefort, M. Anisimova, W. Hordijk, and O. Gascuel. 2010. New algorithms and methods to estimate maximum likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. 59:307-321.

Hall, T.A. 1999. Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/ NT. Nucleic Acids Symp. Ser. 41:95-98.

Hubisz, M.J., D. Falush, M. Stephens, and J.K. Pritchard. 2009. Inferring weak population structure with the assistance of sample group information. Mol. Ecol. Resour. 9:1322-1332.

Jones, G.P., S. Planes, and S.R. Thorrold. 2005. Coral reef fish larvae settle close to home. Curr. Biol. 15:1314-1318.

Jost, L. 2008. GST and its relatives do not measure differentiation. Mol. Ecol. 17:4015-4026.

Katoh, K., and D.M. Standley. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30:772-780.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16:111-120.

Kruskal, J.B. 1956. On the shortest spanning subtree of a graph and the travelling salesman problem. Proc. Am. Math. Soc. 7:48-50.

Kumar, S., K. Tamura, and M. Nei. 2004. Mega3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform. 5:150-163.

Librado, P., and J. Rozas. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451-1452.

Manni, F., and E. Guerard. 2004. Barrier vs. 2.2. Manual of the user. Population genetics team, Museum of Mankind (Musée de l'Homme), Paris [Publication distributed by the authors].

Manni, F., E. Guerard, and E. Heyer. 2004. Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by "Monmonier's algorithm". Hum. Biol. 76:173-190.

Meirmans, P.G., and P.W. Hedrick. 2011. Assessing population structure: F(ST) and related measures. Mol. Ecol. Resour. 11:5-18.

Nei, M. 1987. Molecular evolutionary genetics. Columbia Univ. Press, New York.

Prim, R.C. 1957. Shortest connection networks and some generalizations. Bell Syst. Tech. J. 36:1389-1401.

Pritchard, J.K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945-959.

Saitou, N., and M. Nei. 1987. The neighbour-joining method: a new method for reconstructing phylognetic trees. Mol. Biol. Evol. 4:406-425.

Selkoe, K.A., S.D. Gaines, J.E. Caselle, and R.R. Warner. 2006. Current Shift and Kin Aggregations Explain Genetic Patchiness in Fish Recruits. Ecology 87:3082-3094.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585-595.

Tajima, F. 1993. Measurement of DNA polymorphism,. In: N. Takahata, and A.G. Clark (eds.). Mechanisms of molecular evolution. Introduction to molecular paleopopulation biology, pp. 37–59. Scientific Societies Press, Sinauer Associates, Inc, Tokyo, Sunderland, MA.

Talavera, G., and J. Castresana. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst. Biol. 56:564-577.

Tautz, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Res. 17:6463-6471.

Thompson, J.G., D.G. Higgins, and T.J. Gibson. 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignments through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673-4680.

Wägele, J. 2000. Grundlagen der Phylogenetischen Systematik, pp. 315. Verlag Dr. Friedrich Pfeil, München.

5.
Thesis Chapters

Chapter I

Limited connectivity and a phylogeographic break characterize populations of the pink anemonefish, *Amphiprion perideraion*, in the Indo-Malay Archipelago: inferences from a mitochondrial and microsatellite loci

Tina A. Dohna[1], Janne Timm[1], Lemia Hamid[1] and Marc Kochzius[2]

1) Biotechnology and Molecular Genetics, UFT, University of Bremen, Bremen 28359, Germany

2) Marine Biology, Vrije Universiteit Brussel, Brussel, Belgium

Ecology and Evolution 5(8):1717–1733, 2015

ABSTRACT

To enhance understanding of larval dispersal in marine organisms, species with a sedentary adult stage and a pelagic larval phase of known duration constitute ideal candidates, because inferences can be made about the role of larval dispersal in population connectivity. Members of the immensely diverse marine fauna of the Indo-Malay Archipelago are of particular importance in this respect, as biodiversity conservation is becoming a large concern in this region. In this study, the genetic population structure of the pink anemonefish, *Amphiprion perideraion*, is analyzed by applying 10 microsatellite loci as well as sequences of the mitochondrial control region to also allow for a direct comparison of marker-derived results. Both marker systems detected a strong overall genetic structure (Φ_{ST} = 0.096, p < 0.0001; mean D_{est}= 0.17; F_{ST}= 0.015, p < 0.0001) and best supported regional groupings (Φ_{CT} = 0.199 *p*< 0.0001; F_{CT} = 0.018, *p*< 0.001) that suggested a differentiation of the Java sea population from the rest of the IMA. Differentiation of a New Guinea group was confirmed by both markers, but disagreed over the affinity of populations from west New Guinea. Mitochondrial data suggests higher connectivity among populations with fewer signals of regional substructure than microsatellite data. Considering the homogenizing effect of only a few migrants per generation on genetic differentiation between populations, marker specific results have important implications for conservation efforts concerning this and similar species.

INTRODUCTION

Reproductive population connectivity in spatially separated subpopulations of sessile marine species is shaped primarily through larval dispersal and mortality (Pineda *et al.* 2007). Larval dispersal can achieve population replenishment for exploited or depleted populations, drive colonization of new or abandoned habitats, and diversify the gene pool of isolated populations (Levin 2006, reviewed in Cowen & Sponaugle 2009). Mounting evidence for disproportionately high degrees of restricted and directed larval dispersal in many coastal and offshore species (e.g. Barber *et al.* 2002, Planes & Fauvelot 2002, Swearer *et al.* 2002, Bernardi *et al.* 2003, Taylor & Hellberg 2003, Ovenden *et al.* 2004, Baums *et al.* 2006, Bowen *et al.* 2006, Thacker *et al.* 2007, Schluessel *et al.* 2010) has revealed the potential vulnerability of demographically inter-dependent populations. For sessile marine species, failure of larvae to ensure homogeneous population connectivity throughout the species range produces genetic population structures, which are a valuable source of information for conservation and management efforts, identifying potentially isolated or vulnerable populations and recognizing common gene flow barriers among species. In order to manage populations of marine species for commercial use or under aspects of biodiversity conservation and ecosystem functioning, baseline knowledge of their population dynamics and connectivity needs to be established (Fogarty & Botsford 2007).

The lack of congruency found in the genetic population structure of species with very similar life histories and/or larval ecology/physiology (Barber *et al.* 2011, DiBattista *et al.* 2012) highlights the need to accommodate this variability in research and management (Severance & Karl 2006). Wide geographic (distribution range) and taxonomic coverage (from intraspecific to intergeneric) in sampling of marine fauna and flora is required to develop a clearer picture of the variability inherent in these systems. This is urgently needed

to counteract the steadily increasing pressure on marine resources in degrading coastal habitats (Botsford *et al.* 2001, Palumbi 2003, Hughes *et al.* 2005).

The present study employs sequences of the mitochondrial control region (CR; the hyper variable D-loop; Alvarado *et al.* 1995) and 10 microsatellite markers to investigate the population structure of the Pink Anemonefish, *Amphiprion perideraion* (Bleeker 1855), across the Indo-Malay Archipelago (IMA) and one Philippines site. Non-concordance between nuclear and mitochondrial markers is common in fish and other animals (DiBattista *et al.* 2012 and therein; Toews & Brelsford 2012) supporting the inclusion of both markers types for the recovery of robust phylogenetic relationships (Edwards & Bensch 2009) and to anticipate the inability of either marker to detect genetic structure, where it is present (reviewed in Karl *et al.* 2012).

Populations of *A. perideraion* are commercially harvested for the global marine ornamental trade, placing additional stress on population persistence in light of frequent reef demise and coastal habitat degradation (Wabnitz *et al.* 2003, Shuman *et al.* 2006). Their obligate symbiosis with sea anemones (four potential hosts; Cnidaria; Anthozoa; Hexacorallia; Actiniaria) increases the risk of localized stock depletion due to commercial harvest of the hosts and host vulnerability to high temperature induced bleaching events, projected to increase with climate change (Shuman *et al.* 2006, Jones *et al.* 2008). Though motile by nature, *A. perideraion* is sedentary, as these fish move only within the close vicinity of the sea anemone they inhabit, excluding adult migration as a factor in genetic mixing (Fautin & Allen 1997). The results will shed light on the ability of larval dispersal to connect *A. perideraion* populations on smaller and larger spatial scales.

Studies examining the population structure of *Amphiprion ocellaris*, an *A. perideraion* congener, showed strong structure across the IMA and along a known biogeographic break, the Indo-Pacific Barrier (IPB; Briggs 1974)(Nelson *et al.* 2000, Timm & Kochzius 2008, Timm *et al.* 2012). This barrier, formed by the almost complete fusion of the southern

Indonesian Islands chain, most recently emerged during lowered sea levels in Pleistocene glacial cycles (Voris 2000). The genetic signature of repeated isolation of Pacific and Indian Ocean populations has been detected in quite number of marine species (e.g., Barber *et al.* 2002, Lourie *et al.* 2005, DeBoer *et al.* 2008, Knittweiss *et al.* 2008, Timm & Kochzius 2008). *A. ocellaris* and *A. perideraion* share a very similar life history, demersal egg development, and a short PLD (*A. ocellaris* 8-12 days, Fautin & Allen 1997; *A. perideraion* 18 days, Wellington & Victor 1989), suggesting that geological history and restricted larval dispersal may shape the population structure of *A. perideraion* populations in a similar fashion. In addition, studies on larval recruitment of *A. perideraion* and closely related species have shown high levels of self-recruitment (*Amphiprion chrysopterus,* Beldade *et al.* 2012; *Amphiprion percula,* Almany *et al.* 2007, Buston *et al.* 2012; *Amphiprion polymnus*, Jones *et al.* 2005; *A. perideraion*, Maduppa *et al.* 2014), supporting expectations of a strong genetic population structure. The impact of self-recruitment and sweepstake reproduction (Hedgecock & Pudovkin 2011) can limit migrant exchange between populations, leading to demographic isolation of populations on very small spatial scales (Buston *et al.* 2012), though this is not always the case (Christie *et al.* 2010). In the search for common barriers to dispersal for purposes of conservation planning, the intergeneric comparison is of particular value and can further increase understanding of factors affecting larval dispersal.

The coral reefs of the Indo-Malay Archipelago, which support the highest global marine biodiversity (Roberts *et al.* 2002, Hoeksema 2007, Veron *et al.* 2009), are among the most threatened reef systems worldwide (Burke *et al.* 2002). Coastal degradation, pollution, overexploitation, and climate change all pose serious threats that require prompt action to avert irreversible damage. This region consists primarily of island states, where 350 million people live within 50 km of the coast, relying on ocean resources for their subsistence, transport and trade (Burke *et al.* 2002). Results from this study can be used to further expand the knowledge base available for marine management decisions, which are currently being

installed under the auspices of the Coral Triangle Initiative (CTI), a collective effort at marine resource management by Indonesia, Papua New Guinea, the Solomon Islands, Malaysia, the Philippines, and Timor-Leste (www.coraltriangleinitiative.org).

Figure 5.1 A pair of the pink anemonefish, *Amphiprion perideraion*, in *Heteractis crispa*, one of its four potential sea anemone hosts.

MATERIALS AND METHODS

Sampling, DNA extraction and amplification

With the use of SCUBA, 305 samples of *A. perideraion* were collected from 21 locations spanning the IMA and Japan. Sampling locations where chosen to transverse the IPB, to lie along the strong current of the Indonesian Through Flow (ITF), and to include samples from all major central and peripheral basins of the archipelago. *A. perideraion* individuals could not be found during expeditions in west Sumatra and Singapore (Batam), though these locations lie within the suggested range of this species. Fin clip samples were

stored in 96% ethanol at 4°C. Genomic DNA was extracted with a commercial kit (peqGOLD Tissue DNA Mini Kit, Peqlab, Erlangen).

Control Region A 420bp fragment of the D-loop segment of the mitochondrial control region was amplified with primers CR-A (TTC CAC CTC TAA CTC CCA AAG CTA G) and CR-E (CCT GAA GTA GGA ACC AGA TG) (Lee *et al.* 1995) for 262 individuals (from 19 locations). PCR reactions followed a standard PCR protocol detailed in Timm *et al.* (2008). PCR products were purified with peqGold Cycle-Pure kits (PeqLab, Erlangen). Both strands were sequenced on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Weiterstadt) after amplification with the PCR primers and the Big Dye Terminator Cycle Sequencing Kit (ver. 3.1; Applied Bioscience). *A. perideraion* sequences were subsequently deposited in Genbank.

Microsatellites Primers to amplify 10 microsatellite loci for 289 individuals from 20 locations are listed and described in Table 5.1 along with the observed and expected heterozygosities. Primers were either HEX or FAM labeled and used to amplify sample DNA using the protocol by Timm *et al.* (2012). The amplified fragments were run on an ABI 3100, using an internal 500 Rox Size Standard (Applied Biosystems, Germany). Genemarker (ver. 1.91 Demo) was used to score fragment lengths for all samples. Scoring error between runs was controlled by always including previously analyzed samples with every new 96-sample run and checking the consistency of results for these samples.

Table 5.1 Primers for the amplification of 10 microsatellite loci in *A. perideraion* with their respective motif, PCR product size, number of alleles, PCR annealing temperature, the observed (Ho) and expected (He) heterozygosities, and their biological and literature sources.

Locus	Motif	Product size (bp)	No. alleles	Primers	Ann. temp. (C°)	Source
Ac1578	(AC)9	252-286	13	*F*: 5'-CAGCTCTGTGTGTGTTTAATGC-3' *R*: 5'-CACCCAGCCACCATATTAAC-3'	55,7-57	*A. clarkii* (Liu et al. 2007)
Ac626	(TC)6(AC)20	227-275	20	*F*: 5'-CACACATGCACACACCTTGA-3' *R*: 5'-TAATTGAGGCAGGTGGCTTC-3'	60	*A. clarkii* (Liu et al. 2007)
Ac137	(AC)19	284-332	24	*F*: 5'-GGTTGTTTAGGCCATGTGGT-3' *R*: 5'-TTGAGACACACTGGCTCCT-3'	55,7	*A. clarkii* (Liu et al. 2007)
CF42	(TCTG)18	166-210	24	*F*: 5'-TGCAATTATGCACCTG-3' *R*: 5'-TGGCCAGATTGGTTAC-3'	58.6	*A. percula* Buston et al. 2007)
CF27	(TCTA)16	184-248	14	*F*: 5'-AAGCTCCGGTAACTCAAAACTAAT-3' *R*: 5'-GTCATCTGATCCATGTTGATGTG-3'	60	*A. percula* Buston et al. 2007)
55	(GT)16	418-460	16	*F*: 5'-TTAACTTCCACACCCAGTCT-3' *R*: 5'-ACGCTGTGAGAGTCCATTAT-3'	58,7	*A. polymnus* Quenouille et al. 2004
44	(GT)13	219-253	11	*F*: 5'-TTGGAGCAGCGTACTTAGCT-3' *R*: 5'-AGATGTGTTTACGCACGCTT-3'	58,7	*A. polymnus* Quenouille et al. 2004
61	(GT)49	320-388	28	*F*: 5'-TGAACACATAAACGCTCACTCAC-3' *R*: 5'-AAGACAATGCCTCCACATATCTA-3'	58,7	*A. polymnus* Quenouille et al. 2004

Data analysis

Control region Consensus sequences, produced by editing of forward and reverse sequence strands in Seqman (ver. 4.05 DNAStar) were aligned with Clustal W (1000 bootstrap, minimal manual adjustment of indels) (Thompson *et al.* 1994) as implemented in BioEdit (ver. 7.0.0.1, Hall 1999). After inclusion of a Genbank sequence from the Solomon Islands (DQ343940.1, Santini & Polacco 2006), sequences were trimmed to shortest common sequence length, creating a 382bp alignment of 263 sequences used for all subsequent analyses.

To insure suitability for population genetic analyses the neutrality of the marker was evaluated on the basis of Tajima's D (Tajima 1989, 1993) and Fu's F_S (Fu 1997), which also allows the detection of a recent population expansion or bottleneck. Chakraborty's test of amalgamation (Ewens 1972; Chakraborty 1990) was included to detect potential sample heterogeneity. All tests were carried out in DnaSP (ver.5.0, Librado & Rozas 2009). A sequence of *Amphiprion akallopisos*, a closely related species, was added to allow for rooting of the genealogy.

Unless otherwise stated, all following analyses were carried out with Arlequin (ver. 3.1, Excoffier *et al.* 2005). Nucleotide and haplotype diversities for all populations were calculated according to Nei (1987). Overall genetic population structure in the dataset (Φ_{ST}) and pairwise population differentiation (pairwise Φ_{ST}) were determined with an Analysis of Molecular Variance (AMOVA). Corresponding p-values for pairwise computations were adjusted to control for the false discovery rate (FDR) according to Benjamini and Hochberg (1995) (multtest, R package 2.9.0). Groups for hierarchical AMOVA testing were chosen to represent regional assemblages and/or to reflect gene flow barriers detected in pairwise population comparisons. Interpretation of significant pairwise population differences were expected to add scale to the extent of local or regional population differentiation, otherwise masked by the rather broad groupings achieved in a hierarchical AMOVA. Population

groupings that provided the highest significant between-group differences were applied to test for significant differences in nucleotide and haplotype diversities among these groups using a two-tailed t-test (www.graphpad.com/quickcalcs/ttest1.cfm).

All haplotypes (n = 171) were included in the construction of a Minimum Spanning Tree (MST) (Kruskal 1956, Prim 1957). Clades were defined as containing less mutational steps within, than between clades. Single outlier haplotypes were not defined as clades, as their position in the MST is questionable and may only be resolved with additional data. The relative frequency of clades at each location is visualized in Figure 5.2B with pie charts imposed onto a map of the sampling area.

Microsatellites The suitability of the microsatellite loci for population genetic analysis in *A. perideraion* was evaluated prior to inclusion, since none of the loci had previously been isolated and tested for this species. The expected and observed heterozygosities of loci in each population and overall were resolved in Arlequin, testing for significant deviations from Hardy-Weinberg equilibrium in the distribution of alleles. A likelihood-ratio test was used to detect linkage disequilibrium between pairs of loci (Excoffier & Slatkin 1998). P- values were corrected according to Benjamini and Hochberg (1995), accounting for the FDR. Loci were assessed with Microchecker (ver. 2.2.3; Van Oosterhout *et al.* 2004) to check for null alleles and large allele dropout.

The program FSTAT (ver. 2.9; Goudet 1995) was used to determine the mean gene diversity and allelic richness in each population. The differentiation index D (Jost 2008) was calculated with DEMEtics (ver. 0.8-5 R package; Gerlach *et al.* 2010) to detect average overall (mean D_{est}) and inter-population (pairwise mean D_{est}) genetic differentiation in the dataset (Gerlach *et al.* 2010). The significance of the detected differentiation was described by p-values, estimated from bootstrap resampling (1000) and corrected for the FDR. The inability

of F_{ST} to accurately reflect population differentiation when diversity within populations is high (as with polymorphic microsatellites) has been repeatedly discussed and confirmed (reviewed in Meirmans & Hedrick 2011). F_{ST} is expected to detect significant structure when present, but fails to rank gene flow scenarios correctly (Gerlach *et al.* 2010). This has led to new indices, such as the derivative of Jost's D employed here. Overall F_{ST} and pairwise population F_{ST} values have also been computed and can be found in the supplementary materials. A Mantel's test was conducted between the pairwise F_{ST} and pairwise mean D_{est} values to investigate the correlation computed for all population pairs. Results are in the supplementary material.

A hierarchical AMOVA was run for several different scenarios of population groupings. Inferences drawn from pairwise distance calculations were used for grouping decisions, though only a fraction of all tried groupings is presented here. The population structure within and among *A. perideraion* populations was further investigated with the model-based clustering method implemented in STRUCTURE (ver. 2.3.3.; Pritchard *et al.* 2000). The model applies a Bayesian likelihood approach to estimate the probability of correctly dividing all genotypes in the dataset among *k* number of clusters. All 289 individuals were additionally labeled according to sampling location, so that the LOCPRIOR admixture model could be applied (Hubisz *et al.* 2009). No information about the geographic distance between sampling locations was included. The burn-in period was set to 120,000 with 300,000 repetitions after burn-in. Each *k* (1-20 clusters) was run for 10 iterations and probabilities were calculated on the basis of the median estimated ln probability of the data. The proportion of samples assigned to different clusters at each sampling location was visualized by means of pie chart diagrams, superimposed on the map of the sampling area. An artificial *q*-value threshold difference (≥ 0.25) was enforced for a clear assignment of samples to one of the proposed groups. When this value could not be reached, samples were

treated as potential descendants of mixed ancestry and marked as such in the corresponding pie charts.

Control Region and Microsatellites Geographic distances represent the shortest connection via marine pathways using a Google Earth function. The Isolation by Distance Web Service (Jensen *et al.* 2005) was then used to assess the correlation between geographic and genetic distance (pairwise Φ_{ST} and D_{est}) of sampled populations by applying a Mantel's test, providing a one-tailed p-value for significance of the matrix correlation and the corresponding R-square. A third matrix component was added to include information on whether population pairs stemmed from the same (score = 0), adjoining (score = 1) or non-adjoining (score = 2) "discreet clusters of (genetic) exchange" according to divisions proposed by Kool *et al.* (2011, individual-based biophysical dispersal model). Their model simulates larval dispersal to and from recorded coral reefs in the Indo-West Pacific with the resulting patterns suggesting barriers to dispersal and identifying areas of pronounced admixture. In terrestrial ecology, the inclusion of dispersal-retarding features, such as roads or fences, in otherwise continuous landscapes, is a common addition to calculations assessing the effect of geographic isolation on genetic distances. The geographic complexity of the IMA and previous research on related species suggest that a simple isolation by distance pattern will not apply here, but may become apparent if dominant gene flow barriers are included in the calculation.

RESULTS

*Control Region C*ontrol region sequences (262 individuals from 19 locations) were successfully amplified and subsequently deposited in Genbank (Table S1).

Neutrality testing

Control Region A non-significant test outcome for Tajima's D failed to reject the neutrality of the marker and confirmed its suitability for further analysis (Table 5.2). Fu's F produced a large negative and significant test statistic (considered significant at the 2 % level), implicating departures from population equilibrium (e.g. population expansion). The Mismatch Distribution (Fig. 5.2 C) supported this result by describing a predominantly uni- modal curve of pairwise haplotype differences, expected under a model of sudden population expansion (Rogers & Harpending 1992). Both the sum of squared deviations and Harpending's raggedness index showed no significant deviation from a model of sudden demographic expansion (Table 5.2). The presence of two additional small peaks may underline substructures in the dataset (Ray *et al.* 2003) but they persisted when regional assemblages were analyzed. Chakraborty's test of amalgamation was significant, supporting a scenario of amalgamation of previously separated populations, since the neutrality of the marker was established with other tests (Table 5.2).

Table 5.2 Results for several statistical tests to evaluate the neutrality of the marker (mitochondrial control region). Values in bold are considered significant.

Neutrality Tests		
Tajima's D	-0.979	$p > 0.1$
Fu's FS	**-23.64**	$p < 0.05$
Chakraborty's test	**0.007**	$P < 0.05$
Mismatch Distribution		
SSD	0.0031	$p > 0.1$
Raggedness Index	0.0008	$p >> 0.1$

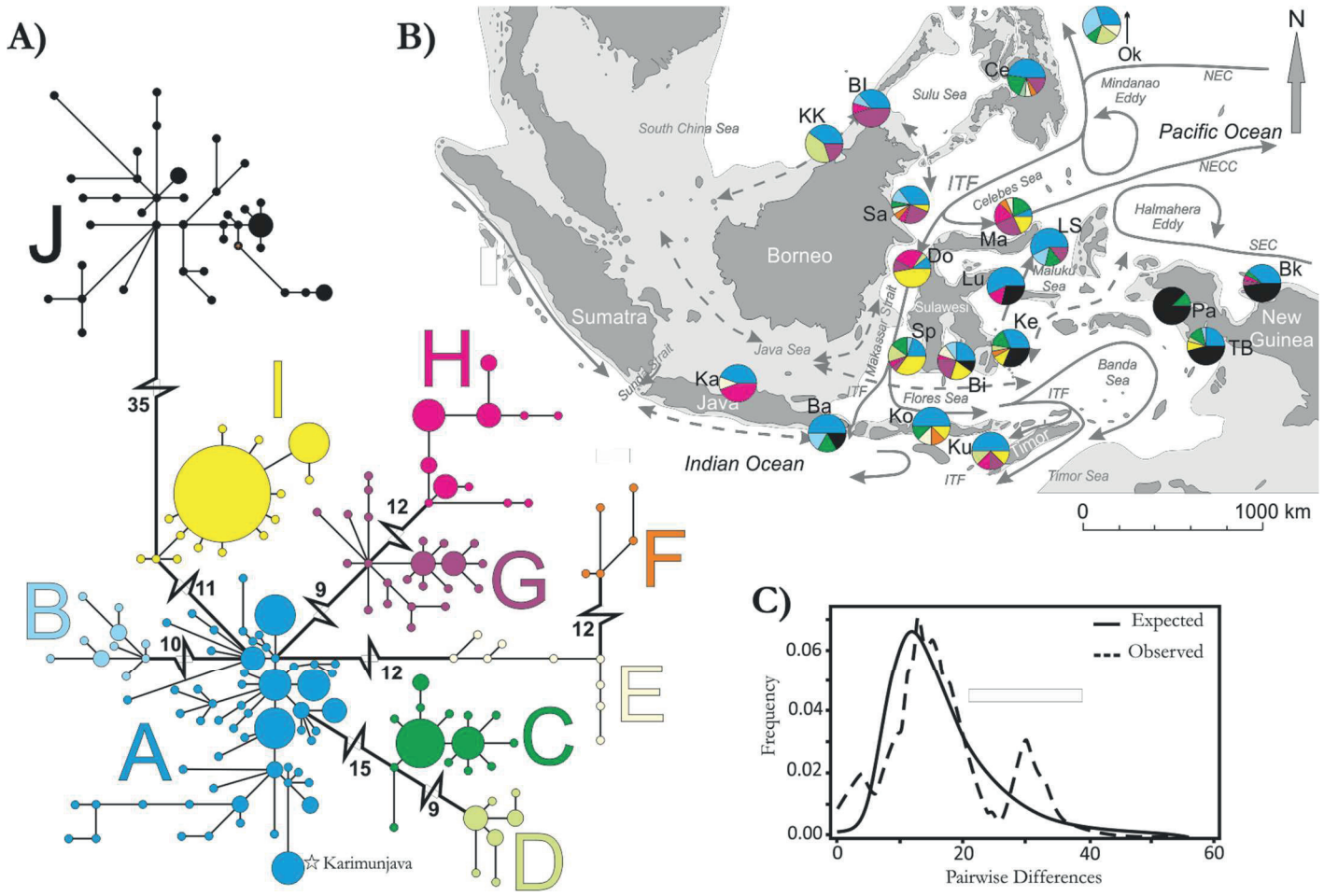


Figure 5.2 All haplotypes identified in 262 CR sequences of A. perideraion were used to **A)** construct a minimum spanning tree (MST) divided into 10 clades (A-J), to **B)** map the fractional contribution of the defined clades to populations at 19 sampling sites within the Indo-Malay Archipelago, and to **C)** display the observed and expected frequencies of pairwise differences (mismatch distribution) for all haplotypes under a model of sudden population ►*cont.*

◀**Fig 5.2** *cont.*

expansion. The size of circles in A is relative to the number of individuals represented by that haplotype, with the smallest circle constituting one and the largest circle 12 individuals. The length of connections between haplotypes is relative to the number of mutational steps between the two (shortest connection represents one mutation), except for connections between clades, where the number of unsampled mutational steps is given. For the map shown in B, major surface currents are indicated with arrows (dashed arrows depict seasonally reversing currents). Dark gray areas are present-day land formations, and light gray shading indicates marine habitat exposed during the Pleistocene glacial maxima, which led to a 120 m drop in sea level (Voris 2000)

Microsatellite characterization and testing

Microsatellites Heterozygosities were high (0.714 - 0.906) in all loci except AC1578 (0.395). The values for observed and expected heterozygosities were close, but observed heterozygosities tended to be lower in most cases (Table 5.1). The highest numbers of alleles were found in loci CF42 and 61, with 45 and 41 alleles, respectively. These two loci also had the most frequent suspected occurrence of null alleles among all populations. Evidence of null alleles was found in 42 % of tested populations for Locus CF42 and in 32% for locus 61. Both loci displayed a larger number of population-specific deviations from HWE than other loci, which further supports suggestions of null allele presence (Table S3). Null alleles are expected to falsely inflate genetic differentiation of populations. However, the overall Φ_{st} remained unchanged (increased by 0.003) when both loci were excluded. Therefore, these loci where included in the further analyses.

None of the tested populations showed a consistent deviation from HWE across loci (Table S3). As a consequence, a gross violation of model assumptions for ideal populations (random mating, no mutation, no drift, and no migration) is unlikely, and the scale of sampling appears to capture discrete populations (Johnson & Black 1984).

Three different loci combinations (44-CF27, Ac626-CF27, Ac137-CF42) indicate linkage disequilibrium in three different populations (Do, Bk, TB). If markers are truly linked, this linkage is expected to carry across populations, which was not the case here. Therefore, all loci were expected to assort independently.

Genetic diversity

Control Region and Microsatellites Haplotype (h = 0.81-1.00) and nucleotide diversities (π = 0.037-0.078) were consistently high among populations, being lowest in Karimunjava (Table 5.3). The population in Karimunjava also had the lowest allelic richness and second to lowest gene diversity. Nucleotide and gene diversities were highest in New Guinea and in

populations lining the eastern Banda Sea. The nucleotide diversity of the New Guinea group (hierarchical AMOVA [Bk, TB, Pa], Table 5.4) in CR data was significantly higher (unpaired t-test: t = 2.121, df = 14, $P = 0.0281$) than in the rest of the archipelago. Most populations located at the northern (Ce, Ok, BI), western (Ka) and southern peripheries (Ku) of the sampling area had nucleotide diversities at the lower end of the spectrum, potentially suggesting that founder events with subsequent expansion may have shaped the diversity of these populations. Allelic richness reflects the same pattern found in gene diversity (Table 5.3).

Table 5.3 Sample sites for *A. perideraion* samples collected from across the IMA with the respective abbreviations (Abbr.) and regional placement. The number of individuals (N_{ind}) analyzed per location for each dataset (CR and Msat) is indicated. Both datasets are composed of the same individuals, with differences in the number of individuals indicating that samples in addition to those constituting the other dataset were incorporated. For the CR dataset, the number of haplotypes (N_{haplo}), the ratio of haplotype number to total individuals sampled (N_{haplo}/N_{ind}), the haplotype (h) and nucleotide (π) diversities are given per site. Msat data are described with gene diversity and allelic richness, including their respective standard deviations (SD).

			Control Region- D-Loop (CR)					Microsatellites- 10 loci (Msat)		
Sample sites	Region	Abbr.	N_{ind}	N_{haplo}	N_{haplo}/N_{ind}	h + SD	π + SD	N_{ind}	Gene diversity ± SD	Allelic richness ± SD
Spermonde	SW Sulawesi	Sp	20	13	0.65	0.95 ± 0.028	0.054 ± 0.028	29	0.798 ± 0.417	3.19 ± 0.55
Donggala	NW Sulawesi	Do	19	14	0.74	0.96 ± 0.031	0.043 ± 0.023	25	0.79 ± 0.414	3.15 ± 0.58
Manado	NE Sulawesi	Ma	16	14	0.88	0.98 ± 0.028	0.053 ± 0.028	24	0.826 ± 0.432	3.21 ± 0.53
Lembeh Strait	NE Sulawesi	LS	7	7	1.00	1.00 ± 0.076	0.046 ± 0.027	8	0.772 ± 0.427	3.09 ± 0.8
Luwuk	E Sulawesi	Lu	14	11	0.79	0.97 ± 0.037	0.066 ± 0.034	14	0.837 ± 0.444	3.24 ± 0.6
Bira	S Sulawesi	Bi	20	19	0.95	0.995 ± 0.018	0.062 ± 0.032	21	0.801 ± 0.421	3.19 ± 0.66
Kendari	E Sulawesi	Ke	19	18	0.95	0.99 ± 0.019	0.078 ± 0.04	18	0.839 ± 0.441	3.24 ± 0.57
Sangalaki	E Borneo	Sa	17	16	0.94	0.99 ± 0.023	0.047 ± 0.025	19	0.826 ± 0.434	3.21 ± 0.65
Karimunjava	off N Java Coast	Ka	9	5	0.56	0.81 ± 0.12	0.037 ± 0.021	8	0.727 ± 0.400	2.8 ± 0.66
Bali	S Bali	Ba	6	6	1.00	1.00 ± 0.096	0.066 ± 0.039	7	0.815 ± 0.455	3.16 ± 0.66
Komodo	Komodo/Flores	Ko	8	8	1.00	1.00 ± 0.063	0.049 ± 0.028	10	0.721 ± 0.392	3.05 ± 0.66
Kupang	Timor	Ku	8	8	1.00	1.00 ± 0.063	0.044 ± 0.025	10	0.777 ± 0.420	3.11 ± 0.75
Banggi Islands	N Borneo	BI	11	11	1.00	1.00 ± 0.039	0.042 ± 0.023	11	0.824 ± 0.441	3.23 ± 0.68
Kota Kinabalu	N Borneo	KK	5	5	1.00	1.00 ± 0.127	0.057 ± 0.035	5	0.849 ± 0.481	3.27 ± 0.53
Biak	E New Guinea	Bk	22	19	0.86	0.97 ± 0.028	0.072 ± 0.037	23	0.83 ± 0.434	3.19 ± 0.52
Cebu	Philippines	Ce	19	16	0.84	0.98 ± 0.027	0.045 ± 0.023	17	0.806 ± 0.426	3.17 ± 0.574
Okinawa	Japan	Ok	10	10	1.00	1.00 ± 0.045	0.044 ± 0.024	0	na	na
Misool	Maluccas	Mi	0	na	na	na	na	2	na	na
Pisang	W New Guinea	Pi	0	na	na	na	na	3	na	na
Papisol	W New Guinea	Pa	0	na	na	na	na	13	0.859 ± 0.455	3.29 ± 0.46
Triton Bay	W New Guinea	Tr	0	na	na	na	na	22	0.825 ± 0.436	3.26 ± 0.53

The ratio of the number of haplotypes found to the number of sampled individuals from populations was high overall, with the lowest ratio seen in Karimunjava (0.56) (Table 5.3). Typical for a control region dataset the fraction of singleton haplotypes found in populations was high, accounting for 100 % of private haplotypes in 13 of the 19 populations analyzed (data not shown). Only populations in Manado, Donggala and Spermonde, situated prominently along the ITF, contained more shared than private haplotypes.

Overall genetic structure

Control Region and Microsatellites AMOVAs with the CR and Msat datasets showed highly significant deviations from panmictic population conditions ($\Phi_{ST} = 0.096$, $p < 0.0001$; mean $D_{est} = 0.17$; $F_{ST} = 0.015$, $p < 0.0001$). Despite the low sample size from Karimunjava, the hierarchical AMOVA's for both datasets best supported a differentiation of Karimunjava from the central Archipelago and eastern populations, as well as an isolation of eastern populations from the center. However, the CR dataset finds the highest between-group variation when the eastern group includes east and west New Guinea populations (Bk, Pa,TB) ($\Phi_{CT} = 0.199$, $p < 0.000$), while the Msat dataset best supports a smaller eastern group with only Biak (and Misool), grouping Papisol and Triton Bay with the central populations ($F_{CT} = 0.018$, $p = 0.003$). A summary of the examined group configurations is provided in Table 5.4. Both datasets support a scenario of central mixing, with pronounced western and eastern population differentiation.

Testing for the effect of isolation by distance produced a very weak correlation ($r = 0.199$, $p = 0.05$) between geographic and genetic distance with Msat data and no significant correlation with the CR dataset. R-squared values were very low (explaining less than 10%) and the regression lines did not describe the spread of the data. Controlling for the effects

of potential dispersal-retarding current features, as modeled by Kool *et al.* (2011), with a third matrix component, did not reveal masked IBD in either dataset.

Table 5.4 Hierarchical AMOVA groupings of *A. perideraion* populations in the Indo-Malay Archipelago based on pair-wise distances of mitochondrial control region sequences (Φ values) and 10 microsatellite loci (F values). Bold values describe the highest index support for the tested combinations.

	Control Region - D-loop		***Microsatellites - 10 loci***	
Groupings	Φ_{CT}	$p \pm SD$	F_{CT}	$p \pm SD$
no groups	0.138	<0.000 ± 0.000	0.015	<0.000 ± 0.000
2 Groups:				
[Ka][all others]	0.0356	0.221 ± 0.015	0.0417	0.056 ± 0.007
[Bk][all others]	0.0789	0.055 ± 0.007	0.0068	0.094 ± 0.009
3 Groups:				
[Bk,Mi][Ka][all others]			**0.0181**	**0.003 ± 0.002**
[Bk,Pa,TB][Ka][all others]	**0.1985**	**<0.000 ± 0.000**	0.0134	0.002 ± 0.001
[Bk,Pa,TB,Ke][Ka][all others]	0.1857	<0.000 ± 0.000		
[Bk,Pa,TB][Ka,Ba][all others]	0.1768	<0.000 ± 0.000		
4 Groups:				
[Bk][Pa,TB][Ka][all others]	0.1831	<0.000 ± 0.000	0.0099	0.018 ± 0.004
[Bk,Pa,TB][Lu;Ke][Ka][all others]	0.1764	<0.000 ± 0.000		
[Bk,Mi][Pi][Ka][all others]			0.0180	0.002 ± 0.001
[Bk,Mi][Ce][Ka][all others]			0.0147	0.005 ± 0.002
[Bk,Pa,TB][Lu;Ke,LS][Ka][all others]	0.1616	<0.000 ± 0.000		

Regional structures

Control Region and Microsatellites Both marker systems revealed extensive regional population substructure in the IMA with patterns in opposition to a simple IBD model and not adhering to dynamics expected from the impact of dominant ocean currents (e.g. ITF) on larval transport. Pairwise population differentiation measures (pairwise Φ_{ST} and mean D_{est}) are listed in Table 5.5 for all populations, with significance adjusted to control for the false discovery rate. Considering only locations with data available from both datasets, the Msat dataset revealed 72 instances of significant population differentiation, as opposed to 29 in the CR dataset. Both datasets agreed on the pronounced differentiations of Biak (range D_{est}= 0.111[Sp] – 0.373[Ka], Φ_{ST}= 0.169 [Pa] – 0.267 [Do]) and Karimunjava (range D_{est} = 0.17[Mi] – 0.401[Sa], Φ_{ST}= 0.109 [Bi] – 0.589 [Pa]) to the rest of the populations, while only

the Msat dataset indicated differentiation of the Philippine population (range D_{est}= 0.106 [Sp] – 0.373 [Ka]) (Table 5.5, Fig. 5.3).

Further evidence for the absence of a simple IBD dispersal mechanism can be seen in the many significant pairwise differences between proximate locations. Donggala, exposed to the strong currents of the ITF in the Makassar Strait, shows a surprisingly high number of significant differentiations to other populations (CR - 11 populations, Msat - 14 populations). Among them are the two most proximate upstream and downstream locations in the datasets, Sangalaki and Spermonde. A similar situation is seen in Luwuk, which is significantly different from its closest northern (LS) and southern (Ke) neighbors in the dataset (Msat data only), and in Kupang and Komodo, which are significantly different from one another, as well as to samples from Bali and populations just to the north (Bi, Ke).

Minimum Spanning Tree and Clade Distribution

Control Region The minimum spanning tree (MST) shown in Figure 5.2 A divides the dataset into ten clades, which are separated by 9-35 nucleotide substitutions (ns). Connections between clades are not drawn to scale, but instead ns separating clades are given by numbers next to the connecting lines. Connections within clades are drawn to scale.

Clade A holds the central position in the star-like topography of the tree, with all other clades directly or indirectly diverging from it. Due to its central position and its presence in all sampled populations (except Papisol) (Fig. 5.2 B), this clade is most likely to contain ancestral haplotypes. The assumed most ancestral (most central and shared) haplotypes of clade A stem from eastern (Ma, Bk, Lu) and southern populations (Bi, Sp) (Fig. 5.2 A&B). The population from Okinawa forms a northern exception here, as it too contains haplotypes of the central clade A. All four individuals from Karimunjava that were placed within clade A carry the same haplotype, which lies on a peripheral, terminal branch, ten ns from the next-closest clade A haplotype.

Most of the sampled populations were found to contain clade C and/or clade D haplotypes, the exceptions being Karimunjava, Luwuk, Bira and the Banggi Islands. Clade E and F haplotypes were found in north-eastern populations (Ce, Ok), along the Celebes Sea (Ma, Sa) and in some of the populations lining the Java and Flores Seas (Ka, Bi, Ko, Ke), but were absent from New Guinea, the Banda and Maluku Seas, the Makassar Strait, and the South China Sea. Both clades contain only singleton haplotypes with internal divergences of up to nine ns.

Clade G haplotypes are found throughout most of the IMA though the haplotype from its most southern expansion in Kupang is 11 ns removed from the next-closest clade member. A similar situation is found in clade H, where the haplotype from Kupang diverges by ten mutational steps. The high number of ns for these and other outliers makes their direct clade association questionable. However, within the given dataset, no alternative connections were suggested by the analysis. Clade H is dominated by haplotypes from Karimunjava, Donggala and Manado, with Karimunjava at the clades' most basal position. The smallest divergence between haplotypes (mostly 2-3 ns) is found in clade I, which is predominantly found along the northern, western, and southern coastline of Sulawesi and in other populations situated along the ITF. Moving into and across the Banda Sea, the presence of clade I haplotypes decreases, while that of clade J haplotypes increases (Fig. 5.2 B). The latter is confined to Bali, east Sulawesi, and New Guinea, and is by far the most divergent clade, removed by 35 ns. Nevertheless, in a phylogeny with its sibling species, *A. sandaracinos* and *A. akallopisos*, these haplotypes clearly group with *A. perideraion* (data not shown). Clade J also includes the haplotype from the Solomon Islands, which was included from Genbank. Haplotypes from Biak and the western New Guinea populations are distributed throughout this clade, occupying both central and very divergent peripheral positions.

Table 5.5 Population pairwise differences in control region sequences (Φ_{ST} index, above diagonal) and microsatellite data (D_{est} index, below diagonal) for *A. perideraion* for all sampling sites are shown (1000 permutations). Bold values denote significance at $P \leq 0.05$ (1000 bootstraps) after correction for multiple testing (Benjamini & Hochberg 1995, False Discovery Rate procedure). Corresponding FST index values for the Msat dataset is available in Supplementary Material, Table S1.

	Sp	**Do**	**Ma**	**LS**	**Lu**	**Bi**	**Ke**	**Sa**	**Ka**	**Ba**	**Ko**	**Ku**	**BI**	**KK**	**Bk**	**Ce**	**Mi**	**Pi**	**Pa**	**TB**	**Ok**
Sp		0.022	0.035	0.047	**0.113**	0.035	**0.093**	**0.07**	**0.16**	0.051	0.025	0.028	**0.135**	0.015	**0.217**	0.049	na	na	**0.538**	**0.171**	0.049
Do	**0.071**		0.044	0.155	**0.161**	0.034	**0.152**	**0.101**	**0.192**	**0.174**	0.085	0.117	**0.155**	**0.167**	**0.267**	**0.139**	na	na	**0.594**	**0.222**	**0.182**
Ma	0.062	0.032		0.042	**0.106**	0.016	**0.104**	0.012	**0.126**	0.075	0.013	0.051	0.038	0.048	**0.217**	0.029	na	na	**0.541**	**0.180**	0.087
LS	**0.116**	**0.146**	0.101		0.043	0.005	0.067	-0.029	**0.154**	-0.065	-0.029	-0.057	0.01	-0.016	0.165	-0.031	na	na	**0.554**	0.147	-0.025
Lu	**0.096**	**0.115**	**0.123**	**0.139**		0.022	-0.018	0.074	0.138	-0.051	0.051	0.045	0.099	0.095	0.001	**0.095**	na	na	**0.325**	0.020	**0.093**
Bi	0.044	**0.068**	0.030	0.06	**0.14**		0.044	0.000	**0.109**	-0.003	-0.011	0.009	0.023	0.067	**0.127**	**0.037**	na	na	**0.453**	**0.116**	0.053
Ke	**0.073**	0.062	0.056	0.023	**0.109**	0.002		**0.103**	**0.155**	-0.035	0.058	0.079	**0.14**	0.082	0.000	**0.098**	na	na	**0.236**	-0.016	0.083
Sa	0.036	**0.073**	0.070	**0.141**	**0.14**	0.037	0.051		**0.132**	0.025	-0.033	0.001	-0.01	0.058	**0.206**	-0.011	na	na	**0.558**	**0.185**	0.035
Ka	**0.339**	**0.299**	**0.258**	**0.316**	**0.296**	**0.299**	**0.328**	**0.401**		**0.143**	**0.115**	**0.148**	**0.176**	0.182	**0.242**	**0.163**	na	na	**0.589**	**0.203**	**0.165**
Ba	**0.161**	**0.205**	**0.151**	0.081	0.109	0.109	0.105	0.093	**0.359**		-0.01	-0.016	0.078	0.024	0.027	0.012	na	na	0.395	0.022	-0.01
Ko	0.088	**0.145**	**0.111**	0.03	**0.118**	**0.122**	**0.133**	**0.147**	**0.276**	**0.154**		-0.035	0.053	0.044	0.182	-0.026	na	na	**0.548**	0.152	0.004
Ku	**0.109**	0.122	**0.197**	0.094	0.038	**0.162**	**0.128**	**0.11**	**0.305**	**0.266**	**0.163**		0.049	0.014	**0.179**	0.007	na	na	**0.578**	**0.163**	0.005
BI	0.031	**0.146**	0.041	0.085	**0.113**	0.027	-0.000	0.055	**0.367**	0.056	**0.137**	**0.113**		0.098	**0.219**	0.046	na	na	**0.586**	**0.209**	**0.114**
KK	0.001	0.099	0.067	0.029	0.062	0.086	-0.038	0.005	**0.385**	0.114	0.027	0.032	-0.100		0.2	0.006	na	na	**0.545**	0.149	-0.013
Bk	**0.111**	**0.204**	**0.164**	**0.131**	**0.12**	**0.166**	**0.135**	**0.139**	**0.373**	**0.188**	**0.203**	**0.161**	0.102	0.103		0.197	na	na	**0.169**	-0.124	**0.203**
Ce	**0.106**	**0.115**	**0.184**	**0.223**	**0.109**	**0.162**	**0.145**	0.048	**0.37**	0.128	**0.214**	0.067	**0.117**	**0.177**	**0.165**		na	na	**0.566**	-0.01	0.002
Mi	0.154	0.201	0.161	0.116	0.125	0.132	**0.187**	0.135	**0.17**	-0.204	0.189	0.075	**0.220**	0.077	0.1	0.247		na	na	na	na
Pi	0.059	**0.16**	0.139	**0.198**	**0.170**	**0.184**	0.128	0.103	**0.326**	0.179	0.269	**0.219**	0.086	**0.186**	0.097	0.041	0.207		na	na	na
Pa	0.081	**0.132**	**0.119**	0.086	0.102	0.082	0.077	0.077	**0.362**	0.070	**0.136**	0.043	0.075	0.046	0.080	**0.127**	-0.038	-0.072		0.133	**0.555**
TB	**0.073**	**0.124**	0.078	**0.128**	0.057	0.078	0.042	0.079	**0.312**	**0.213**	**0.136**	**0.119**	0.088	-0.077	0.023	**0.178**	0.054	0.196	0.090		**0.163**

STRUCTURE analysis

Microsatellites Bayesian likelihood analysis implemented in STRUCTURE, suggests that a division of the dataset into four clusters (k=4) is most probable (Figure S1, supplementary materials). Karimunjava consists exclusively of red cluster genotypes, which are only again detected as a small fraction in Komodo with genotypes of potential mixed ancestry (red/black checkered fraction of pie chart, Fig. 5.3). The Philippine samples are similarly characterized by consisting only of pure blue cluster genotypes, while here the connectivity to the rest of the Archipelago is still visible with primarily mixed blue genotypes (exception in Luwuk) detected in central, eastern and southern populations, though completely absent from the Makassar Strait (Sp, Do) and northern Sulawesi populations (Ma, LS). The black cluster is confined to the central IMA (Sulawesi, Bali, Komodo) and to sampling locations on the north and east coast of Borneo (KK, BI, Sa). Moving up the east coast of Sulawesi, black cluster members are displaced by grey cluster members and samples suggesting a combined black, grey and blue (Philippine cluster) ancestry. Crossing the Banda Sea, only samples of potential grey/black mixed ancestry remain and no pure black genotypes can be found. Samples collected at the most eastern locations, Bk (North East New Guinea) and TB (Central West New Guinea), are all members of the grey cluster, with only one sample of mixed or unclear ancestry in TB (blue/black).

The overall pattern suggests 1) a genetic break between the Java Sea population (Karimunjava) and all other locations, 2) a unidirectional (north to south) connectivity of the Philippines to the rest of the archipelago, 3) mixing of central populations along the ITF and within and across the Sulu Sea, and 4) a gradual eastern displacement of the black cluster genotypes by grey genotypes.

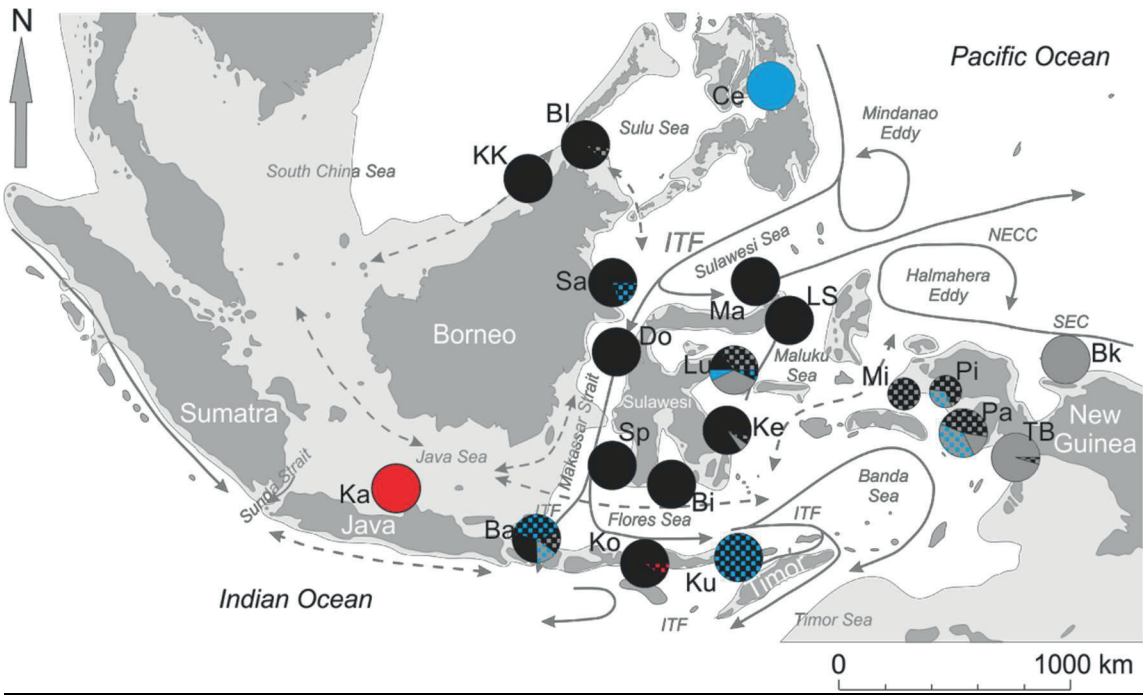


Figure 5.3 Map of the study area with pie charts depicting the fractional assignment of *A. perideraion* individuals from each sampling location to one or more of the four (k=4) genotype clusters defined by STRUCTURE (ver. 2.2., Pritchard *et al.* 2000), based on 10 microsatellite loci. Red, blue, black or grey pie slice colorations represent one of the four clusters each. Chequered pie slices depict potential scenarios of mixed ancestry of the two colors used for the pattern. This was applied when a threshold value difference (≥ 0.25) between two alternative probabilities of group assignments could not be reached.

DISCUSSION

Restricted gene flow across the IMA

The present study used 10 microsatellite loci and sequence data of the mitochondrial CR to investigate the population structure of *A. perideraion* in the IMA. Potential barriers to gene flow acting on the sampled populations were identified and the found structure was placed in its historic and phylogenetic context. The study found substantial population structure overall for both marker types (Φ_{ST} = 0.096, p < 0.0001; mean D_{est} = 0.17; F_{ST} = 0.015, p < 0.0001), confirming expectations derived from genetic population structuring seen in other anemone fish (*A. ocellaris*; Nelson *et al.* 2000, Timm *et al.* 2012) and other reef dwelling species with a pelagic larval phase (e.g. Bay *et al.* 2004; DeBoer *et al.* 2008; Leray *et al.* 2010; reviewed in Carpenter *et al.* 2011). Demersal egg development (Riginos *et al.* 2011), a

relatively short PLD (18 days) (Wellington & Victor 1989), site attachment of adult fishes (Fautin & Allen 1997), and high rates of self-recruitment (Maduppa *et al.* 2014) are all expected to contribute to the observed structure and highlight the vulnerability of this and similar species from a conservation standpoint.

Population structure and genetic diversity within the IMA

Population breaks between eastern, central and western IMA populations detected in this study mirror similar breaks in a congeners of *A. perideraion* (*A. ocellaris*, Timm & Kochzius 2008; Timm *et al.* 2012). Hierarchal AMOVA found that the highest significant genetic differentiation between regional groups is achieved when Karimunjava and Biak (with Misool) form western and eastern groups respectively, though disagreement among markers exists in assigning west New Guinea populations to the central or the eastern group. In addition, both marker types showed a large number of significant pairwise differences between populations not adherent to a simple isolation by distance model or following prominent oceanographic features (e.g. ITF). Significant population differentiation between geographically proximate locations, e.g. along and across the Makassar Strait (D_{est} = 0.071 - 0.073, Table 5.5), across and along the Flores Sea (D_{est} = 0.154-0.266), and along other coastlines could indicate that barriers to gene flow are acting on these populations. The analysis revealed significant substructures within the IMA and barriers to gene flow that may need to be considered for conservation purposes.

Amalgamation of previously isolated and secondarily admixed divergent gene pools, which is characteristic for populations in highly fragmented and repeatedly fused habitats produced during Pleistocene glaciations, is here supported by a significant Chakraborty's test of amalgamation and a large negative Fu's F (Table 5.2). Excluding the population from Karimunjava, high nucleotide diversities found in all locations support the proposed

mechanism of amalgamation of populations previously isolated in individual basins of the IMA (McManus 1985).

Both the sum of squared deviations and Harpending's raggedness index indicate non-significant deviation from expectations under a simulated sudden demographic expansion model, though the additional small peaks in the observed distribution may indicate a gradual move towards demographic equilibrium (Table 5.2, Fig. 5.2 C). Mismatch distributions for mitochondrial CR data in *A. ocellaris* at three different spatial scales (south- east Sulawesi/Sulawesi/entire IMA) also produced "trimodal" mismatch distributions at all scales (Timm & Kochzius 2012). Colonization of newly forming habitats with the gradual rise of seawater levels would explain a pattern of sudden population expansion as indicated here and as also found in other species populating the IMA.

The Eastern IMA

The hierarchical AMOVA grouped all west and east New Guinea locations (Bk, TB, Pa) with CR data, but isolates east New Guinea (Bk) (and Misool) from all other populations using Msat data. The low sample number from Misool does not allow conclusions to be drawn with any reliability about this population, but its association to east New Guinea, instead of more proximate west New Guinea locations, may give some indication that this population could be subject to other dynamics (Barber *et al.* 2011). Population genetic analysis with a hierarchical AMOVA of *A. clarkii* Msat data (sibling species, unpublished data) marked Misool as a divergent population, forming its own group. In *A. ocellaris*, the population from Misool grouped with other west New Guinea populations, and did not appear as distinct (Timm *et al.* 2012). Misool's association to Pacific populations in *A. ocellaris* perideraion could not be ascertained, as the distribution of this species does not extend that far. A study by Timm *et al.* (2008) sets the speciation process of *A. perideraion* from an ancestral type well within the Pleistocene glacial oscillations, approx. 1.6 mya, starting at

the Pacific fringes of the IMA and within some of its basins (South China Sea, Sulu Sea and Celebes Sea). Considering that the central position of the haplotype network is dominated by east New Guinea and Banda Sea haplotypes (Fig. 5.2 A&B), one could speculate that CR data is showing signals of an invasion pathway of *A. perideraion* from Pacific populations into the central IMA.

The STRUCTURE analysis revealed a clear association of west New Guinea with the east of the Island, though inspection under the mixed ancestry model diffused the clear delineation, indicating increased connectivity between the southern (TB) population and Biak, and increasing connectivity across the Banda Sea moving up the west coast. Pairwise differences in the CR data identified the eastern IMA group (Bk, TB, Pa) as the most divergent population (Φ_{ST} = 0.116 – 0.594, Table 5.5), also indicated by the large distance (35 ns) of black clade haplotypes (Fig. 5.2 B) dominating in eastern locations. Papisol (west New Guinea) stands out as the most divergent population in this group, a trend not reflected in the Msat data where Biak (east New Guinea) produces the highest pairwise differences. Overall, Pacific populations of New Guinea should be considered separate from those lining the Banda Sea. Further sampling in Misool and the surrounding islands could clarify which mechanisms are shaping populations there.

The Western IMA

Measures of pairwise population divergence and hierarchical AMOVA also highlight the strong differentiation of the Java Sea (Ka) samples from the central and eastern IMA populations. Despite the seasonally oscillating currents (Fig. 5.2 B) that appear to be connecting the Java Sea to the central IMA, a genetic break has been detected here for quite a number of species, including several fish (Bay *et al.* 2004; Winters *et al.* 2010; Gaither *et al.* 2011). Since present-day current patterns often fail to explain the population structure found in the IMA for species with a pelagic larval phase, common genetic signals for barriers to

connectivity are often attributed to population fragmentation caused by eustatic sea level fluctuations. Extreme sea-level low stands (up to -130 m) during glacial maxima of the Pleistocene (most recently approx. 20,000 ya) led to the formation of an almost uninterrupted land barrier known as the Indo-Pacific barrier (IPB)(Fleminger 1986) along the southern chain of islands that now form part of Indonesia (Fig. 5.2 B, light shading around land structures). This led to a massive reduction of the ITF and Indian and Pacific Ocean basin connectivity (Voris 2000). Vicariance, driven by repeated marine habitat reduction and fragmentation, is believed to have pushed allopatric speciation within and along the IMA, as well as enabling genetic drift to manifest itself within separated populations (McMillan & Palumbi 1995; Williams 2000; Kochzius *et al.* 2003). The genetic structure detected in *A. perideraion* populations may also show remnant signs of the Indo- Pacific barrier as both marker types confirm a significantly reduced gene flow between the Java Sea population and all other locations sampled in the IMA, with the exception of Luwuk (CR) and the Kota Kinabalu (Msat) site (Table 5.5).

From a conservation standpoint it is also important to know whether phylogeographic barriers still persist today in order to adjusted ecosystem management strategies accordingly. Model simulations by Kool *et al.* (2011) of larval dispersal (15-30 day PLD) in the IMA under contemporary oceanographic conditions demonstrated that larvae released in the Makassar Strait and western Flores Sea would not enter the Java Sea, thereby failing to reach Karimunjava. The predicted PLD of *A. perideraion* is only 18 days, much less than the max. PLD (30 days) used for virtual larvae in the model, so that the trajectory of *A. perideraion* larvae can be expected to be even more restricted. This strengthens the case for a divergence of the *A. perideraion* population in Karimunjava even under contemporary oceanic conditions, suggesting that a continued isolation of the Java Sea population from the rest of the IMA should be considered and accounted for in management plans.

Two studies investigating the population structure of *A. ocellaris* (control region sequences Timm *et al.* 2008; six microsatellite loci, Timm *et al.* 2012) found that samples from Karimunjava were more strongly associated with more western (including Indian Ocean) locations than with the proximate Islands of Bali, Komodo and Sulawesi, which also agrees with model predictions for this area. The Karimunjava sampling site describes the most western location where *A. perideraion* could be found so that the affinity of Karimunjava to Indian Ocean haplo- and genotypes could not be ascertained. Efforts to sample *A. perideraion* populations in the 1000 Islands Marine National Park north-east of Jakarta, in Padang on the west coast of Sumatra, and in Batam (Malakka Strait) were unsuccessful, although all three locations lie within the suggested distribution of *A. perideraion* (Fautin & Allen 1997). In comparison with other sampling locations, reduced genetic diversity for both markers, in addition to its absence at more western sites, may suggest that *A. perideraion* populations in the western expansion of the species range may be especially vulnerable to disturbance and/or exploitation. More extensive sampling in this area is needed to strengthen these conclusions.

The Northern IMA

The effect of a massive reduction in gene flow through repeated and nearly complete closure of seaways connecting the South China and Sulu Seas during the Pleistocene eustatic oscillations could not be detected in either dataset, as haplo- and genotypes in the two populations from northern Borneo did not indicate consorted divergence from the central IMA. This may indicate that the re-colonization of the northern coastline of Borneo radiated from the Celebes and Sulu Seas, once the coastline became again submerged and connecting seaways reopened (at approx. 10,000 ya; Crandall *et al.* 2012). Analysis of the false clown anemonefish, *A. ocellaris*, population structure (CR, Timm & Kochzius 2008) produced a similar result, with no apparent emergence of a distinct South China Sea clade present

in Kota Kinabalu or the Banggi Islands. Several population-genetic studies dealing with invertebrates also suggest a similar invasion succession: giant clams, *Tridacna crocea* and *T. maxima* (Kochzius & Nuryanto 2008; Nuryanto & Kochzius 2009) and the blue starfish *Linckia laevigata* with its ectoparasite, *Thyca crystalline* (Kochzius *et al.* 2009). Predictions of connectivity under contemporary oceanic conditions according to Kool *et al.* (2011) classify the South China Sea, Sulu Sea and Celebes Sea as belonging to the same "discreet cluster of exchange among populations".

According to model predictions of simulated larval dispersal, the Philippines are expected to show considerable retention of larvae, with very limited larval exchange in and out of the Sulu Sea (Kool *et al.* 2011). The population groupings best supported by either marker system do not, however, predict the isolation of the Cebu population, though pairwise comparisons do show a considerable amount of differentiation, including a barrier across the Sulu Sea towards the north coast of Borneo (Banggi Islands, Kota Kinabalu). STRUCTURE analysis very clearly defines the isolated status of the Philippines, corroborating model predictions and indicating the need to place special attention on the management of the coral reef fauna resident there. The special status of Philippine populations was also specified for other fish species, such as two species of seahorses (Lourie *et al.* 2005) and the three-spot damsel *Dascyllus trimaculatus* (Ablan *et al.* 2002), but was not detectable in the analysis of *A. ocellaris*, independent of the markers applied (CR or Msat)(Timm *et al.* 2012).

Conclusions and implications for management

The population structure found in *A. perideraion* in the IMA is marked by Pleistocene isolation and persisting barriers to gene flow. Considering that even very small numbers of migrants every few generations can lead to population homogeneity and produce misleading

signs of demographically relevant population connectivity, the detection of significant differentiation between populations should be taken quite seriously from a conservation standpoint. Conservation efforts for the protection of marine resources are often concerned with understanding the scale at which efforts are most effective to ensure population persistence through demographic connectivity in networks of marine reserves.

The many instances of detected significant differentiation and the strong overall population structure detected with both marker systems suggest that local populations may need to be managed at a local scale, as successful intermediate or long distance dispersal could be rare. Despite the obvious need for the installation of marine reserve networks in the IMA, which are being developed in part under the management of the Coral Reef Initiative, other factors such as coastal development, climate-change-induced reef demise (bleaching), and the continued degradation of coastal habitats through pollution and unchecked exploitation, may override any conservation efforts. The lack of suitable settlement habitats outside of reserves can prevent an overspill effect from reserve areas and negate efforts to increase marine resources for local populations. In the case of *A. perideraion* and other anemonefish, harvesting of sea anemones removes suitable settlement habitat, preventing any larvae from settling. Without management strategies targeting vital components of the life history of these and other fishes, reserves may solely act as "islands of bounty" in an otherwise desolate environment.

Species response to Pleistocene sea level oscillations and more recent examples of complete marine habitat annihilation (Barber *et al.*, 2002, volcanic eruption on Krakatau) demonstrate the very high re-colonization potential of marine species in the IMA. However, the decreasing overall quality of marine habitat can prevent these mechanisms from working properly while increasing the importance of large-scale local and global efforts to reduce

marine pollution, control unchecked exploitation, and support an ecologically sensible use and management of marine resources.

REFERENCES

Ablan, M.C.A., J.W. McManus, C.A. Chen, K.T. Shao, J. Bell, A.S. Cabanban, *et al.* 2002. Meso-scale trans boundary units for the management of coral reefs in the South China sea area. NAGA Rep. World Fish Cent. Q. 25:4-9.

Almany, G.R., M.L. Berumen, S.R. Thorrold, S. Planes, and G.P. Jones. 2007. Local replenishment of coral reef fish populations in a marine reserve. Science 316:742-744.

Alvarado, B.J.R., A.J. Baker, and J. Mejuto. 1995. Mitochondrial DNA control region sequences indicate extensive mixing of swordfish (*Xiphias gladius*) populations in the Atlantic Ocean. Can. J. Fish Aquat. Sci. 52:1720-1732.

Barber, P.H., M.K. Moosa, and S.R. Palumbi. 2002. Rapid recovery of genetic diversity of stomatopod populations on Krakatau: temporal and spatial scales of marine larval dispersal. Proc. R. Soc. Lond. B 269:1591-1597.

Barber, P.H., S.H. Cheng, M.V. Erdmann, and K. Tengardjaja. 2011. Evolution and conservation of marine biodiversity in the Coral Triangle: insights from stomatopod Crustacea. Crustac. Issues 19:129-156.

Baums, I.B., C.B. Paris, and L.M. Cherubin. 2006. A biooceanographic filter to larval dispersal in a reef-building coral. Limnol. Oceanogr. 51:1969-1981.

Bay, L.K., J.H. Choat, L. van Herwerden, and D.R. Robertson. 2004. High genetic diversities and complex genetic structure in an Indo-Pacific tropical reef fish (*Chlorurus sordidus*): evidence of an unstable evolutionary past? Mar. Biol. 144:757-767.

Beldade, R., S.J. Holbrook, R.J. Schmitt, S. Planes, D. Malone, and D. Bernardi. 2012. Larger female fish contribute disproportionately more to self-replenishment. Proc. Biol. Sci. 279:2116–2121.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. Series B 57:289–300.

Bernardi, G., S.J. Holbrook, R.J. Schmitt, and N.L. Crane. 2003. Genetic evidence for two distinct clades in the French Polynesian population of coral reef three-spot damselfish *Dascyllus trimaculatus*. Mar. Biol. 143:485-490.

Botsford, L.W., A. Hastings, and S. Gaines. 2001. Dependence of sustainability on the configuration of marine reserves and larval dispersal distance. Ecol. Lett. 4:144-150.

Bowen, B.W., A.L. Bass, A. Muss, J. Carlin, and D.R. Robertson. 2006. Phylogeography of two Atlantic squirrelfishes (family Holocentridae): exploring links between pelagic larval duration and population connectivity. Mar. Biol. 149:899-913.

Briggs, J.C. 1974. Marine zoogeography. McGraw-Hill, New York, NY. Burke, L., L. Selig, and M. Spalding. 2002. Reefs at risk in Southeast Asia. UNEP-WCMC, Cambridge, UK.

Buston, P.M., G.P. Jones, S. Planes, and S.R. Thorrold. 2012. Probability of successful larval dispersal declines fivefold over 1 km in a coral reef fish. Proc. Biol. Sci. 279:1883-1888.

Buston, P.M., S.M. Bogdawicz, A. Wong, and R.G. Harrison. 2007. Are clownfish groups composed of close relatives? An analysis of microsatellite DNA variation in *Amphiprion percula*. Mol. Ecol. 16:3671–3678.

Carpenter, K.E., P.H. Barber, E.D. Crandall, M.C. Ablan-Lagman, G.N. Mahardika, B.M. Manjaji-Matsumoto, *et al.* 2011. Comparative phylogeography of the Coral Triangle and implications for marine management. J. Mar. Biol. 2011:1–14.

Chakraborty, R. 1990. Mitochondrial DNA polymorphism reveals hidden heterogeneity within some Asian populations. Am. J. Hum. Genet. 47:87-94.

Christie, M.R., D.W. Johnson, C.D. Stallings, and M.A. Hixon. 2010. Self-recruitment and sweepstakes reproduction amid extensive gene flow in a coral-reef fish. Mol. Ecol. 19:1042-1057.

Cowen, R.K., and S. Sponaugle. 2009. Larval dispersal and marine population connectivity. Annu. Rev. Mar. Sci. 1:443-466.

Crandall, E.D., E.J. Sbrocco, T.S. Deboer, P.H. Barber, and K.E. Carpenter. 2012. Expansion dating: calibrating molecular clocks in marine species from expansions onto the Sunda Shelf following the last glacial maximum. Mol. Biol. Evol. 29:707-719.

DeBoer, T., M. Subia, K. Kovitvonga, Ambaryanto, M. Erdmann, and P.H. Barber. 2008. Phylogeography and limited genetic connectivity in the endangered giant boring clam, *Tridacna crocea*, across the Coral Triangle. Conserv. Biol. 22:1255-1266.

DiBattista, J.D., L.A. Rocha, M.T. Craig, K.A. Feldheim, and B.W. Bowen. 2012. Phylogeography of two closely related Indo-Pacific butterflyfishes reveals divergent evolutionary histories and discordant results from mtDNA and microsatellites. J. Hered. 103:617-629.

Edwards, S.V., and S. Bensch. 2009. Looking forwards or looking backwards in avian phylogeography? A comment on Zink and Barrowclough 2008. Mol. Ecol. 18:2930-2933.

Ewens, W.J. 1972. The sampling theory of selectively neutral alleles. Theor. Popul. Biol. 3:87-112.

Excoffier, L., and M. Slatkin. 1998. Incorporating genotypes of relatives into a test of linkage disequilibrium. Am. J. Hum. Genet. 62:171-180.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol. Bioinform Online 1:47-50.

Fautin, D.G., and G.R. Allen. Revised edition 1997. Anemone fishes and their host sea anemones. Western-Australian Museum, Perth, Australia.

Fleminger, A. 1986. The Pleistocene equatorial barrier between the Indian and Pacific Oceans and a likely cause for Wallace's line. UNESCO Tech. Pap. Mar. Sci. 49:84-97.

Fogarty, M.J., and L. Botsford. 2007. Population connectivity and spatial management of marine fisheries. Oceanography 20:112-123.

Fu, Y.-X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915-925.

Gaither, M.R., B.W. Bowen, T.-R. Bordenave, L.A. Rocha, S.J. Newman, J.A. Gomez, *et al.* 2011. Phylogeography of the reef fish *Cephalopholis argus* (Epinephelidae) indicates Pleistocene isolation across the indo-pacific barrier with contemporary overlap in the coral triangle. BMC Evol. Biol. 11:189.

Gerlach, G., A. Jueterbock, P. Kraemer, J. Deppermann, and P. Harmand. 2010. Calculations of population differentiation based on G(ST) and D: forget G(ST) but not all of statistics! Mol. Ecol. 19:3845-3852.

Goudet, J. 1995. Fstat version 1.2: a computer program to calculate F-statistics. J. Hered. 86:485-486.

Hall, T.A. 1999. Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/ NT. Nucleic Acids Symp. Ser. 41:95-98.

Hedgecock, D., and A.I. Pudovkin. 2011. Sweepstakes reproduction success in highly fecund marine fish and shellfish: a review and commentary. Bull. Mar. Sci. 87:971-1002.

Hoeksema, B.W. 2007. Delineation of the Indo-Malayan centre of maximum marine biodiversity: the Coral Triangle. Pp. 117-178 in W. Renema, ed. Biogeography, time and place: distributions, barriers and Islands. Springer, Dordrecht, The Netherlands.

Hubisz, M.J., D. Falush, M. Stephens, and J.K. Pritchard. 2009. Inferring weak population structure with the assistance of sample group information. Mol. Ecol. Resour. 9:1322-1332.

Hughes, T.P., D.R. Bellwood, C. Folke, R.S. Steneck, and J. Wilson. 2005. New paradigms for supporting resilience of marine ecosystems. Trends Ecol. Evol. 20:380-386.

Jensen, J.L., A.J. Bohonak, and S.T. Kelley. 2005. Isolation by distance, web service. BMC Genetics 6: 13. v.3.23 http:// ibdws.sdsu.edu/

Johnson, M.S., and R. Black. 1984. The Wahlund effect and the geographical scale of variation in the intertidal limpet *Siphonaria* sp. Mar. Biol. 79:295-302.

Jones, G.P., S. Planes, and S.R. Thorrold. 2005. Coral reef fish larvae settle close to home. Curr. Biol. 15:1314-1318.

Jones, A.M., S. Gardner, and W. Sinclair. 2008. Losing 'Nemo': bleaching and collection appear to reduce inshore populations of anemonefishes. J. Fish Biol. 73:753-761.

Jost, L. 2008. GST and its relatives do not measure differentiation. Mol. Ecol. 17:4015-4026.

Karl, S.A., R.J. Toonen, W.S. Grant, and B.W. Bowen. 2012. Common misconceptions in molecular ecology: echoes of the modern synthesis. Mol. Ecol. 21:4171-4189.

Knittweis, L., W.E. Krämer, J. Timm, and M. Kochzius. 2008. Genetic structure of *Heliofungia actiniformis* (Scleractinia: Fungiidae) populations in the Indo-Malay Archipelago: implications for live coral trade management efforts. Conserv. Genet. 10:241-249.

Kochzius, M., and A. Nuryanto. 2008. Strong genetic population structure in the boring giant clam, *Tridacna crocea*, across the Indo-Malay Archipelago: implications related to evolutionary processes and connectivity. Mol. Ecol. 17:3775-3787.

Kochzius, M., R. Söller, M.A. Khalaf, and D. Blohm. 2003. Molecular phylogeny of the lionfish genera *Dendrochirus* and *Pterois* (Scorpaenidae, Pteroinae) based on mitochondrial DNA sequences. Mol. Phylogenet. Evol. 28:396-403.

Kochzius, M., C. Seidel, J. Hauschild, S. Kirchhoff, P. Mester, I. Meyer-Wachsmuth, *et al.* 2009. Genetic population structure of the blue starfish *Linckia laevigata* and its gastropod ectoparasite *Thyca crystallina*. Mar. Ecol. Prog. Ser. 396:211-219.

Kool, J.T., C.B. Paris, P.H. Barber, and R.K. Cowen. 2011. Connectivity and the development of population genetic structure in Indo-West Pacific coral reef communities. Glob. Ecol. Biogeogr. 20:695-706.

Kruskal, J.B. 1956. On the shortest spanning subtree of a graph and the travelling salesman problem. Proc. Am. Math. Soc. 7:48-50.

Lee, W.J., W.H. Howell, and T.D. Kocher. 1995. Structure and evolution of teleost mitochondrial control regions. J. Mol. Evol. 41:54-66.

Leray, M., R. Beldade, S.J. Holbrook, R.J. Schmitt, S. Planes, and G. Bernardi. 2010. Allopatric divergence and speciation in coral reef fish: the three-spot Dascyllus, *Dascyllus trimaculatus*, species complex. Evolution 64:1218-1230.

Levin, L.A. 2006. Recent progress in understanding larval dispersal: new directions and digressions. Integr. Comp. Biol. 46:282-297.

Librado, P., and J. Rozas. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451-1452.

Liu, S.Y.V., H.T. Yu, and C.F. Dai. 2007. Eight microsatellite loci in Clark's anemonefish, *Amphiprion clarkii*. Mol. Ecol. Notes 7:1169-1171.

Lourie, S., D.M. Green, and C.J. Vincent. 2005. Dispersal, habitat differences, and comparative phylogeography of Southeast Asian seahorses (Syngnathidae: *Hippocampus*). Mol. Ecol. 14:1073-1094.

Madduppa, H.H., J. Timm, and M. Kochzius. 2014. Interspecific, spatial and temporal variability of selfrecruitment in anemonefishes. PLoS ONE 9:e90648.

McManus, J.W. 1985. Marine speciation, tectonics and sealevel changes in Southeast Asia. Proceedings of the Fifth International Coral Reef Congress, Tahiti 4:133-138.

McMillan, W.O., and S.R. Palumbi. 1995. Concordant evolutionary patterns among Indo-West Pacific butterflyfishes. Proc. Biol. Sci. 260:229-236.

Meirmans, P.G., and P.W. Hedrick. 2011. Assessing population structure: F(ST) and related measures. Mol. Ecol. Resour. 11:5–18.

Nei, M. 1987. Molecular evolutionary genetics. Columbia Univ. Press, New York.

Nelson, J.S., R.J. Hoddell, L.M. Chou, W.K. Chan, and V.P.E. Wang. 2000. Phylogeographic structure of false clownfish, *Amphiprion ocellaris*, explained by sea-level changes on the Sunda shelf. Mar. Biol. 137:727-736.

Nuryanto, A., and M. Kochzius. 2009. Highly restricted gene flow and deep evolutionary lineages in the giant clam *Tridacna maxima*. Coral Reefs 28:607-619.

Ovenden, J.R., J. Salini, S. O'Connor, and R. Street. 2004. Pronounced genetic population structure in a potentially vagile fish species (*Pristipomoides multidens*, Teleostei; Perciformes; Lutjanidae) from the East Indies triangle. Mol. Ecol. 13:1991-1999.

Palumbi, S.R. 2003. Population genetics, demographic connectivity, and the design of marine reserves. Ecol. Appl. 13:146-158.

Pineda, J., J. Hare, and S. Sponaugle. 2007. Marine population connectivity: larval transport and dispersal in the coastal ocean and consequences for population connectivity. Oceanography 20:22-39.

Planes, S., and C. Fauvelot. 2002. Isolation by distance and vicariance drive genetic structure of a coral reef fish in the Pacific Ocean. Evolution 56:378-399.

Prim, R.C. 1957. Shortest connection networks and some generalizations. Bell Syst. Tech. J. 36:1389-1401.

Pritchard, J.K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945-959.

Quenouille, B., Y. Bouchenak-Khelladi, C. Hervet, and S. Planes. 2004. Eleven microsatellite loci for the saddleback clownfish *Amphiprion polymnus* (Teleosti: Pomacentridae). Mol. Ecol. Notes 4:291-293.

Ray, N., M. Currat, and L. Excoffier. 2003. Intra-deme molecular diversity in spatially expanding populations. Mol. Biol. Evol. 20:76-86.

Riginos, C., K.E. Douglas, Y. Jin, D.F. Shanahan, and E.A. Treml. 2011. Effects of geography and life history traits on genetic differentiation in benthic marine fishes. Ecography 34:566-575.

Roberts, C.M., C.J. McClean, and J.E.N. Veron. 2002. Marine biodiversity hotspots and conservation priorities for tropical reefs. Science 295:1280-1284.

Rogers, A.R., and H. Harpending. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Mol. Biol. Evol. 9:552-569.

Santini, S., and G. Polacco. 2006. Finding Nemo: molecular phylogeny and evolution of the unusual life style of anemonefish. Gene 385:19-27.

Schluessel, V., D. Broderick, S.P. Collin, and J.R. Ovenden. 2010. Evidence of extensive population structure in the white spotted eagle ray, *Aetobatus narinari*, within the Indo-Pacific inferred from mitochondrial gene sequences. J. Zool. 281:46-55.

Severance, E.G., and S.A. Karl. 2006. Contrasting population genetic structures of sympatric, mass spawning Caribbean corals. Mar. Biol. 150:57-68.

Shuman, C.S., G. Hodgson, and R. Ambrosen. 2006. Population impacts of collecting sea anemones and anemonefish for the marine aquarium trade in the Philippines. Coral Reefs 24:564-573.

Swearer, S.E., J.S. Shima, M.E. Hellberg, S.R. Thorrold, G.P. Jones, D.R. Robertson, *et al.* 2002. Evidence of selfrecruitment in demersal marine populations. Bull. Mar. Sci. 70:251-272.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585-595.

Tajima, F. 1993. Measurement of DNA polymorphism. Pp. 37–59 in N. Takahata, A.G. Clark, eds. Mechanisms of molecular evolution. Introduction to molecular paleopopulation biology. Scientific Societies Press, Sinauer Associates, Inc, Tokyo, Sunderland, MA.

Taylor, M.S., and M.E. Hellberg. 2003. Genetic evidence for local retention of pelagic larvae in a Caribbean reef fish. Science 299:107–109.

Thacker, C.E., A.R. Thompson, D.M. Roje, and E.Y. Shaw. 2007. New expansions in old clades: population genetics and phylogeny in a *Gnatholepis* species (Teleostei: Gobiodei) in the Pacific. Mar. Biol. 153:375-385.

Thompson, J.G., D.G. Higgins, and T.J. Gibson. 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignments through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673-4680.

Timm, J., and M. Kochzius. 2008. Geological history and oceanography of the Indo- Malay Archipelago shape the genetic population structure in the false clown anemonefish (*Amphiprion ocellaris*). Mol. Ecol. 17:3999-4014.

Timm, J., M. Figiel, and M. Kochzius. 2008. Contrasting patterns in species boundaries and evolution of anemonefishes (Amphiprioninae, Pomacentridae) in the centre of marine biodiversity. Mol. Phylogenet. Evol. 49:268-276.

Timm, J., S. Planes, and M. Kochzius. 2012. High similarity of genetic population structure in the false clown anemonefish (*Amphiprion ocellaris*) found in microsatellite and mitochondrial control region analysis. Conserv. Genet. 13:693-706.

Toews, D.P.L., and A. Brelsford. 2012. The biogeography of mitochondrial and nuclear discordance in animals. Mol. Ecol. 21:3907-3930.

Van Oosterhout, C., W.F. Hutchinson, D.P.M. Wills, and P. Shipley. 2004. Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4:535-538.

Veron, J.E.N., L.M. DeVantier, E. Turak, A.L. Green, S. Kininmoth, M. Stafford-Smith, *et al.* 2009. Delineating the Coral Triangle. Galaxea 11:91-100.

Voris, H.K. 2000. Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. J. Biogeogr. 27:1153-1167.

Wabnitz, C., M. Taylor, E. Green, and T. Razak. 2003. From ocean to aquarium. UNEP WCMC, Cambridge, UK.

Wellington, G.M., and B.C. Victor. 1989. Planktonic larval duration of one hundred species of Pacific and Atlantic damselfishes (Pomacentridae). Mar. Biol. 101:557-567.

Williams, S.T. 2000. Species boundaries in the starfish genus *Linckia*. Mar. Biol. 136:137-148.

Winters, K.L., L. van Herwerden, H.J. Choat, and D.R. Robertson. 2010. Phylogeography of the Indo-Pacific parrotfish *Scarus psittacus*: isolation generates distinctive peripheral populations in two oceans. Mar. Biol. 157:1679-1691

SUPPORTING INFORMATION - Online Version of the Article

Table S5.1 Genbank accession ID's for CR sequences of *A. perideraion* used in this study.

Sampling Location	Genbank Accession ID
Indonesia:	
Makassar Strait, Sulawesi, Spermonde Archipelago	JX513647 - JX513666
Makassar Strait, Sulawesi, Donggala	JX513667 - JX513685
Celebes Sea, Sulawesi, Manado	JX513686 - JX513701
Celebes Sea, Borneo (Kalimantan Timur), Sangalaki	JX513762 - JX513778
Lembeh Strait, Sulawesi	JX513702 - JX513708
Maluku Sea, Sulawesi, Luwuk	JX513709 - JX513722
Flores Sea, Sulawesi, Bira	JX513723 - JX513742
Flores Sea, Komodo	JX513794 - JX513801
Banda Sea, Sulawesi, Kendari	JX513743 - JX513761
Banda Sea, New Guinea, Padang	
Banda Sea, New Guinea, Triton Bay	
Java Sea, Java, Karimunjava	JX513779 - JX513787
Java Sea, Bali	JX513788 - JX513793
Savu Sea, Timor, Kupang	JX513802 - JX513809
Pacific Ocean, New Guinea, Biak	JX513826 - JX513846
Malaysia:	
South China Sea, Banggi Islands	JX513810 - JX513820
South China Sea, Borneo, Kota Kinabalu	JX513821 - JX513826
Philippines : Cebu Strait, Cebu	JX513847 - JX513865
Solomon Islands	DQ343940 Santini & Polacco, 2006
Japan : Okinawa	JX513866 - JX513875

Table S5.2 Population pairwise differences (F_{st}, below diagonal) between all *A. perideraion* sampling sites using data from ten microsatellite loci. Bold values denote significance at $P \leq 0.05$ (above diagonal) after correction for multiple testing (Benjamini and Hochberg 1995, False Discovery Rate procedure).

	Sp	Do	Ma	LS	Lu	Bi	Ke	Sa	Ka	Ba	Ko	Ku	BI	KK	Bk	Ce	Mi	Pi	Pa	Tr
Sp		*0.081*	*0.109*	*0.063*	***0.027***	*0.317*	*0.120*	***0.000***	***0.000***	***0.000***	*0.063*	*0.063*	*0.081*	*0.596*	***0.000***	***0.027***	*0.415*	*0.360*	*0.120*	*0.171*
Do	0.007		*0.446*	***0.000***	***0.000***	***0.000***	*0.063*	***0.046***	***0.000***	***0.000***	***0.000***	***0.027***	***0.000***	*0.235*	***0.000***	***0.000***	*0.527*	*0.145*	***0.000***	***0.000***
Ma	0.006	0.002		*0.170*	*0.063*	*0.415*	*0.264*	*0.184*	***0.000***	***0.027***	*0.109*	***0.000***	*0.531*	*0.371*	***0.000***	***0.000***	*0.474*	*0.170*	*0.081*	*0.317*
LS	0.013	**0.019**	0.011		*0.171*	*0.340*	*0.630*	***0.046***	***0.000***	*0.145*	*0.624*	*0.317*	*0.531*	*0.333*	***0.046***	*0.063*	*0.317*	*0.204*	*0.120*	*0.273*
Lu	**0.011**	**0.014**	0.012	0.013		*0.096*	*0.120*	***0.027***	***0.000***	*0.235*	*0.120*	*0.170*	*0.171*	*0.538*	***0.000***	***0.000***	*0.602*	*0.192*	***0.027***	*0.531*
Bi	0.003	**0.010**	0.004	0.007	0.014		*0.584*	*0.364*	***0.000***	*0.109*	*0.096*	***0.046***	*0.340*	*0.364*	***0.000***	***0.000***	*0.441*	*0.096*	***0.046***	*0.171*
Ke	0.006	0.009	0.007	0.002	0.012	0.001		*0.171*	***0.000***	*0.192*	***0.000***	***0.046***	*0.379*	*0.492*	***0.000***	***0.000***	*0.283*	*0.273*	*0.171*	*0.703*
Sa	**0.009**	**0.010**	0.007	0.017	**0.018**	0.003	0.008		***0.000***	***0.027***	***0.000***	*0.109*	*0.160*	*0.446*	***0.000***	*0.316*	*0.326*	*0.245*	*0.109*	*0.133*
Ka	**0.057**	**0.054**	**0.048**	**0.055**	**0.051**	**0.049**	**0.056**	**0.063**		***0.027***	***0.000***	***0.000***	***0.000***	***0.000***	***0.000***	***0.000***	*0.415*	***0.000***	***0.000***	***0.000***
Ba	**0.028**	**0.032**	**0.023**	0.022	0.015	0.022	0.014	**0.022**	**0.070**		***0.046***	***0.000***	*0.670*	*0.145*	***0.027***	***0.000***	*0.865*	*0.171*	***0.027***	*0.063*
Ko	0.011	**0.020**	0.014	0.000	0.012	0.014	**0.018**	**0.020**	**0.050**	**0.028**		***0.046***	*0.171*	*0.235*	***0.027***	***0.027***	*0.264*	***0.027***	***0.000***	***0.046***
Ku	0.016	**0.020**	**0.025**	0.012	0.017	**0.021**	**0.019**	0.012	**0.055**	**0.043**	**0.022**		***0.027***	*0.214*	***0.027***	*0.245*	*0.531*	*0.171*	*0.192*	*0.120*
BI	0.011	**0.017**	0.002	0.004	0.011	0.006	0.006	0.009	**0.063**	0.005	0.011	**0.027**		*0.293*	***0.046***	***0.027***	*0.596*	*0.371*	*0.096*	*0.264*
KK	0.000	0.011	0.009	0.009	0.002	0.007	0.004	0.002	**0.065**	0.026	0.007	0.014	0.011		*0.170*	*0.133*	*0.531*	***0.027***	*0.214*	*0.869*
Bk	**0.017**	**0.029**	**0.024**	**0.025**	**0.017**	**0.024**	**0.021**	**0.025**	**0.067**	**0.033**	**0.031**	**0.027**	**0.019**	0.022		***0.000***	*0.842*	*0.433*	*0.133*	*0.655*
Ce	**0.015**	**0.019**	**0.019**	0.028	**0.019**	**0.019**	**0.020**	0.004	**0.066**	**0.024**	**0.027**	0.008	**0.020**	0.020	**0.026**		*0.364*	*0.333*	***0.046***	***0.000***
Mi	0.011	0.022	0.017	0.027	0.015	0.016	0.014	0.013	0.058	0.000	0.043	0.023	0.016	0.000	0.001	0.033		*0.527*	*0.773*	*0.618*
Pi	0.017	0.027	0.026	0.033	0.030	0.031	0.015	0.018	**0.090**	0.045	**0.067**	0.035	0.019	**0.041**	0.012	0.012	0.022		*0.901*	*0.192*
Pa	0.010	**0.018**	0.014	0.019	**0.019**	**0.017**	0.010	0.012	**0.066**	**0.025**	**0.030**	0.012	0.018	0.013	0.011	**0.017**	0.000	0.000		*0.317*
Tr	0.004	**0.012**	0.005	0.008	0.002	0.007	0.000	0.007	**0.055**	**0.023**	**0.015**	0.014	0.006	0.000	0.001	**0.018**	0.006	0.022	0.005	

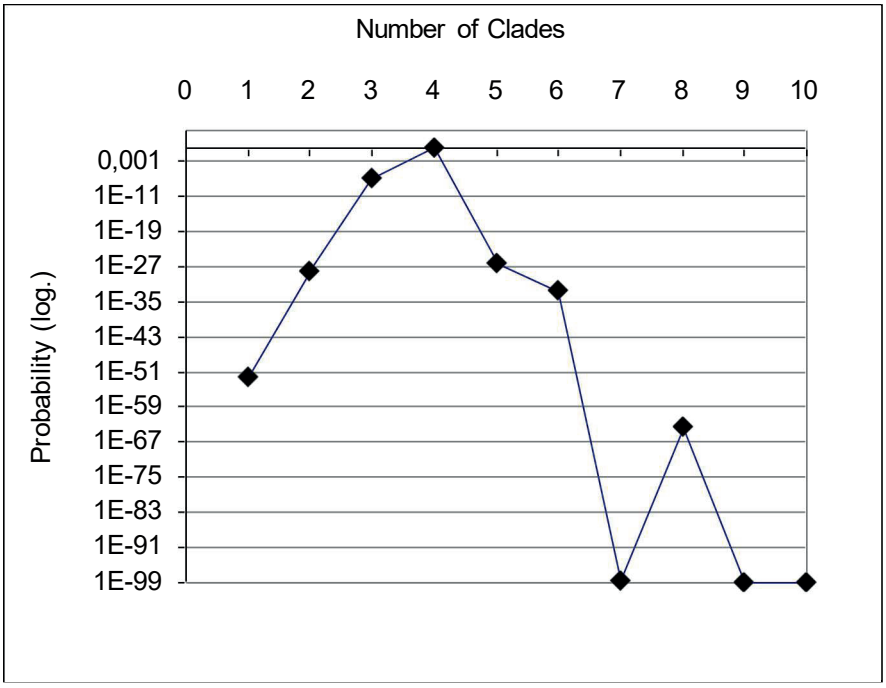


Figure S5.1 Data from 10 microsatellite loci were used to produce groupings for 290 samples of *A. perideraion* in STRUCTURE (ver. 2.2., Pritchard *et al.* 2000). Depicted here is the probability that each number of groupings applied during the analysis (k = 1-10) constitutes the correct subdivision of the dataset (Bayesian likelihood). The length of the burn-in period was set at 120000 and the number of MCMC reps to 300000, with 10 iterations for each k. Zero values were entered as 1E-99.

Chapter II

Phylogeography of the orange anemonefish, *Amphiprion sandaracinos*, as a basis for a synergized genetic landscape of four congeners (*Amphiprion* spp.)

Tina A. Dohna[1], Marc Kochzius[2], Maria Liebsch[3], Melina Rodríguez Moreno[4] and Janne Timm[1]

1) Biotechnology and Molecular Genetics, UFT, University of Bremen, Bremen 28359, Germany

2) Marine Biology, Vrije Universiteit Brussel, Brussel, Belgium

3) Max Planck Institute for Molecular Genetics, Berlin, Germany

4) Centro de Excelencia en Ciencias Marinas, Universidad del Valle, Cali, Colombia

Anticipated submission to Diversity and Distributions

ABSTRACT

Amphiprion sandaracinos is a popular aquarium fish, easily collected from its sea anemone host, where it spends the entirety of its post larval life in a mutually obligate symbiosis. A short pelagic larval duration, adult site fidelity, highly specialized host use (only two sea anemones species), fishery pressure on fish and hosts, and detection of pronounced population structure in other anemonefish, justify a detailed analysis of the phylogeography of this charismatic reef specialist. Mitochondrial Control Region sequences (CR) and eight microsatellite loci (Msat) were analyzed to determine overall and population pairwise genetic differentiation and to assess genetic diversity gradients. Similar data for congeners, *Amphiprion ocellaris*, *Amphiprion perideraion*, and *Amphiprion clarkii*, were included to search for common diversity patterns and to produce a synergized genetic landscape of shared geneflow barriers. Populations of *A. sandaracinos* from locations in the Indo-Malay Archipelago were characterized by a strong population structure (Control R, $\Phi_{ST} = 0.219$, $p < 0.0001$; Msat, $D_{est} = 0.123$, $p = 0.013$) and a significant increase in haplotype diversity moving south and east across the sampling region. Diversity patterns among congenerics were species specific, with a general tendency of nucleotide diversities being higher in southern and eastern populations. Concatenation of species specific barrier maps produced a genetic landscape with scaled barriers to geneflow, affecting populations of the anemonefish studied here. The geographic placement of these barriers and their ranked impact are an important spatial component for marine resource management, where single species barriers can often not be fully considered.

INTRODUCTION

Patterns of genetic differentiation within and among populations of marine species in the Indo-Malay Archipelago (IMA) are currently receiving added attention as a way to identify vulnerabilities in taxa and biotic regions based on species-specific and shared barriers to connectivity (Carpenter *et al.* 2011, von der Heyden *et al.* 2014, Treml *et al.* 2015). Population genetic studies covering fish (e.g. Lourie *et al.* 2005, Horne *et al.* 2008, Timm & Kochzius 2008, Gaither *et al.* 2011, Lord *et al.* 2012, Timm *et al.* 2012, Dohna *et al.* 2015) and invertebrate species (e.g. Barber *et al.* 2000, 2002; Crandall *et al.* 2008, DeBoer *et al.* 2008, Kochzius *et al.* 2009, Nuryanto & Kochzius 2009, Duda *et al.* 2012) in and across the IMA have been conducted, and a limited overlap in the internal patterns has been found (Carpenter *et al.* 2011, von der Heyden *et al.* 2014). Understanding the mechanisms regulating the magnitude and directionality of connectivity among populations and regions is the key to effective resource management (Almany *et al.* 2009, McCook *et al.* 2009, Beger *et al.* 2010, Kininmonth *et al.* 2011, Olds *et al.* 2012). This is of particular urgency for the coral reefs of the IMA, which support the highest global marine biodiversity (Roberts *et al.* 2002, Hoeksema 2007, Veron *et al.* 2009) while also ranking among the most threatened reef systems worldwide (Burke *et al.* 2002, 2011; Wilkinson 2002, Allen 2008, Peñaflor *et al.* 2009).

Many sessile marine species rely on the dispersing capabilities of their pelagic larvae to ensure connectivity among both distant and proximate subpopulations. Their population connectivity is shaped primarily through larval dispersal and mortality (Pineda *et al.* 2007). Larvae are carried by ocean currents for the pelagic larval duration (PLD) until settlement, though active swimming behavior (Leis *et al.* 1996, Fisher *et al.* 2000) and local current regimes (circular currents, eddies and fronts) (Cowen *et al.* 2000, Kool *et al.* 2011) can prevent a simple calculation of the dispersal based on current speed, direction and PLD (Bradbury & Bentzen 2007, Bradbury *et al.* 2008, Shanks 2009, Weersing & Toonen 2009, Riginos *et al.*

2011). Additionally, the availability of suitable settlement habitat, egg type (Riginos *et al.* 2011), relative productivity of larval sources (source/sink dynamics), larval size (e.g., Litvak & Leggett 1992, Meekan *et al.* 2006), and maternal traits (e.g., Beldade *et al.* 2012, *Amphiprion chrysopterus*) have all been implicated in population connectivity, resulting in a complex and poorly understood dynamic (reviewed in Cowen & Sponaugle 2009). Coral reef fish usually have PLDs on the order of several weeks to months (e.g. 22-26 days in *Dascyllus trimaculatus,* 24-33 days in *Chromis multilineata,* 30-56 days in *Bodianus rufus,* Wellington & Robertson 2001). The PLD of 8-18 days in anemonefish (Wellington & Victor 1989, Fautin & Allen 1997) is therefore considered short and can lead to a genetic population structure at smaller spatial scales despite their pelagic larvae, as has been shown for *A. ocellaris* (Nelson *et al.* 2000, Timm & Kochzius 2008, Timm *et al.* 2012) and *A. perideraion* (Dohna *et al.* 2015). This results in an ongoing need to investigate individual connectivity patterns, especially for species reliant on pelagic larvae for population subsistence and reseeding in the absence of adult migration.

Population genetic studies are of particular importance for species under added stress from commercial harvesting, such as the anemonefish species found in the IMA (Wabnitz *et al.* 2003). The orange skunk anemonefish, *Amphiprion sandaracinos* (Allen 1972) (Pomacentridae, Amphiprioninae), studied here, is a popular aquarium fish collected for the ornamental trade in much of its range. All members of the Amphiprioninae are site-bound, living in obligate symbiotic mutualism with tropical sea anemones (Cnidaria, Hexacorallia, Actiniaria) in which they settle as juveniles (Fautin & Allen 1997), thereby excluding adult migration in genetic mixing. Host sea anemones are also reliant on dinoflagellate endosymbionts (*Symbodinium* sp.)(Baker 2003) which are expelled under conditions of toxic, light and temperature stress (Hobbs *et al.* 2013), in addition to sea anemones being collected from the wild for the marine ornamental trade (Shuman *et al.* 2005, Turton & Otomo 2007, Maduppa *et al.* 2014a). This type of multi-species symbioses is common in reef communities, highlighting the vulnerability of these and similar species systems under climate

change and increasing anthropogenic impacts.

While efforts have focused on establishing patterns common among similar species, the strategy of extrapolating community patterns from exemplar species has been questioned, because the validity of the resulting generalizations is largely unknown (Bird *et al.* 2007, Toonen *et al.* 2011) and they have even shown to fail for species with very similar life histories and/or larval ecology/physiology (Rocha *et al.* 2002, Reid *et al.* 2006, Bird *et al.* 2007, Barber *et al.* 2011, DiBattista *et al.* 2012). The result was a call for multi-species studies so as to prevent erroneous extrapolation of management units for whole communities from single-species structures (Toonen *et al.* 2011). Multi-species studies can aid in the identification of geneflow - inhibiting geographical features which produce concordant patterns of genetic discontinuity (Avise 2000, Carpenter *et al.* 2011, von der Heyden *et al.* 2014), although the theoretical framework for this type of approach is poorly developed (but see Hickerson & Meyer 2008). The present study aims to provide evidence of shared genetic barriers and geographical trends in genetic diversity among four anemonefishes, *A. sandaracinos, Amphiprion perideraion* (Bleeker 1855), *Amphiprion ocellaris* (Cuvier 1830), and *Amphiprion clarkii* (Bennett 1830), with overlapping distributions and very similar life histories. Studying closely related species helps to reduce some of the biological complexity and may substantiate inferences concerning geographic barriers and diversity that are drawn from genetic evidence (Dawson 2012).

Anemonefishes spawn throughout the year in most tropical locations (Allen 1975, Ross 1978), so that larvae are assumed to meet similar conditions upon hatching. All four species recruit to the same reef habitat with location specific degrees of vertical stratification (Elliott & Mariscal 2001, Ricciardi *et al.* 2010, Litsios *et al.* 2014). A distinguishing feature of the fishes studied here is their degree of host specialization, with *A. sandaracinos* being found with two, *A. ocellaris* with three, *A. perideraion* with four, and *A. clarkii* with ten sea anemone species.

Host generalists, such as *A. clarkii* recruits, are expected to encounter suitable settlement substrate at higher frequencies than host specialists, such as *A. sandaracinos* recruits. This could lead to greater recruitment success and reduced genetic structure for the generalist, if host specialization does in fact act on genetic structure as predicted.

Another distinguishing feature is the PLD length of these four species. Although there is some overlap, *A. clarkii* has the shortest PLD, while *A. ocellaris* and *A. perideraion* have successively longer projected PLD's (PLD of *A. sandaracinos* is unknown). While some studies found no correlation between PLD and genetic structure in other coral reef fish (Bay *et al.* 2006, Riginos *et al.* 2011, Portnoy *et al.* 2013), PLD has also been argued to be a good predictor for genetic structure (Faurby & Barber 2012, Weersing & Toonen 2009, Selkoe & Toonen 2011). The intrageneric comparison of these highly similar species can add to this discussion, which is of major consequence for spatial planning in marine resource management.

By mapping the genetic landscape of *A. sandaracinos* and integrating the results with existing datasets of congenerics, this study will address the following questions: (1) does the population structure of *A. sandaracinos* follow expectations of a strong differentiation among sites/regions, based on results from congenerics and other sessile marine species; (2) does the genetic diversity of *A. sandaracinos* follow a homogeneous distribution or can diversity gradients or islets be identified; (3) where can genetic barriers be detected within the IMA and are they congruent across species and molecular markers; (4) is the PLD length or the number of host sea anemones a better predictor of connectivity between populations of anemonefishes? The results are expected to contribute to our understanding of the dynamics that shape diversity and connectivity in this global biodiversity hotspot.

MATERIALS AND METHODS

Sampling and Sequencing

Control Region (CR) - *A. sandaracinos*

Specimens of *A. sandaracinos* (n = 89) were visually identified, caught with hand nets, and fin-clipped from locations in the IMA. Subsequently they were released back to their anemones. Fin clip samples were stored in 96 % ethanol at 4 °C. Genomic DNA was extracted with a commercial kit (peqGOLD Tissue DNA Mini Kit, Peqlab, Erlangen, Germany). Universal primers CR-E 5'-CCT GAA GTA GGA ACC AGA TG-3' and CR-A 5'-TTC CAC CTC TAA CTC CCA AAG CTA G-3' (Lee *et al.* 1995) were employed to amplify a 420-bp fragment of the hypervariable D-loop segment of the mitochondrial control region (CR) for 89 individuals from 12 locations. PCR reactions followed a standard PCR protocol detailed in Timm and Kochzius (2008). PCR products were purified with a QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany). Both strands were sequenced on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Weiterstadt, Germany) after cycle PCR with the PCR primers and the Big Dye Terminator Cycle Sequencing Kit (ver. 3.1; Applied Biosystems).

Microsatellites (Msat) - *A. sandaracinos*

Several samples were lost during the construction of the control region dataset, making all samples from Manado and most from Biak and Kupang unavailable for microsatellite genotyping. The primers used to amplify eight microsatellite loci are listed in the supplementary materials (Table S5.4), along with their source and characterization. Samples were amplified following the protocol by Timm *et al.* (2012). The amplified fragments were run on an ABI 3100 Genetic Analyzer, using an internal 500 Rox Size Standard (Applied Biosystems). Genemarker (ver. 1.91 Demo; Softgenetics, State College, USA) was used to score fragment lengths for all samples.

Data Analysis

A. sandaracinos

Forward and reverse sequence strands of *A. sandaracinos* CR sequences were assembled and edited in Seqman (ver. 4.05 DNAStar). The sequences were aligned with Clustal W (Thompson *et al.* 1994), as implemented in BioEdit (ver. 7.0.0.1, Hall 1999) after including one CR-sequence of *A. sandaracinos* (GenBank DQ343943.1, Santini & Polacco 2006) from the Solomon Islands. Sequences were trimmed to the shortest common sequence length (375bp) and the resulting alignment was used for all subsequent analyses.

To insure suitability for population genetic analyses, the neutrality of the marker was evaluated on the basis of Tajima's D (Tajima 1989, 1993) and Fu's FS (Fu 1997), which also allows the detection of a recent population expansion or bottleneck. Chakraborty's test of amalgamation (Ewens 1972; Chakraborty 1990) was included to detect potential sample heterogeneity. All tests were carried out in DnaSP (ver.5.0, Librado & Rozas 2009).

Haplotype diversities (*h,* Nei 1987), nucleotide diversities (π, Nei & Jin 1989), and their standard deviations were calculated in ARLEQUIN, as were all following tests unless otherwise stated. An Analysis of Molecular Variance (AMOVA) for the whole dataset was conducted to test for genetic structure among populations (locus-by-locus AMOVA, 10000 permutations). Divergence between population pairs (pairwise Φ_{ST}) was also determined to investigate subtler population differentiation within groups or regions. Respective significance values were corrected according to Benjamini & Hochberg (1995) (multtest, R package 2.9.0), to control for the False Discovery Rate (FDR), inherent in multiple comparisons. Groups for hierarchical AMOVA testing were chosen to represent regional assemblages and/or to reflect gene flow barriers detected in pairwise population comparisons. A Minimum Spanning Tree (MST), based on pairwise differences of CR haplotypes, was estimated and drawn by hand. Clade assignments were made based on

greater number of mutations between clades than within. An identical haplotype network generated with TCS (ver. 1.21, Clement *et al.* 2000) (data not shown) identified the most probable common ancestral haplotype. The relative frequency of clades at each location was visualized with pie charts imposed onto a map of the sampling area.

To determine significant differences in population haplotype and nucleotide diversities at a regional scale, unpaired t-tests were executed using the online tool GraphPad (http://www.graphpad.com/quickcalcs/ttest1.cfm) between groups of populations for all four species.

A. perideraion*, *A. ocellaris*, and *A. clarkii

Measures of overall and regional genetic structure in *A. perideraion* and *A. ocellaris* were taken or generated from previously published datasets (*A. perideraion* - Dohna *et al.* 2015 and *A. ocellaris* - Timm & Kochzius 2008; Timm *et al.* 2012). The differentiation index D (Jost 2008) was calculated with DEMEtics (ver. 0.8-5 R package; Gerlach *et al.* 2010) to detect average overall (mean D_{ST}) and inter-population (pairwise mean D_{ST}) genetic differentiation in the datasets (Gerlach *et al.* 2010). The significance of the detected differentiation was described by *P*-values, estimated from bootstrap resampling (1000), and corrected to control for the FDR according to Benjamini and Hochberg (1995) (multtest, R package 2.9.0).

Measures of population structure for *A. clarkii* were derived from CR data generated and treated with methods identical to those described below for *A. sandaracinos*, leading to a matrix of pairwise Φ_{ST} values for 194 samples from 18 sites. Pairwise F_{ST} values were generated by analysis of a microsatellite dataset (8 loci, 226 samples from 15 sites; Rodríguez Moreno 2009) with ARLEQUIN (ver. 3.1, Excoffier *et al.* 2005) after the suitability of the markers for this type of analysis had been established with standard methods.

Common Genetic Barriers - *A. sandaracinos*, *A. clarkii*, *A. ocellaris*, and *A. perideraion*

Sampling locations were not shared among all species and across markers, so individual Voronoï tessellation maps were constructed in Barrier (ver. 2.2, Manni *et al.* 2004) for each of the four species and each marker type, where necessary. The maps consisted of Voronoï tessellations generated from XY coordinates for sampling locations. The tessellations represent individual polygonal neighborhoods for each of the included sites (population samples) and determine which sites are neighbors (i.e. adjacent). A Delaunay triangulation (Brassel & Reif 1979) is applied to connect all neighboring sites on the map. Only one possible Delaunay triangulation can be generated for a set of known geographic locations. The Monmonier's (1973) maximum difference algorithm is run on these maps to identify polygon edges where the distance (here pairwise Φ_{ST}, F_{ST} and De_{ST}) between populations within adjacent polygons is the greatest. By generating bootstrapped distance matrixes and applying them to the map, the robustness of the emerging patterns can be evaluated.

Distance matrixes for *A. sandaracinos* (CR - pairwise Φ_{ST}), *A. perideraion* (CR - pairwise Φ_{ST}, Msat - pairwise De_{ST}), *A. ocellaris* (CR - pairwise Φ_{ST}, Msat - pairwise De_{ST}), and *A. clarkii* (CR - pairwise Φ_{ST}, Msat - pairwise F_{ST}) were bootstrapped (1000) independently (boot, R package 2.9.0). Non-significant values were set to zero prior to bootstrapping. The Monmonier's algorithm does not perform well when a large number of zero values are included in the distance matrix (Manni & Guérard 2004). Microsatellite data for *A. sandaracinos* were therefore not included, as only three significant values remained after corrections for multiple testing. Triangulations differed slightly between maps due to missing or added intermediate populations, but were adjusted to minimize these differences. The Islands of Borneo, Sulawesi, and the northern tip of New Guinea were included as structures in the triangulation with the help of virtual points. This allows interruption of Delaunay

triangulations between locations that are adjacent within the triangulation, but are separated by physical structures in the real world, like lakes, deserts, or in this case, land.

To identify high order barriers prior to bootstrapping, maps were run with the original distance matrix for up to nine barriers. Order and position of the barriers were recorded prior to running the 1000 bootstrapped matrixes across the map. Bootstrap support values for the previously identified barriers were recorded. Potential shared barriers, their species composition, and cumulative bootstrap support were determined by overlaying all derived maps. All barriers detected in two or more species were included in a final map containing all sampling locations in order to visualize the resultant genetic landscape.

Pelagic Larval Duration (PLD) and Host Specialization - *A. sandaracinos*, *A. clarkii*, *A. ocellaris*, and *A. perideraion*

Records for the pelagic larval duration (PLD) of anemonefishes were acquired by literature search, as were references for host anemone use. Unfortunately, no published data for *A. sandaracinos* PLD could be found. PLD measurements for *A. ocellaris* could also not been acquired, so that the PLD of its sibling species *Amphiprion percula* was used instead. The data was inspected to make inferences about their influence on the genetic structure found in the studied species.

RESULTS

Genetic diversity- *A. sandaracinos*, *A. clarkii*, *A. ocellaris*, and *A. perideraion*

A total of 375 base pairs (bp) of the mitochondrial CR (D-loop) could be resolved for 89 *A. sandaracinos* individuals from 12 sampling locations situated in the IMA (Fig. 5.4A).

Several tests confirmed the suitability of the marker for all following analysis by failing to reject neutral evolution at this locus (Table 5.6). The ratio of the number of transitions to transversions was approximately 6 to1, double than in *A. ocellaris* (Timm & Kochzius 2008) and equal to results for *A. perideraion* (Dohna *et al.* 2015) and *A. clarkii* (*this study*). This provided 52 unique haplotypes, of which 46 (88 %) were private haplotypes (restricted to one location). With only 5 of the 46 private haplotypes found in more than one individual, the large majority were singletons. The percentage of private haplotypes at each sampling location, and the ratio of singleton haplotypes to total private haplotypes for all four fish species is shown in Fig. 5.5.

Table 5.6 Results for several statistical tests to evaluate the neutrality of the marker (mitochondrial control region) and indices describing results for the bootstrapped mismatch distribution of haplotype pairs. Values in bold are considered significant.

Neutrality Test		
Tajima's D	-1.412	$P > 0.05$
Fu's FS	**-22.97**	$P < 0.001$
Chakraborty's test	**25.96**	$P < 0.000$
Mismatch Distribution		
SSD	0.027	$P > 0.1$
Raggedness Index	0.010	$P > 0.1$

Haplotype diversities were high in *A. sandaracinos* (h = 0.86-1.00), with similar values found in *A. perideraion* (h = 0.81-1.00), *A. ocellaris* (h = 0.97-1.00), and *A. clarkii* (h = 0.91-1.00) and a mean haplotype diversity of h = 0.95 (*A. sandaracinos* in Table 5.7, all species in Fig. 5.5). Unpaired t-test in *A. sandaracinos* detected significantly lower haplotype diversity in a northern population group [KK, BI, Sa, Ma, LS] compared to a southern and eastern population group [Sp, Bi, Ke, Ku, Pi, Bk]. Nucleotide diversities were similarly high (π = 0.011 – 0.114) as in the other anemone fish, increasing in a southerly and easterly direction, with their highest in Biak, east New Guinea (Fig. 5.5). The very high upper range value in *A. sandaracinos* is produced by a highly divergent haplotype (54 unsampled mutational steps)

found in Biak, which is closely associated with the haplotype found in the sample from the Solomon Islands by Santini and Polacco (2006) (Clade V, Fig. 5.4B).

Table 5.7 Sample sites for *A. sandaracinos* collected from across the IMA with the respective site abbreviations (Abbr.) (see Fig. 5.4 A for regional placement of sites). The number of individuals (N_{ind}) analyzed per location is indicated for each dataset. For CR region data the haplotype (*h*) and nucleotide (π) diversities are given per site with their respective standard deviations (SD). Microsatellite data are presented in terms of gene diversity and SD.

		Control Region (CR)				***Microsatellites- 8 loci***	
Sample sites	Abbr.	N_{ind}	N_{haplo}	*h* + SD	π + SD	N_{ind}	Gene Diversity + SD
Spermonde	**Sp**	22	11	0.918 ± 0.0372	0.0132 ± 0.0075	19	0.807 ± 0.447
Manado	**Ma**	5	4	0.900 ± 0.161	0.0309 ± 0.0198	0	na
Lembeh Strait	**LS**	7	6	0.952 ± 0.096	0.0262 ± 0.0157	7	0.812 ± 0.462
Bira	**Bi**	9	8	0.972 ± 0.064	0.0372 ± 0.021	8	0.857 ± 0.475
Kendari	**Ke**	10	8	0.956 ± 0.059	0.0288 ± 0.016	5	0.774 ± 0.465
Sangalaki	**Sa**	11	8	0.891 ± 0.092	0.0231 ± 0.013	11	0.805 ± 0.440
Kupang	**Ku**	5	5	1.000 ± 0.127	0.0204 ± 0.013	1	na
Banggi Islands	**BI**	3	3	1.000 ± 0.272	0.0111 ± 0.009	3	0.842 ± 0.526
Kota Kinabalu	**KK**	7	5	0.857 ± 0.137	0.0163 ± 0.010	5	0.747 ± 0.437
Biak	**Bk**	3	4	1.000 ± 0.177	0.1137 ± 0.075	1	na
Misol	**Mi**	5	5	1.000 ± 0.127	0.0365 ± 0.023	5	0.822 ± 0.476
Pisang	**Pi**	2	2	na	na	2	na

Comparing haplotype and nucleotide diversities of all four anemonefish species (Fig. 5.5) uncovers some common patterns, despite the fact that many locations do not have data for all three species. *A. ocellaris* has the most homogeneous distribution of haplotype and nucleotide diversities across the sampling range, with a gradual increase in nucleotide diversities moving south, confirmed by a significant unpaired t-test ($t = 3.133$, $df = 14$, $p = 0.007$) between northern and more southern populations. This culminates in highest nucleotide diversities in populations fringing the Java, Flores and Banda Seas, the same general pattern noted for *A. clarkii*, *A. perideraion,* and *A. sandaracinos*. However, no significant differences between northern and southern populations were detected for the ladder three. High nucleotide diversity in east New Guinea is shared by *A. clarkii*, *A. perideraion,* and *A. sandaracinos*. *Amphiprion ocellaris* does not occur here, but Timm *et al.* (2008) found highly divergent haplotypes (82 bp) in Biak for *A. percula*, its sibling species.

A distinguishing feature is that *A. perideraion* haplotype diversities follow no apparent pattern across the sampling range with higher and lower diversities spread randomly, in contrast to the clear north (low) - south (high) gradient in *A. sandaracinos* ($t = 3.1359$, $df = 10$, $P = 0.011$), the western (high) - eastern (low) haplotype diversities in populations of *A. clarkii* ($t = 3.4039$, $df = 16$, $p = 0.004$), and the homogenously high haplotype diversity found in *A. ocellaris* (Fig. 5.5). While a general nucleotide diversity gradient is common to all four species, haplotype diversity patterns appear species specific.

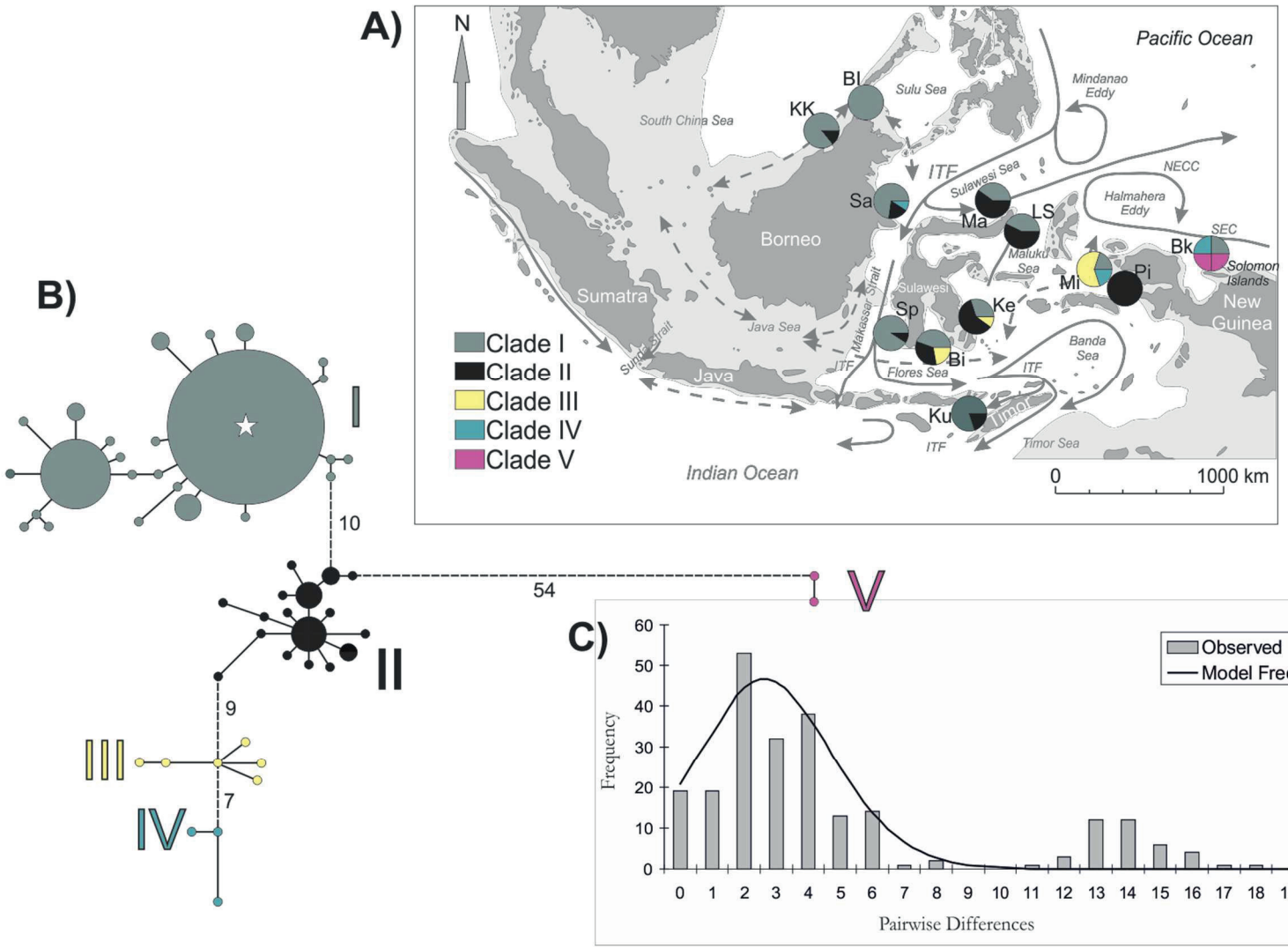


Figure 5.4 A) Map of the study area with clade frequencies (identified in B) as fractions of the total population sample. **B)** Minimum spanning tree (MST) of 53 *Amphiprion sandaracinos* mitochondrial control region haplotypes from 12 locations across the Indo-Malay Archipelago. Circle sizes in the MST reflect total sample size for that haplotype with the smallest circle representing one individual. The length of connecting lines represents mutation steps between haplotypes, shortest connection is one mutation. Mutation steps separating clades are given next to the dashed connecting lines. Haplotype marked by a white star was identified as the most probable common ancestor haplotype (MPCAH). **C)** Observed and expected frequencies of pairwise differences (mismatch distribution) for all haplotypes under a model of sudden population expansion

Minimum Spanning Tree (MST) - *A. sandaracinos*

Haplotypes of *A. sandaracinos* grouped into five clades (I-V), separated by 7-54 mutational steps (ms) (Fig. 5.4B). Though not positioned at the most central position, the most probable common ancestral haplotype (MPCAH, marked with white star in Fig. 5.4B) of *A. sandaracinos* is present in the grey clade (I), which also contains the two haplotypes shared among the largest numbers of sample sites. The MPCAH also constitutes the most common haplotype, as it was found in 18 individuals from eight different locations. This haplotype network displays a very linear character in the topography of the clade arrangement, with each clade sequentially giving rise to the next, except for the very divergent clade V, splitting off from a more central position.

The relative frequency of clade occurrence for the sampled populations was mapped onto their geographic position in the IMA (Fig. 5.4A). The grey clade (I) is most dominant in North Borneo, but decreases in frequency along the ITF from Sanggalaki to Spermonde, and Bira. Moving south and east, it is sequentially replaced by the black clade (II), which is present in nine of the 12 populations, but shows a general pattern of increasing frequency moving into and across the Banda Sea. It is absent from Misool and Biak, which are dominated by more peripheral (yellow and purple) and ancestral (grey) clades. The blue clade (III) contains only three haplotypes, namely from Misool, Biak and Sanggalaki, with no detected occurrences in intermediate populations. The purple clade (V), removed by 54 ms, is found exclusively in Biak and the Solomon Islands, contributing one haplotype each. This high degree of divergence corresponds to interspecific divergence found between *A. sandaracinos* and its sibling species, *A. perideraion*, in a molecular phylogeny based on CR sequences from IMA populations (Timm *et al.* 2008). In a phylogeny these sequences do however clearly group with other *A. sandaracinos* sequences.

AMOVA and hierarchal AMOVA - *A. sandaracinos*

AMOVA detected a strong and significant population structure in the sampled area for both the CR (Φ_{ST} = 0.219, p < 0.0001) and the Msat dataset (D_{est}= 0.123, p = 0.013, F_{ST}= 0.031 p < 0.0001) (Table 5.10). Hierarchical AMOVA (Table 5.8) saw similar but not identical grouping of populations between the two markers, probably due to the lower number of individuals in the Msat dataset. The analysis of variance components of different groupings of geographically associated populations (hierarchical AMOVA) based on CR haplotypes suggested a three-groups division into 1) Biak (with the Solomon Islands) 2) Misool, and 3) all other populations, placing 41 % of the total variation among groups, only 8 % among populations within groups, and 51 % within populations. The microsatellite dataset, which does not include the very divergent individual from Biak (CR haplotype in clade V) and samples from the Solomon Islands, supported a statistically significant two group scenario of 1) Biak and Pisang and 2) all other populations. This explains 48 % of the total variation as variation between groups. While an identical grouping as in the CR region dataset was non-significant (p = 0.084), it would also attribute more than 44 % of the total variation to between group variation (Table 5.8).

Table 5.8 Hierarchical AMOVA groupings of *A. sandaracinos* populations in the Indo-Malay Archipelago based on pair-wise distances of mitochondrial control region sequences (Φ values) and 8 microsatellite loci (F values). Rerunning the analysis without the highly divergent clade V haplotypes was done to test grouping of the East New Guinea population [Bk] with the west of the island in the absence of clade V. Bold values describe the highest index support for the tested combinations.

Regional Groupings	**Control Region**		**8 Msat Loci**		**Control Region - without Clade V haplotypes**	
	Φ_{CT}	*p*	F_{CT}	*p*	Φ_{CT}	*p*
no groups	0.219	0.000	0.03110	0.001	0.16484	0.000
[**Bk**][*all others*]	0.51617	0.08376	0.15958	0.05963	na	na
[**Bk**][**Mi**][*all others*]	**0.41363**	**0.02802**	0.04475	0.08407	0.20039	0.04203
[**Bk,Mi**][*all others*]	0.26317	0.03129	0.00926	0.16716	**0.20690**	**0.03910**
[**Bk,Pi**][*all others*]	0.04116	0.27077	**0.04839**	**0.01662**	0.04116	0.26295
[**Bk,Mi,Pi**][*all others*]	0.21613	0.00475	0.01914	0.03030	0.16630	0.00587
[**Bk,Pi**][**Mi**][*all others*]	na	na	0.02982	0.03226	na	na
[**Bk,Pi**][**Mi**][**TI**][*all others*]	na	na	0.02624	0.01955	na	na
[**Bk,Pi,Ke**][**Mi**][*all others*]	na	na	0.02006	0.02346	na	na
[**Bk,Pi**][**TI**][*all others*]	na	na	0.03528	0.03519	na	na
[**Bk,Mi,Ke**][*all others*]	0.11631	0.03099	0.00174	0.18964	na	na
[**Bk,Mi**][**Ke,Pi,LS**][*all others*]	0.20443	0.00069	na	na	na	na
[**Bk,Mi**][**Ke,LS**][*all others*]	0.16472	0.01366	na	na	na	na
[**Bk,Mi**][**KK,BI**][*all others*]	0.14608	0.02158	na	na	na	na
[**Bk,Pi**][**Ke,Lu,TI**][**Mi**][*all others*]	na	na	0.02056	0.00782	na	na
[**Bk,Pi**][**Ke,Lu,TI**][*all others*]	na	na	0.01923	0.00684	na	na

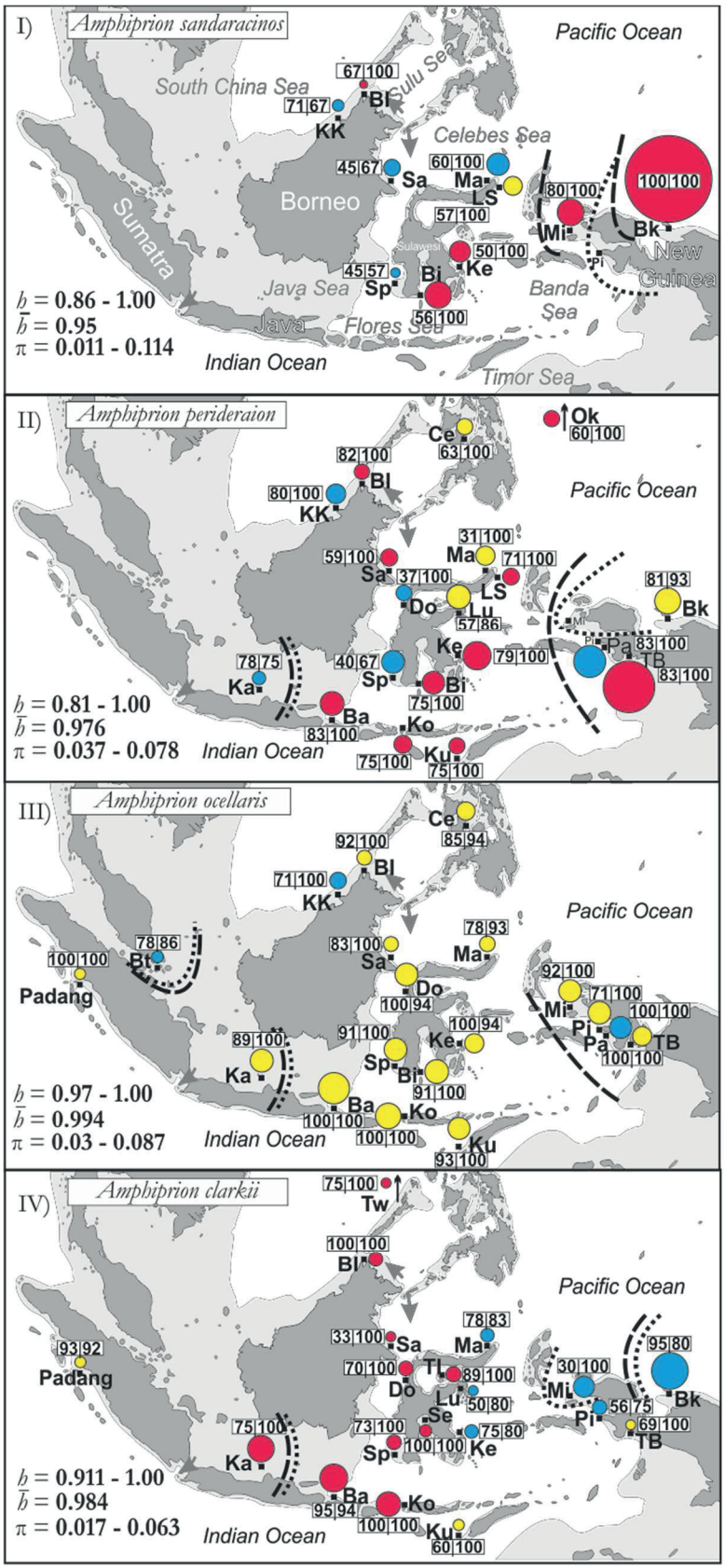


Figure 5.5 Maps I-IV depict nucleotide and haplotype diversities in four species of anemonefish, projected onto the geographic sampling region. The percentage of private haplotypes found at each sampling location and the fraction of singleton in private haplotypes is also depicted. See figure legend below for other projection details.

Legend:

Relative size:
Nucleotide diversity (π)

Haplotype diversity (h)
$\bar{h}$ = mean haplotype diversity
blue = below mean
red = above mean
yellow = less than 0.01 from mean

Species specific values and ranges are given in species maps

Hierarchical AMOVA Divisions:
– – – CR
..... Msat

100|100 ← Percent singleton/private haplotypes
↑ Percentage of samples with private haplotypes

Common Genetic Barriers - *A. sandaracinos*, *A. clarkii*, *A. ocellaris*, and *A. perideraion*

Cumulative genetic barriers shared by two or more species were recorded and visualized on a map of the sampling area (Fig. 5.6). Nine barriers (1-9) were detected, that were shared by three or more fish species and their cumulative bootstrap support was used to scale the barrier thickness on the map proportionately (Table 5.9, Fig. 5.6). An additional ten barriers (a-j) shared by only two species were detected and marked on the map as described above (Fig. 5.6).

The best supported barrier transects the Maluku and Banda Sea, separating Papua New Guinea populations from East Sulawesi. This barrier is actually composed of the five highest ranking barriers shared by three or more species. Barrier six formed between Manado in the extreme Northeast of Sulawesi and Cebu in the Philippines. The least supported three-species barrier was found to be located between Misool (Maluku Sea) and the population in Biak (East Papua New Guinea). Two-species barriers are spread throughout the archipelago, but those found to have the highest support, tend to expand existing three-species barriers and add subdivision across and along the Flores Sea. More western barriers are restricted to support from *A. ocellaris* and *A. clarkii*, because the other two species could not be sampled in those locations for the present study.

Table 5.9 Synergy of results from individual BARRIER maps for all four species with microsatellite (Msat) and control region (CR) datasets (except *A. sandaracinos*, only CR dataset). For each of the nine highest ranking barriers shared by three or more species the added bootstrap support (∑ bs), mean bootstrap support, minimum and maximum bootstrap support, the number of species sharing this barrier (N_{Sp}) and number of datasets (N_{CR} or N_{Msat}) contributing to the overall barrier are given (map in Fig. 5.6). Anemonefishes were abbreviated as follows: *As* - *A. sandaracinos*; *Ap* - *A. perideraion*; *Ac* - *A. clarkii*; *Ao* - *A. ocellaris*.

	cummulative					Datasets with barrier	
					Species		Control
Barrier	bootstrap support		min. bootstrap	max. bootstrap	with barrier	Microsatellite	Region
on Map	(∑ bs)	mean	support	support	(N_{Sp})	(N_{Msat})	(N_{CR})
1	691	99	66 (*Ao* , Msat)	126 (*Ap* , Msat)	4	3	4
2	530	133	111 (*Ap* , Msat)	145 (*Ao* , Msat)	3	2	2
3	483	161	153 (*Ap* , CR)	166 (*Ac* , CR)	3	0	3
4	482	161	143 (*Ap* , CR)	177(*Ac* , CR)	3	0	3
5	462	154	129 (*Ao* , CR)	169 (*Ap* , CR)	3	0	3
6	321	80	43 (*Ac* , Msat)	106 (*Ap* , Msat)	3	2	2
7	251	84	48 (*As* , CR)	125 (*Ac* , Msat)	3	2	1
8	243	81	36 (*Ap* ,CR)	121 (*Ac* , CR)	3	0	3
9	182	36	22 (*Ao* , CR)	61 (*As* , CR)	3	2	3

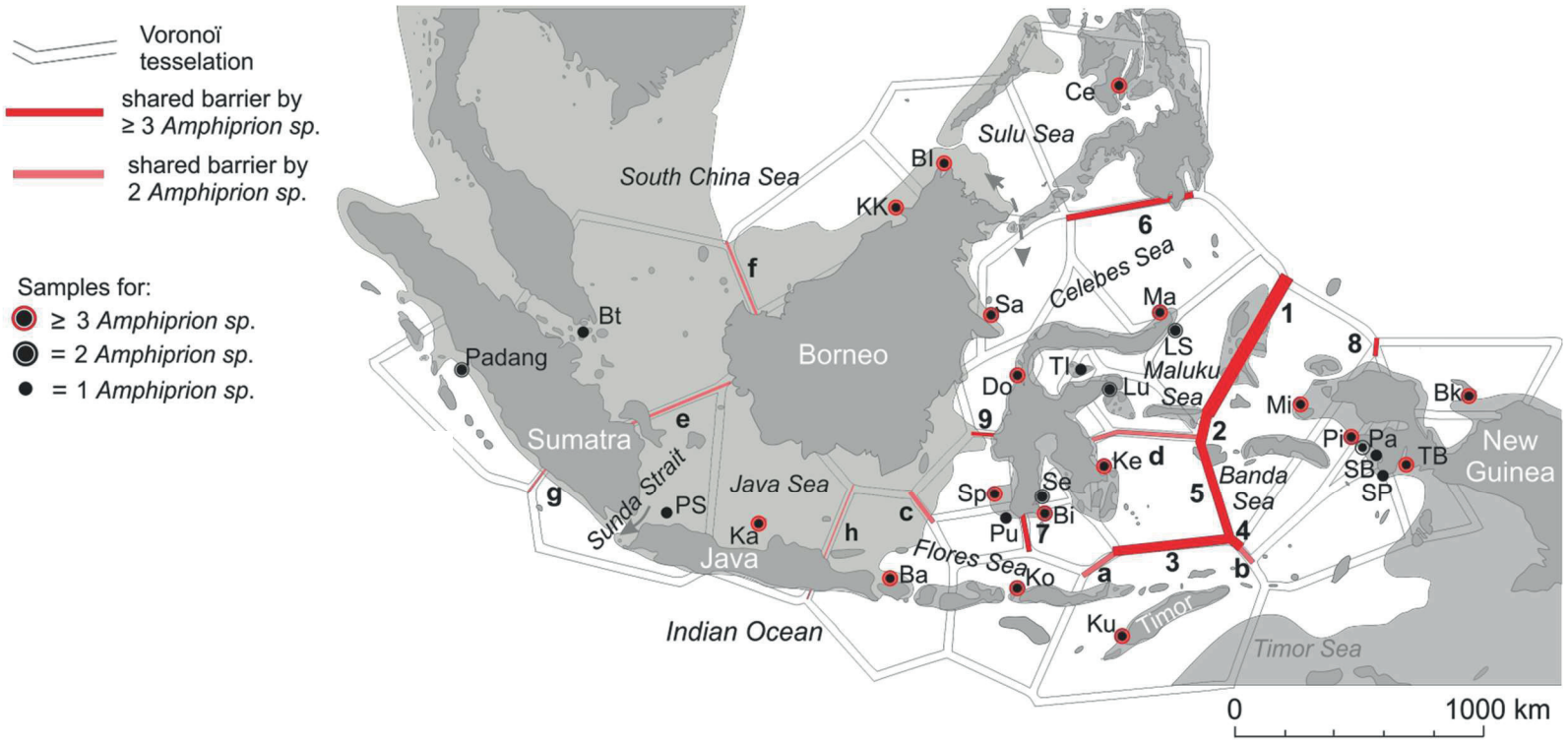


Figure 5.6 Map of the Indo-Malay Archipelago with all sampling locations that were included for the four anemonefishes, *A. sandaracinos*, *A. perideraion*, *A. clarkii*, and *A. ocellaris*. At each sampling point one to four species were collected. Markings for sampling locations include species number information (see legend). Species and markers specific maps were superimposed, identifying common barriers to geneflow among species and the respective cumulative bootstrap support for these barriers. Barrier thickness is scaled according to bootstrap support. Red barriers signify genetic breaks shared by at least three of the species, while light red barriers denote a two species overlap. Grey double lines constitute the Voronoï tessellation built in BARRIER.

PLD and host anemone use - *A. sandaracinos*, *A. clarkii*, *A. ocellaris*, and *A. perideraion*

A. sandaracinos showed the third highest population structure (Φ_{ST} = 0.219) found among the four anemonefish when CR data are inspected, following *A. clarkii* (Φ_{ST} = 0.42, *this study*) and *A. ocellaris* (Φ_{ST} = 0.241, Timm *et al.* 2008) and followed only by *A. perideraion* (Φ_{ST} = 0.093, Dohna *et al.* 2015). A comparison of the detected structure (F_{ST}) in the microsatellite datasets of *A. sandaracinos* (F_{ST}= 0.031, 8 loci), *A. ocellaris* (F_{ST} = 0.048, 6 loci), *A. clarkii* (F_{ST} = 0.051, 8 loci) and *A. perideraion* (F_{ST} = 0.016, 10 loci) matches the ranking established by the CR datasets.

Ranking the fish according to the number of potential host anemones does not follow this same order ([Ac][Ao][As][Ap] vs [As][Ao][Ap][Ac]). However, when maximum PLD estimates are considered, the species order established by the relative strength of genetic structure is maintained in an inverse relationship. The significance of this relationship was not tested because PLD estimates are based on few data and do not reflect regional variation (Wellington & Victor 1989, Thresher *et al.* 1989, Almany *et al.* 2007).

Table 5.10 Pelagic larval duration (PLD) and host use (N hosts) for all four species of anemonefish studied here. Sources for the PLD estimates are [1]Wellington & Victor 1989, [2]Thresher *et al.* 1989, or [3]Almany *et al.* 2007. Also included are the resulting indices of genetic structure determined by sequences of the mitochondrial control region (CR, Φ_{ST}) and nuclear microsatellite markers (Msat, F_{ST}). A species list of the potential host anemones is given with X signifying the use of this host by the respective fishes.

	PLD (days)			N	CR	Msat	Host sea anemones									
							Heteractis	*H.*	*H.*	*H.*	*Entacmaea*	*Stichodactyla*	*S.*	*S.*	*Macrodactyla*	*Cryptodendrum*
Species	[1]	[2]	[3]	hosts	Φ_{ST}	F_{ST}	*magnifica*	*crispa*	*aurora*	*malu*	*quadricolor*	*mertensii*	*gigantea*	*haddoni*	*doreensis*	*adhaesivum*
A. clarkii	15-16	8-9	n/a ~11	10	0.42	0.051	X	X	X	X	X	X	X	X	X	X
A. ocellaris	n/a	n/a	(*A. percula*)	3	0.241	0.048	X					X	X			
A. sandaracinos	n/a	n/a	n/a	2	0.219	0.031		X				X				
A. perideraion	18	10-12	n/a	4	0.093	0.016	X	X					X		X	

DISCUSSION

The strong overall population structure detected in *A. sandaracinos* (Φ_{ST} und D_{est}) meets study expectations based on its relatively short PLD and the absence of adult migration in a highly complex seascape. Among the four congeners studied here, it ranks third in overall population genetic structure. However, the ranking could change if more western populations were added, as these populations add considerably to the overall genetic population differentiation found in *A. ocellaris* (west Sumatra, Batam, Karimunjava; Timm & Kochzius 2008), *A. perideraion* (Karimunjava; Dohna *et al.* 2015) and *A. clarkii* (west Sumatra, Karimunjava; data not shown).

Samples from more western regions would be of particular interest here, due to the genetic break occurring across the Indo-Pacific Barrier (IPB; Briggs 1974) in congeners (Fig. 5.5; Timm *et al.* 2008, Timm *et al.* 2012, Dohna *et al.* 2015) and other marine taxa (e.g. Benzie 1999, Barber *et al.* 2000, 2002, Kochzius & Nuryanto 2008, Hobbs *et al.* 2009, Gaither *et al.* 2011, DeBoer *et al.* 2014, Raynal *et al.* 2014), although definitions of the exact location of the barrier vary (Winters *et al.* 2010). Absence of this very dominant genetic break in *A. sandaracinos* could weaken inferences drawn from concatenating regional patterns of differentiation among congeners, because in that case, dynamics ruling geneflow in *A. sandaracinos* may be very different on an evolutionary and temporal timescale. AMOVA grouping of regional population clusters, however, revealed the presence of an "eastern barrier" (Barber *et al.* 2006) also found in its congeners (Fig. 5.5) and suggesting that, where investigated, the evolutionary, spatial and temporal dynamics affecting populations of these congeners may be similar. This warrants the construction of a concatenated genetic landscape for the four anemonefish species, highlighting shared genetic barriers across the IMA.

Genetic diversities in marine taxa are often characterized by high haplotype, but medium and low nucleotide diversities (reviewed in Grant & Bowen 1998), thought to evidence their long stable evolutionary history (Grant & Bowen 1998) or resulting from secondary contact between divergent lineages (Bay *et al.* 2004). The geological history of the IMA with its repeated marine habitat reductions and extensive fragmentation during glacial cycles (Voris 2000, Sathiamurthy & Voris 2006) suggests that the second scenario is most likely. Differences in the timing of speciation events in these fishes and a relatively young species history (Santini & Polacco 2006, Timm *et al.* 2008), further supports the assumption of allopatric lineage divergence during population fragmentation caused by the highly stochastic geological history of the IMA. High nucleotide diversities, as found in the species studied here, would also support a scenario of secondarily admixed divergent lineages from glacial refugia (Lewis & Crawford 1995, Hewitt 2000, 2004).

Genetic diversity estimates in *A. sandaracinos* are high overall (Table 5.7), agreeing with expectations based on congeners and other reef fishes in the IMA (e.g. Bay *et al.* 2004, Timm *et al.* 2012, Raynal *et al.* 2014, Dohna *et al.* 2015). The strong and significant gradient of increasing haplotype diversity moving south and east across the sampling area fits well with postulated regions of increased diversity in the Banda, Flores and Ceram Seas, as modeled by Kool *et al.* (2011) in an individual-based biophysical dispersal model spanning the IMA. The model also projects that the high diversity in the central regions of the IMA is fed by upstream populations from the Sulu and South China Sea, operating as important larval sources while maintaining a reduced genetic diversity. The absence of more peripheral clades III, IV and V haplotypes (Fig. 5.4 B) from the northern sampling locations supports this result, as do average or reduced nucleotide diversities in all four species inspected here (Fig. 5.5). Larval source/sink dynamics are an important aspect in conservation planning and need to be accounted for in efforts to preserve genetic diversity.

Regional nucleotide diversity maxima differ among the four species, while adhering to a generally increasing North-South(east) gradient. *Amphiprion ocellaris* and *A. clarkii* populations sampled in West Sumatra (Padang) showed low relative nucleotide and mean haplotype diversity, adhering to model predictions of reduced diversity at the edges of the IMA (Kool *et al.* 2011). The development of such high resolution models is a great asset for the field of phylogeography and population genetics, and they will be increasingly used to extrapolate general patterns based on species-specific input parameters, replacing some of the costly and time consuming field and laboratory work required to generate real world data, which are often difficult to interpret.

Anemone fishes are of particular relevance for a conservation-oriented approach because both fish and host anemones are collected for the marine aquarium trade, amplifying the fishing pressure on anemonefishes through removal of suitable settlement substrate and adult habitat. In addition, sea anemones are susceptible to bleaching, a temperature-stress induced expulsion of symbiotic dinoflagellates, which is expected to increase in frequency with projected climate change scenarios (Saenz-Agudelo *et al.* 2011). The data compiled for this study allow locating commonalities among the population genetic patterns found in four congenerics with near identical life histories.

Although detailed single-species studies have not lost their relevance and intrinsic value, it remains problematic to generate valid generalizations from genetic patterns that can be of use to spatial planning in marine resource management (Beger *et al.* 2010). Biophysical larval dispersal models of the IMA (Kool *et al.* 2011, Treml *et al.* 2012, Treml *et al.* 2015), have been extremely helpful in linking biophysical larval advection, PLD and other early life history characteristics (e.g. homing behavior, active larval swimming). However, model validations and improvements also depend on actual data (e.g. genetic, otolith metrics, chemical tags) to check the accuracy of input parameters, errors in model output, and

agreement between model simulations and patterns observed by other means (Cowen 2006, Treml *et al.* 2012). The approach taken in the present study was to derive from nuclear and mitochondrial markers a geographic placement and a scaling of common genetic discontinuities needed to develop a multi-species genetic landscape, which can be interpreted against the backdrop of modeled scenarios for larval dispersal in the IMA (Kool *et al.* 2011, Treml *et al.* 2012, Treml *et al.* 2015).

The five best supported genetic barriers (1 to 5) shared by ≥ 3 of the species studied here, connect to transverse the Banda Sea, separating Northwest and East New Guinean populations from the rest of the IMA. This barrier agrees with model predictions of connectivity below the migration rate threshold (MRT) for anemonefish from Misool to Sulawesi and the lesser Sunda Islands (Treml *et al.* 2012). The MRT metric was introduced to describe critical recruitment or connectivity levels for demographically relevant population mixing (Cowen *et al.* 2006). The model indicated a stepping stone connectivity via more western Molucca and Ceram Sea reefs, but due to a lack of additional sample sites between New Guinea and Sulawesi, this could not be investigated in more detail here. Overall, this barrier is predicted to filter 10 to 30 % of the 99 model taxa tested (Treml *et al.* 2015) and was detected in all four study species here (Fig. 5.6).

A connectivity barrier between East New Guinea populations and locations further west has been detected in quite a number of species to date (reviewed in Carpenter *et al.* 2011, DeBoer *et al.* 2014), although its exact position is usually defined through species ranges (e.g. no Pacific populations of *A. ocellaris*) or the scale of the study. A further barrier (8, ≥ 3) separates the populations in eastern New Guinea (Biak) from those on its western coast. AMOVA results for the four species studied here are similar, although they disagree (interspecific and/or intragenomic) as to the exact position of the 'eastern' barrier. This is seen in all three anemonefishes with ranges extending into the Pacific (Fig. 5.5; As, Ap, Ac), indicating that differentiating biological factors (e.g. host availability, reproductive output,

PLD) may be causing the observed disparities. Parameters for PLD, reproductive output, and spawning phenology were found to be most instrumental in shaping modeled genetic connectivity (Treml *et al.* 2015), but could also be driving the differences found among the species investigated here. At least in tropical latitudes, anemonefishes were found to spawn throughout the year with some lunar periodicity (Allen 1975, Ross 1978), so phenology is probably not a main factor in this system. Interspecific differences in the size and output of the adult reproductive population segment or dispersal based on PLD may well be causing the observed population structure divergence and this warrants further investigation.

No interspecific mito-nuclear discordance pattern in respect to the eastern barrier could be observed among the anemonefish, adding little to the discussion of which markers (nuclear vs. mitochondrial) produce the most contemporary picture of connectivity. It does, however, add to the discussion about the value of single mitochondrial marker studies, which has been repeatedly questioned (Fauvelot *et al.* 2007) and has led many relevant journals to discontinue publishing single marker studies (Bowen *et al.* 2014). Without questioning the utility of multi-marker studies, it should be pointed out, that in a comparative context, as was applied here, a single marker is much more applicable. Direct interspecific comparisons are possible without having to blindly equate the output from different genetic loci (e.g. microsatellites), that may well have been subject to different forces due their structure and their location in the genome.

The Philippines are an archipelago with a high degree of endemism and species richness, contributing significantly to the high biodiversity found in the IMA (Roberts *et al.* 2002, Carpenter & Springer 2005). A genetic barrier, supported by all anemonefishes except *A. ocellaris*, formed south of the Philippines, signifying a genetic break across the Celebes Sea, but connectivity towards the west across the Sulu Sea. This barrier has been detected in other invertebrate and fish species (Lourie *et al.* 2005, DeBoer *et al.* 2014), but is not a dominant barrier shared by many species (reviewed in von der Heyden *et al.* 2014). It may, however,

indicate that anemonefishes and similar benthic brooding species are restricted by this barrier and are dependent on regional self-seeding under strong fishing pressure (Shuman *et al.* 2005) or other threats. Kool *et al.* (2011) projected low genetic diversity for the Philippine islands, which are functioning as a larval source for populations further south and contribute to the high genetic diversity at the center of the IMA while having only low import levels from the outside. This barrier also agrees with modeled connectivity below MRT across the Celebes Sea for anemonefish (Treml *et al.* 2012) and a projected barrier to demographically relevant connectivity for 5 to 10 % of modeled taxa (Treml *et al.* 2015). When concatenating the patterns of genetic differentiation across species and markers, the most pronounced barriers should be given the highest priority in spatial planning for these and similarly distributed coral reef species, placing special attention on the Philippines. An essential next step would include a more comprehensive sampling of the region to investigate additional internal barriers, which have also been detected in other species (reviewed in Carpenter *et al.* 2011; DeBoer *et al.* 2014, van der Heyden *et al.* 2014).

Additional barriers found in less than three species in the Makassar Straight and the south-western tip of Sulawesi suggest that the ITF does not guarantee coastline connectivity for these and similar species. This is the only instance where data generated by this study conflict with modeled results by Treml *et al.* (2012), who predicted high connectivity (above MRT) along the south-western tip of Sulawesi. The exceptional concordance between the patterns recognized here via Voronoï tessellation for four anemonefish species and those modeled by Treml *et al.* (2012) is 1) additional confirmation for the accuracy of their model parameters and output, and 2) confirms the utility of the method applied here to produce a multispecies genetic landscape, highlighting major and minor genetic discontinuities.

Population connectivity in anemonefish is achieved through the dispersal of larval fish hatched from brooded eggs, which settle on to suitable reef habitat once competency is reached. Anemonefish have different degrees of specialization in regards to their obligate symbiont host, which also implies that recruitment may be differentially successful, depending on their degree of specialization. Host specialization and the length of the PLD are among few distinguishing features of the four fish studied here and invite a closer look.

Ecological specialization has been linked to increased genetic structure in terrestrial systems (Brouat *et al.* 2003, Hoehn *et al.* 2007, Alcaide *et al.* 2009, DiLeo *et al.* 2010) but has received little attention in the marine realm. A study investigating four species of Atlantic wrasses, found higher population differentiation in habitat specialists (Rocha *et al.* 2005). Other studies focusing on the effect of dietary specialization on population connectivity found no effect in Hawaiian endemic butterflyfishes (Craig *et al.* 2010), but a higher population structure in dietary generalists, rather than specialists, in congeners of butterflyfishes on the Great Barrier Reef (Lawton *et al.* 2011). So far no clear consensus has been reached as to the role of ecological specialization on structuring marine populations.

A. clarkii is known to take up residence in ten different species of sea anemones, a multiple of what has been observed for the other three species studied here, leading to expectations of reduced genetic differentiation in this host generalist. However, population differentiation for *A. clarkii* was highest among the four congeners, suggesting that early life history and population connectivity may not be shaped by this aspect of their ecology or that the mechanism is not a simple linear relationship and/or is skewed by other more dominant forces. Ricciardi *et al.* (2010) demonstrated a partial niche overlap of *A. sandaracinos* and *A. perideraion* with *A. clarkii*, while *A. perideraion* and *A. ocellaris* niches were shown to overlap to a very large degree in reef assemblages around Manado (Sulawesi, Indonesia). The study concluded that despite the niche overlap, competitive exclusion among anemonefish is not a dominant factor in shaping anemonefish assemblages, but that the structure may rest on

random events ("who got there first") and may change due to anthropogenic impact (removal for ornamental fishery) (Shuman *et al.* 2005). Another recent study (Litsios *et al.* 2014) concluded that host specialist anemonefishes are environmental niche generalists, compensating the cost of specialization with a greater tolerance to environmental conditions (pH, temperature, and salinity). This mechanism could be dampening the effect of host specialization based differential habitat encounters for juveniles, possibly explaining why the relationship between host specialization and genetic structure was not apparent here.

Although *A. sandaracinos* is most specialized in host use, in the presence of host competition it is often found to cohabitate, usually as bachelor, with other bigger and more dominant species (*A. clarkii* and *A. chrysopterus*; Elliott & Mariscal 2001, Ricciardi *et al.* 2010, Bos 2011). The same strategy is known for *A. perideraion*, which is often found in association with *A. clarkii* (Hattori 1995, Ricciardi *et al.* 2010). *A. clarkii* was also observed to cohabitate with *Amphiprion melanopus*, adding energetic costs for competition with this and other cohabitants. This illustrates that ecological and behavioral strategies have developed in response to niche overlap that may affect population connectivity and reproductive output to a large degree. A causative relationship between habitat specialization and degree of population structure was not indicated here, because increasing specialization in anemonefish (Ac, Ap, Ao, As) did not match the pattern of either sequentially increasing or decreasing population structure (Ac, Ao, As, Ap or reverse) (Table 5.10).

The (max) length of the PLD, on the other hand, can be said to maintain an inverse relationship with the detected population structure, with longer PLDs leading to reduced genetic differentiation among populations. This is a very tentative observation, given that there is no PLD estimate for *A. sandaracinos*, PLDs of the other three species overlap, have produced different observations among studies and are in part based on limited data (e.g.

two otolith ring counts in *A. perideraion*). Despite these shortcomings, the results warrant further investigation in light of the active debate on whether PLD can be used as a reliable proxy for connectivity in marine populations. The genetic population differentiation in high dispersal species is often expectedly low (Palumbi 1994, Bohonak 1999, Kinlan & Gaines 2003), but species with high potential for dispersal through long PLDs have also been found to have a pronounced genetic structure (e.g. Barber *et al.* 2000; Planes & Fauvelot 2002, Swearer *et al.* 2002, Bernardi *et al.* 2003, Taylor & Hellberg 2003, Baums *et al.* 2006; Bowen *et al.* 2006; Thacker *et al.* 2007, Iacchei *et al.* 2014), suggesting that unforeseen forces are shaping these populations to a larger degree. This study was able to show that populations of anemonefish show overlapping genetic discontinuities with varying degrees of genetic structure, suggesting a correspondence to differences in their projected PLD and matching results from modeled larval dispersal in the IMA (Treml *et al.* 2012).

Conclusions

Through their obligate symbiosis with sea anemones, all anemonefish can generally be considered habitat specialists, which have been shown to be most vulnerable to change (Munday 2002, 2004). High levels of gene flow are assumed to counteract population extirpation through population replenishment and the sustenance of high levels of genetic diversity, important for resilience to changing conditions and disease. Where investigated, anemonefish show strong barriers to connectivity (Nelson *et al.* 2000, Timm & Kochzius 2008, Bay & Caley 2011, Timm *et al.* 2012, Dohna *et al.* 2015; *this study*) and high levels of self-recruitment (Jones *et al.* 2005, Almany *et al.* 2007, Beldade *et al.* 2012, Buston *et al.* 2012, Madduppa *et al.* 2014b), indicating an amplified vulnerability to exploitation (fish and hosts) which is extrapolated by an increasing risk of temperature induced bleaching of sea anemones (Hobbs *et al.* 2013). This can lead to a loss of hosts (Hobbs *et al.* 2013), removing settlement

substrate and adult habitat (Hattori 2002, 2005). Regardless of the total number of anemonefish within a given area, with only one breeding pair/sea anemone, host abundance is inextricably linked to the reproductive output of anemonefish populations and may well represent the Achilles heel for their survival under current and projected anthropogenic climate change impacts (Saenz-Agudelo *et al.* 2011, Hobbs *et al.* 2013). A logical next step would be to identify areas where host survival is most probable, so called refuges (Marshall & Baird 2000, Iluz *et al.* 2008, Bongaerts *et al.* 2010, Keppel *et al.* 2012), and incorporating these spatial components with the known genetic barriers for anemonefish, ideally constructing hierarchical prioritization schemes accounting for all components of the symbiosis, the fish, the host, and the host's endosymbionts.

REFERENCES

Alcaide, M., D. Serrano, J.J. Negro, J.L. Tella, T. Laaksonen, C. Müller, *et al.* 2009. Population fragmentation leads to isolation by distance but not genetic impoverishment in the philopatric Lesser Kestrel: a comparison with the widespread and sympatric Eurasian Kestrel. Heredity 102:190-198.

Allen, G.R. 1975. Anemonefishes - their classification and biology. TFH, Neptune City.

Allen, G. R. 2008. Conservation hotspots of biodiversity and endemism for Indo-Pacific coral reef fishes. Aquatic Conserv: Mar. Freshw. Ecosyst., 18: 541-556.

Almany, G.R., M.L. Berumen, S.R. Thorrold, S. Planes, and G.P. Jones. 2007. Local replenishment of coral reef fish populations in a marine reserve. Science 316:742-744.

Almany, G.R., S.R. Connolly, D.D. Heath, J.D. Hogan, G.P. Jones, L.J. McCook, *et al.* 2009. Connectivity, biodiversity conservation and the design of marine reserve networks for coral reefs. Coral Reefs 28:339-351.

Avise, J.C. 2000. Phylogeography: the history and formation of species. Cambridge MA: Harvard University Press.

Baker, A.C. 2003. Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of Symbiodinium. Annu. Rev. Ecol. Evol. S. 34:661-689.

Barber, P.H., M.V. Erdmann, S.R. Palumbi. 2006. Comparative phylogeography of three codistributed stomatopods: origins and timing of regional lineage diversification in the coral triangle. Evolution 60:1825-1839.

Barber, P.H., S.H. Cheng, M.V. Erdmann, K. Tengardjaja, and Ambariyanto. 2011. Evolution and conservation of marine biodiversity in the Coral Triangle: insights from stomatopod Crustacea. Crustacean Iss. 19:129-156.

Barber, P.H., S.R. Palumbi, M.V. Erdmann, and M.K. Moosa. 2000. A marine Wallace's line? Nature 406:692-693.

Barber, P.H., S.R. Palumbi, M.V. Erdmann, and M.K. Moosa. 2002. Sharp genetic breaks among populations of *Haptosquilla pulchella* (Stomatopoda) indicate limits to larval transport: patterns, causes, and consequences. Mol. Ecol. 11:659-674.

Baums, I.B., C.B. Paris, and L.M. Cherubin. 2006. A biooceanographic filter to larval dispersal in a reef-building coral. Limnol. Oceanogr. 51:1969-1981.

Bay, L.K., and M.J. Cale. 2011. Greater Genetic Diversity in Spatially Restricted Coral Reef Fishes Suggests Secondary Contact among Differentiated Lineages. Diversity 3:483-502 doi:10.3390/d3030483.

Bay, L.K., J. H. Choat, L. van Herwerden, and D.R. Robertson. 2004. High genetic diversities and complex genetic structure in an Indo-Pacific tropical reef fish (*Chlorurus sordidus*): evidence of an unstable evolutionary past? Mar. Biol. 144:757-767.

Bay, L.K., R.H. Crozier, and M.J. Caley. 2006. The relationship between population genetic structure and pelagic larval duration in coral reef fishes on the Great Barrier Reef. Mar. Biol. 149:1247-1256.

Beger, M., L. Simon, E. Game, I. Ball, E. Treml, M. Watts, and H.P. Possingham. 2010. Incorporating functional ecological connectivity into spatial decision making for conservation. Conserv. Lett. 3:359-368.

Beldade, R., S.J. Holbrook, R.J. Schmitt, S. Planes, D. Malone, and G. Bernardi. 2012. Larger female fish contribute disproportionately more to self-replenishment. Proc. Biol. Sci. 279:2116-2121.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. Series B 57:289-300.

Benzie, J.A.H. 1999. Major genetic differences between crown-of-thorns starfish (*Acanthaster planci*) populations in the Indian and Pacific Oceans. Evolution 53:1782-1795.

Bernardi, G., S.J. Holbrook, R.J. Schmitt, and N.L. Crane. 2003. Genetic evidence for two distinct clades in the French Polynesian population of coral reef three-spot damselfish *Dascyllus trimaculatus*. Mar. Biol. 143:485-490.

Bird, C.E., B.S. Holland, B.W. Bowen, and R.J. Toonen. 2007. Contrasting phylogeography in three endemic Hawaiian limpets (*Cellana* spp.) with similar life histories. Mol. Ecol. 16:3173-3186.

Bohonak, A.J. 1999. Dispersal, Gene Flow, and Population Structure. Q. Rev. Biol. 74:21-45.

Bongaerts, P., T. Ridgway, E.M. Sampayo, and O. Hoegh-Guldberg. 2010. Assessing the 'deep reef refugia' hypothesis: focus on Caribbean reefs. Coral Reefs 29:309-327.

Bowen, B.W., A.L. Bass, A. Muss, J. Carlin, and D.R. Robertson. 2006. Phylogeography of two Atlantic squirrelfishes (family Holocentridae): exploring links between pelagic larval duration and population connectivity. Mar. Biol. 149:899-913.

Bowen, B.W., K. Shanker, N. Yasuda, M.C.D. Malay, S. von der Heyden, G. Paulay, *et al.* 2014. Phylogeography unplugged: comparative surveys in the genomic era. Bull. Mar. Sci. 90:13-46.

Bos, A. R. 2011. Clownfishes *Amphiprion clarkii* and *A. sandaracinos* (Pomacentridae) coexist in the sea anemone *Stichodactyla mertensii*. Coral Reefs 30:369.

Bradbury, I.R., and P. Bentzen. 2007. Non-linear genetic isolation by distance: implications for dispersal estimation in anadromous and marine fish populations. Mar. Ecol. Prog. Ser. 340:245-257.

Bradbury, I.R., B. Laurel, P.V.R. Snelgrove, P. Bentzen, and S.E. Campana. 2008. Global patterns in marine dispersal estimates: the influence of geography, taxonomic category and life history. Proc. R. Soc. Lond. 275:1803-1809.

Brassel, K.E., and D. Reif. 1979. A procedure to generate Thiessen polygons. Geogr. Anal. 325:31-36.

Briggs, J.C. 1974. Marine Zoogeography. McGraw-Hill, New York, NY.

Brouat, C., F. Sennedot, P. Audiot, R. Leblois, and J.Y. Rasplus . 2003. Fine-scale genetic structure of two carabid species with contrasted levels of habitat specialization. Mol. Ecol. 12:1731-1745.

Burke, L., K. Reytar, M. Spalding, and A. Perry. 2011. Reefs at risk revisited. Washington, DC: World Resources Institute.

Burke, L., L. Selig, and M. Spalding. 2002. Reefs at risk in Southeast Asia. UNEP-WCMC, Cambridge, UK.

Buston, P.M., S.M. Bogdawicz, A. Wong, and R.G. Harrison. 2007. Are clownfish groups composed of close relatives? An analysis of microsatellite DNA variation in *Amphiprion percula*. Mol. Ecol. 16:3671–3678.

Buston, P.M., G.P. Jones, S. Planes, and S.R. Thorrold. 2012. Probability of successful larval dispersal declines fivefold over 1 km in a coral reef fish. Proc. Biol. Sci. 279:1883-1888.

Carpenter, K.E., and V.G. Springer. 2005. The center of the center of marine shore fish biodiversity: the Philippine Islands. Environ. Biol. Fish. 72:467-480.

Carpenter, K.E., P.H. Barber, E.D. Crandall, M.C.A. Ablan-Lagman, Ambariyanto, G.N. Mahardika, *et al.* 2011. Comparative phylogeography of the coral triangle and implications for marine management. J. Mar. Biol. 2011:1-14.

Chakraborty, R. 1990. Mitochondrial DNA polymorphism reveals hidden heterogeneity within some Asian populations. Am. J. Hum. Genet. 47:87-94.

Clement, M., D. Posada, *et al.* 2000. "TCS: a computer program to estimate gene genealogies." Mol. Ecol. 9:1657-1660.

Cowen, R.K., K.M.M. Kamazima, S. Sponaugle, C.B. Paris, and D.B. Olson. 2000. Connectivity of Marine Populations: Open or Closed? Science 287:857.

Cowen, R.K., C.B. Paris, and A. Srinivasan. 2006. Scaling of connectivity in marine populations. Science 311:522-527.

Cowen, R.K., and S. Sponaugle. 2009. Larval dispersal and marine population connectivity. Annu. Rev. Mar. Sci. 1:443-466.

Craig, M.T., J.A. Eble, and B.W. Bowen. 2010. Origins, ages and population histories: comparative phylogeography of endemic Hawaiian butterflyfishes (genus Chaetodon). J. Biogeogr. 37: 2125-2136.

Crandall, E.D., Frey, M.A., R. K. Grosberg, and P.H. Barber. 2008. Contrasting demographic history and phylogeographical patterns in two Indo-Pacific gastropods. Mol. Ecol. 17:611-626.

Dawson, M.N. 2012. Parallel phylogeographic structure in ecologically similar sympatric sister taxa. Mol. Ecol. 21:987-1004.

DeBoer, T.S, M.R.A. Naguit, M.V. Erdmann, M.C.A. Ablan-Lagman, Ambariyanto, K.E. Carpenter, *et al.* 2014. Concordance between phylogeographic and biogeographic boundaries in the Coral Triangle: conservation implications based on comparative analyses of multiple giant clam species. Bull. Mar. Sci. 90:277-300.

DeBoer, T., M. Subia, K. Kovitvonga, Ambaryanto, M. Erdmann, and P.H. Barber. 2008. Phylogeography and limited genetic connectivity in the endangered giant boring clam, *Tridacna crocea*, across the Coral Triangle. Conserv. Biol. 22:1255-1266.

DiBattista, J.D., M.T. Craig, L.A. Rocha, K.A. Feldheim, B.W. Bowen. 2012. Phylogeography of the Indo-Pacific butterflyfishes, *Chaetodon meyeri* and *Chaetodon ornatissimus*: sister species reveal divergent evolutionary histories and discordant results from mtDNA and microsatellites. J. Hered. 103:617-629.

DiLeo, M.F., J.R. Row, and S.C. Lougheed. 2010. Discordant patterns of population structure for two co-distributed snake species across a fragmented Ontario landscape. Divers. Distrib. 16:571-581.

Dohna, T.A., J. Timm, L. Hamid, and M. Kochzius. 2015. Limited connectivity and a phylogeographic break characterize populations of the pink anemonefish, *Amphiprion*

perideraion, in the Indo-Malay Archipelago: inferences from a mitochondrial and microsatellite loci. Ecol. Evol. 5:1717-1733.

Duda, T.F., M. Terbio, G. Chen, S. Phillips, A.M. Olenzek, D. Chang, *et al.* 2012. Patterns of population structure and historical demography of Conus species in the tropical Pacific. Am. Malacological Bull. 30:175-187.

Elliott, J.K., and Mariscal R.N. 2001. Coexistence of nine anemonefish species: differential host and habitat utilization, size and recruitment. Mar. Biol. 138:23-36.

Ewens, W.J. 1972. The sampling theory of selectively neutral alleles. Theor. Popul. Biol. 3:87-112.

Faurby, S., and P.H. Barber. 2012. Theoretical limits to the correlation between pelagic larval duration and population genetic structure. Mol. Ecol. 21:3419-3432.

Fautin, D.G., and G.R. Allen. Revised edition 1997. Anemone fishes and their host sea anemones. Western-Australian Museum, Perth, Australia.

Fauvelot, C., C. Lemaire, S. Planes, and F. Bonhomme. 2007. Inferring gene flow in coral reef fishes from different molecular markers: which loci to trust? Heredity 99:331.339.

Fisher, R., D.R. Bellwood, and S.D. Job. 2000. Development of swimming abilities in reef fish larvae. Mar. Ecol. Prog. Ser. 202:163-173.

Fu, Y.-X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915-925.

Gaither, M.R., B.W. Bowen, T.-R. Bordenave, L.A. Rocha, S.J. Newman, J.A. Gomez, *et al.* 2011. Phylogeography of the reef fish *Cephalopholis argus* (Epinephelidae) indicates Pleistocene isolation across the indo-pacific barrier with contemporary overlap in the coral triangle. BMC Evol. Biol. 11:189.

Gerlach, G., A. Jueterbock, P. Kraemer, J. Deppermann, and P. Harmand. 2010. Calculations of population differentiation based on G(ST) and D: forget G(ST) but not all of statistics! Mol. Ecol. 19:3845-3852.

Grant, W.S., and B.W. Bowen. 1998. Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. Heredity 89:415-426.

Hall, T.A. 1999. Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acid S. 41:95-98.

Hattori, A. 1995. Coexistence of 2 anemonefishes, *Amphiprion clarkii* and *A. perideraion*, which utilize the same host sea anemone. Environ. Biol. Fish. 42:345-353.

Hattori, A. 2002. Small and large anemonefishes can coexist using the same patchy resources on a coral reef, before habitat destruction. J. Anim. Ecol. 71: 824-831.

Hattori, A. 2005. High mobility of the protandrous anemonefish *Amphiprion frenatus*: nonrandom pair formation in limited shelter space. Ichthyol. Res. 52: 57-63.

Hewitt, G.M. 2000. The genetic legacy of the Quaternary ice ages. Nature 405:907-913.

Hewitt, G.M. 2004. Genetic consequences of climatic oscillations in the Quaternary. Phil. Trans. R. Soc. B 359:183-195.

Hickerson, M.J., and C.P. Meyer. 2008. Testing comparative phylogeographic models of marine vicariance and dispersal using a hierarchical Bayesian approach. BMC Evol. Biol. 8:322.

Hobbs, J.P.A., A.J. Frisch, G.R. Allen, and L. van Herwerden. 2009. Marine hybrid hotspot at Indo-Pacific biogeographic border. Biol. Lett. 5:258-261.

Hobbs, J.-P.A., A.J. Frisch, B.M. Ford, M. Thums, P. Saenz-Agudelo, K.A. Furby, and M.L. Berumen. 2013. Taxonomic, Spatial and Temporal Patterns of Bleaching in Anemones Inhabited by Anemonefishes. PLoS ONE 8: e70966. doi:10.1371/journal.pone.0070966

Hoehn, M., S.D. Sarre, and K. Henle. 2007. The tales of two geckos: does dispersal prevent extinction in recently fragmented populations? Mol. Ecol. 16:3299-3312.

Hoeksema, B.W. 2007. Delineation of the Indo-Malayan centre of maximum marine biodiversity: the Coral Triangle. Pp.117–178 in W. Renema, ed. Biogeography, time and place: distributions, barriers and Islands. Springer, Dordrecht, The Netherlands.

Horne, J.B., L. van Herwerden, J.H. Choat, and D.R. Robertson. 2008. High population connectivity across the Indo-Pacific: Congruent lack of phylogeographic structure in three reef fish congeners. Mol. Phylogenet. Evol. 49:629-638.

Iacchei, M., J.M. O'Malley, and R.J. Toonen. 2014. After the gold rush: population structure of spiny lobsters in Hawaii following a fishery closure and the implications for contemporary spatial management**.** Bull. Mar. Sci. 90:331-357.

Iluz, D., R. Vago, N. Chadwick, R. Hoffman, and Z. Dubinsky. 2008. Seychelles lagoon provides corals with a refuge from bleaching. Int. J. Ecol. doi:10.1155/2008/281038.

Jones, G.P., S. Planes, and S.R. Thorrold. 2005. Coral reef fish larvae settle close to home. Curr. Biol. 15:1314-1318.

Jost, L. 2008. GST and its relatives do not measure differentiation. Mol. Ecol. 17:4015-4026.

Keppel, G., K.P. Van Niel, G.W. Wardell-Johnson, C.J. Yates, M. Byrne, L. Mucina, *et al.* 2012. Refugia: identifying and understanding safe havens for biodiversity under climate change. Global Ecol. Biogeogr. 21:393-404.

Kininmonth, S., M. Beger, M. Bode, V. Adams, D. Brumbaugh, D. Dorfman, *et al.* 2011. Dispersal connectivity and reserve selection for marine conservation. Ecol. Model. 222:1272-1282.

Kinlan, B.P., and S.D. Gaines. 2003. Propagule Dispersal in Marine and Terrestrial Environments: A Community Perspective. Ecology 84:2007-2020.

Kochzius, M., C. Seidel, J. Hauschild, S. Kirchhoff, P. Mester, I. Meyer-Wachsmuth, *et al.* 2009. Genetic population structure of the blue starfish *Linckia laevigata* and its gastropod ectoparasite, *Thyca crystallina*. Mar. Ecol. Prog. Ser. 396:211-219.

Kochzius, M., and A. Nuryanto. 2008. Strong genetic population structure in the boring giant clam, *Tridacna crocea*, across the Indo-Malay Archipelago: implications related to evolutionary processes and connectivity. Mol. Ecol. 17:3775-3787.

Kool, J.T., C.B. Paris, P.H. Barber, and R.K. Cowen. 2011. Connectivity and the development of population genetic structure in Indo-West Pacific coral reef communities. Global Ecol. Biogeogr. 20:695-706.

Lawton, R.J., MV. Messmer, M.S. Pratchett, and L.K. Bay. 2011. High gene flow across large geographic scales reduces extinction risk for a highly specialised coral feeding butterflyfish. Mol. Ecol. 20:3584-3598.

Lee, W.J., W.H. Howell, and T.D. Kocher. 1995. Structure and evolution of teleost mitochondrial control regions. J. Mol. Evol. 41:54-66.

Lewis, P.O., and D.J. Crawford. 1995. Pleistocene refugium endemics exhibit greater allozymic diversity than widespread congeners in the genus *Polygonella* (Polygonaceae). Am. J. Bot. 82:141-149.

Leis, J.I.M., H.P.A. Sweatman, and S.E. Reader. 1996. What the pelagic stages of coral reef fishes are doing out in blue water: day-time field observations of larval behavioural capabilities. Mar. Freshwat. Res. 47:401-41.

Librado, P., and J. Rozas. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451-1452.

Litsios, G., A. Kostikova, and N. Salamin. 2014. Host specialist clownfishes are environmental niche generalists. Proc. R. Soc. 281:20133220.

Litvak, M. K., and W. C. Leggett. 1992. Age and size-selective predation on larval fishes: the bigger-is-better hypothesis revisited. Mar. Ecol. Prog. Ser. 81:13-24.

Liu, S.Y.V., H.T. Yu, and C.F. Dai. 2007. Eight microsatellite loci in Clark's anemonefish, *Amphiprion clarkii*. Mol. Ecol. Notes 7:1169-1171.

Lord, C., J. Lorion, A. Dettai, S. Watanabe, K. Tsukamoto, C. Cruaud, and P. Keith. 2012. From endemism to widespread distribution: phylogeography of three amphidromous *Sicyopterus species* (Teleostei: Gobioidei: Sicydiinae). Mar. Ecol. Prog. Ser. 455:269-285.

Lourie, S.A., D.M. Green, and A.C.J. Vincent. 2005. Dispersal, habitat differences, and comparative phylogeography of Southeast Asian seahorses (Syngnathidae: Hippocampus). Mol. Ecol. 14:1073-1094.

Madduppa, H.H., J. Timm, and M. Kochzius. 2014b. Interspecific, spatial and temporal variability of selfrecruitment in anemonefishes. PLoS ONE 9:e90648.

Madduppa, H.H., K. von Juterzenka, M. Syakir, M. Kochzius. 2014a. Socio-economy of marine ornamental fishery and its impact on the population structure of the clown anemonefish *Amphiprion ocellaris* and its host anemones in Spermonde Archipelago, Indonesia. Ocean Coast. Manage. 100:41-50.

Manni, F., E. Guerard, and E. Heyer. 2004. Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by "Monmonier's algorithm". Hum. Biol. 76:173-190.

Manni, F., and E. Guerard. 2004. Barrier vs. 2.2. Manual of the user. Population genetics team, Museum of Mankind (Musée de l'Homme), Paris [Publication distributed by the authors].

Marshall, P.A., and A.H. Baird. 2000. Bleaching of corals on the Great Barrier Reef: differential susceptibilities among taxa. Coral Reefs 19:155-163.

McCook, L.J., G.R. Almany, M.L. Berumen, J.C. Day, A.L. Green, G.P. Jones, *et al.* 2009. The challenge of incorporating connectivity science into coral reef management now: principles and practice. Coral Reefs 28:353-366.

Meekan, M. G., Bradshaw, C. J. A., Press, M., McLean, C., Richards, A., *et al.* 2006. Population size and structure of whale sharks *Rhincodon typus* at Ningaloo Reef, Western Australia. Mar. Ecol. Prog. Ser. 319:275-28.

Monmonier, M. 1973. Maximum-difference barriers: an alternative numerical regionalization method. Geogr. Anal. 3:245-61.

Munday, P.L. 2002. Does habitat availability determine geographical-scale abundances of coral-dwelling fishes? Coral Reefs 21:105-116.

Munday, P.L. 2004. Habitat loss, resource specialization, and extinction on coral reefs. Glob. Change Biol. 10: 1642-1647.

Nei, M. 1987. Molecular evolutionary genetics. Columbia Univ. Press, New York.

Nei, M., and L. Jin. 1989. Variances of the average numbers of nucleotides substitutions within and between populations. Mol. Biol. Evol. 6:290-300.

Nelson, J.S., R.J. Hoddell, L.M. Chou, W.K. Chan, and V.P.E. Wang. 2000. Phylogeographic structure of false clownfish, *Amphiprion ocellaris*, explained by sea-level changes on the Sunda shelf. Mar. Biol. 137:727-736.

Nuryanto, A., and M. Kochzius. 2009. Highly restricted gene flow and deep evolutionary lineages in the giant clam Tridacna maxima. Coral Reefs 28:607-619.

Olds, A.D., R.M. Connolly, K.A. Pitt, and P.S. Maxwell. 2012. Habitat connectivity improves reserve performance. Conserv. Lett. 5:56-63.

Palumbi, S.R. 1994. Genetic divergence, reproductive isolation and marine speciation. A. Rev. Ecol. Syst. 25:547-572.

Peñaflor, E.L., W.J. Skirving, A.E. Strong, S.F. Heron, and L.T. David. 2009. Sea-surface temperature and thermal stress in the Coral Triangle over the past two decades. Coral Reefs 28:841-850.

Pineda, J., J. Hare, and S. Sponaugle. 2007. Marine population connectivity: larval transport and dispersal in the coastal ocean and consequences for population connectivity. Oceanography 20:22-39.

Planes, S., and C. Fauvelot. 2002. Isolation by distance and vicariance drive genetic structure of a coral reef fish in the Pacific Ocean. Evolution 56:378-399.

Portnoy, D.S., C.M. Hollenbeck, M.A. Renshaw, N.J. Cummings, and J.R. Gold. 2013. Does mating behaviour affect connectivity in marine fishes? Comparative population genetics of two protogynous groupers (Family Serranidae). Mol. Ecol. 22:301-313.

Quenouille, B., Y. Bouchenak-Khelladi, C. Hervet, and S. Planes. 2004. Eleven microsatellite loci for the saddleback clownfish *Amphiprion polymnus*. Mol. Ecol. Notes 4:291-293.

Raynal, J.M., E.D. Crandall, P.H. Barber, G.N. Mahardika, M.C. Lagman, and K.E. Carpenter. 2014. Basin isolation and oceanographic features influencing lineage divergence in the humbug damselfish (*Dascyllus aruanus*) in the Coral Triangle. Bull. Mar. Sci. 90:513-532.

Reid, D.G., K. Lal, J. Mackenzie-Dodds, F. Kaligis, D.T.J. Littlewood, and S.T. Williams. 2006. Comparative phylogeography and species boundaries in Echinolittorina snails in the central Indo-West Pacific. J. Biogeogr. 33:990-1006.

Ricciardi, F., M. Boyer, and J. Ollerton. 2010. Assemblage and interaction structure of the anemonefish-anemone mutualism across the Manado region of Sulawesi, Indonesia. Environ. Biol. Fish 87:333-347.

Riginos, C., K.E. Douglas, Y. Jin, D.F. Shanahan, and E.A. Treml. 2011. Effects of geography and life history traits on genetic differentiation in benthic marine fishes. Ecography 34:566-575.

Roberts, C.M., C.J. McClean, J.E.N. Veron, J.P. Hawkins, G.R. Allen, D.E. McAllister, *et al.* 2002. Marine Biodiversity Hotspots and Conservation Priorities for Tropical Reefs. Science 295:1280.

Rocha, L.A., A.L. Bass, D.R. Robertson, and B.W. Bowen. 2002. Adult habitat preferences, larval dispersal, and the comparative phylogeography of three Atlantic surgeonfishes (Teleostei: Acanthuridae). Mol. Ecol. 11:243-252.

Rocha, L.A., D.R. Robertson, J. Roman, and B.W. Bowen. 2005. Ecological speciation in tropical reef fishes. Proc. Biol. Sci. 272:573-579.

Rodríguez Moreno, Melina. 2009. Mikrosatelliten Analyse der genetischen Populationsstruktur des Anemonenfisches *Amphiprion clarkii* im Indo-Malayischen Archipel und Nordwestpazifik. MSc Thesis, University of Bremen

Ross, R.M. 1978. Reproductive behavior of the anemonefish *Amphiprion melanopus* on Guam. Copeia 1978: 103-107.

Saenz-Agudelo, P., G.P. Jones, S.R. Thorrold, and S. Planes. 2011. Detrimental effects of host anemone bleaching on anemonefish populations. Coral Reefs 30: 497-506.

Santini, S., and G. Polacco. 2006. Finding Nemo: molecular phylogeny and evolution of the unusual life style of anemonefish. Gene 385:19-27.

Sathiamurthy, E., and H.K. Voris. 2006. Maps of Holocene sea level transgression and submerged lakes on the Sunda Shelf. Natl. Hist. J. Chulalongkorn University, Supplement. 2:1-43.

Selkoe, K.A., and R.J. Toonen. 2011. Marine connectivity: an new look at pelagic larval duration and genetic metrics of dispersal. Mar. Ecol. Prog. Ser. 436: 291-305.

Shanks, A.L. 2009. Pelagic larval duration and dispersal distance revisited. Biol. Bull. 216:373-385.

Shuman, C.S., G. Hodgson, and R.F. Ambrose. 2005. Population impacts of collecting sea anemones and anemonefish for the marine aquarium trade in the Philippines. Coral Reefs 24:564-573.

Swearer, S.E., J.S. Shima, M.E. Hellberg, S.R. Thorrold, G.P.Jones, D.R. Robertson, *et al.* 2002. Evidence of self recruitment in demersal marine populations. Bull. Mar. Sci. 70:251-272.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585-595.

Tajima, F. 1993. Measurement of DNA polymorphism. Pp. 37–59 in N. Takahata, A.G. Clark, eds. Mechanisms of molecular evolution. Introduction to molecular paleopopulation biology. Scientific Societies Press, Sinauer Associates, Inc, Tokyo, Sunderland, MA.

Taylor, M.S., and M.E. Hellberg. 2003. Genetic Evidence for Local Retention of Pelagic Larvae in a Caribbean Reef Fish. Science 299:107.

Thacker, C.E., A.R. Thompson, D.M. Roje, and E.Y. Shaw. 2007. New expansions in old clades: population genetics and phylogeny in a Gnatholepis species (Teleostei: Gobiodei) in the Pacific. Mar. Biol. 153:375-385.

Thompson, J.G., D.G. Higgins, and T.J. Gibson. 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignments through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673-4680.

Thresher, R.E., P. L. Colin, and L.J. Bell. 1989. Planktonic Duration, Distribution and Population Structure of Western and Central Pacific Damselfishes (Pomacentridae). Copeia 2:420-434.

Timm, J., and M. Kochzius. 2008. Geological history and oceanography of the Indo- Malay Archipelago shape the genetic population structure in the false clown anemonefish (*Amphiprion ocellaris*). Mol. Ecol. 17:3999-4014.

Timm, J., Figiel, M., and M. Kochzius. 2008. Contrasting patterns in species boundaries and evolution of anemonefishes (Amphiprioninae, Pomacentridae) in the centre of marine biodiversity. Mol. Phylogenet. Evol. 49:268-276.

Timm, J., S. Planes, and M. Kochzius. 2012. High similarity of genetic population structure in the false clown anemonefish (*Amphiprion ocellaris*) found in microsatellite and mitochondrial control region analysis. Conserv. Genet.13:693-706.

Toonen, R.J., K.R. Andrews, I.B. Baums, C.E. Bird, C.T. Concepcion, T.S. Daly-Engel, *et al.* 2011. Defining boundaries for applying ecosystem-based management: a multispecies case study of marine connectivity across the Hawaiian Archipelago. J. Mar. Biol. 2011:460173.

Treml, E.A., J.J. Roberts, Y. Chao, P.N. Halpin, H.P. Possingham, and C. Riginos. 2012. Reproductive output and duration of the pelagic larval stage determine seascape-wide connectivity of marine populations. Integr. Comp. Biol. 52:525-537.

Treml, E.A., J.J. Roberts, P.N. Halpin, H.P. Possingham and C. Riginos. 2015. The emergent geography of biophysical dispersal barriers across the Indo-West Pacific. Divers. Distrib. 21:465-476.

Turton, C., and Y. Otomo. 2007. Tending our reefs: a review and assessment of the marine aquarium trade in Australia. Australian Marine Aquarium Trade Report Earth Economics Available: http://www.earth.economics.com.au/aquariumtrade.pdf.

Veron, J.E.N., L.M. DeVantier, E. Turak, A.L. Green, S. Kininmoth, M. Stafford-Smith, *et al.* 2009. Delineating the Coral Triangle. Galaxea 11:91-100.

Voris, H.K. 2000. Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. J. Biogeo. 27:1153-1167.

von der Heyden, S., M. Beger, R.J. Toonen, L. van Herwerden, M.A. Juinio-Meñez, R. Ravago-Gotanco, *et al.* 2014. The application of genetics to marine management and conservation: examples from the Indo-Pacific. Bull. Mar. Sci. 90:123-158.

Wabnitz, C., M. Taylor, E. Green, and T. Razak. 2003. From ocean to aquarium. UNEP-WCMC, Cambridge, UK.

Weersing, K., and Toonen R.J. 2009. Population genetics, larval dispersal, and connectivity in marine systems. Mar. Ecol. Prog. Ser. 393:1-12.

Wellington, G.M., and D.R. Robertson. 2001. Variation in larval life-history traits among reef fishes across the Isthmus of Panama. Mar. Biol. 138:11-22.

Wellington, G.M., and B.C. Victor. 1989. Planktonic larval duration of one hundred species of Pacific and Atlantic damselfishes (Pomacentridae). Mar. Biol. 101:557-567.

Wilkinson, C. 2002. Status of coral reefs of the world. Townsville, Australia: Australian Institute of Marine Science.

Winters, K.L., L. van Herwerden, H.J. Choat, and D.R. Robertson. 2010. Phylogeography of the Indo-Pacific parrotfish *Scarus psittacus*: isolation generates distinctive peripheral populations in two oceans. Mar. Biol. 157:1679-1691.

SUPPLEMENTARY MATERIALS

Table S5.3 Primers for the amplification of 8 microsatellite loci in *A. sandaracinos* with their respective motif, PCR product size, number of alleles, PCR annealing temperature, and their biological and literature sources.

Locus	Motif	Product size (bp)	No. alleles	Primers	Ann. temp. (C°)	Source
Ac1578	(AC)9	252-286	13	*F*: 5'-CAGCTCTGTGTGTGTTTAATGC-3' R: 5'-CACCCAGCCACCATATTAAC-3'	55.7-57	*A. clarkii* (Liu *et al*. 2007)
Ac626	TC)6(AC)20	227-275	20	*F*: 5'-CACACATGCACACACCTTGA-3' R: 5'-TAATTGAGGCAGGTGGCTTC-3'	60	*A. clarkii* (Liu *et al*. 2007)
Ac137	(AC)19	284-332	24	*F*: 5'-GGTTGTTTAGGCCATGTGGT-3' R: 5'-TTGAGACACACTGGCTCCT-3'	55.7	*A. clarkii* (Liu *et al*. 2007)
CF42	(TCTG)18	166-210	24	*F*: 5'-TGCAATTATGCACCTG-3' R: 5'-TGGCCAGATTGGTTAC-3'	58.6	*A. percula* (Buston *et al*. 2007)
CF27	(TCTA)16	184-248	14	*F*: 5'-AAGCTCCGGTAACTCAAAACTAAT-3' R: 5'-GTCATCTGATCCATGTTGATGTG-3'	60	*A. percula* (Buston *et al*. 2007)
55	(GT)16	418-460	16	*F*: 5'-TTAACTTCCACACCCAGTCT-3' R: 5'-ACGCTGTGAGAGTCCATTAT-3'	58.7	*A. polymnus* (Quenouille *et al*. 2004)
44	(GT)13	219-253	11	*F*: 5'-TTGGAGCAGCGTACTTAGCT-3' R: 5'-AGATGTGTTTACGCACGCTT-3'	58.7	*A. polymnus* (Quenouille *et al*. 2004)
61	(GT)49	320-388	28	*F*: 5'-TGAACACATAAACGCTCACTCAC-3' R: 5'-AAGACAATGCCTCCACATATCTA-3'	58.7	*A. polymnus* (Quenouille *et al*. 2004)

Chapter III

Obstacles to Molecular Species Identification in Sea Anemones (Hexacorallia: Actiniaria) with COI, a COI Intron and ITS II

Tina A. Dohna[1] and Marc Kochzius[2]

1) Biotechnology and Molecular Genetics, UFT, University of Bremen, 28359 Germany
2) Marine Biology, Vrije Universiteit Brussel, Belgium

Marine Biodiversity: DOI 10.1007/s12526-015-0329-5, 201

ABSTRACT

DNA barcoding has been successfully applied to a very large number of taxa, but remains problematic for basal diploblasts and debates about suitable molecular markers are ongoing. Sea anemones (Anthozoa: Hexacorallia: Actiniaria) populate most any marine environment and often play an irreplaceable role as hosts to other animals. Three genetic markers were tested to assess their utility for molecular species identification in members of the Actiniaria, namely the cytochrome oxidase subunit I (COI), a COI Intron with a Homing Endonuclease Gene (HEG), and the Internal Transcribed Spacer II (ITS II). Both the power of COI and the COI Intron to distinguish species is limited by events of very low inter-specific sequence differences and not by high intra-specific diversity. This finding implies that more comprehensive taxon sampling will not resolve this problem and other markers need to be investigated in several families. Results should discourage the use of ITS II as an alternative to COI for barcoding in Actiniarians, since it shows similar limitations as COI.

INTRODUCTION

DNA barcoding is an international effort to record and catalogue species-specific DNA sequence data (barcodes), by which unknown specimens can be identified, new or cryptic species discovered (e.g., Hebert *et al.* 2003a, b, 2004, Hajbabaei *et al.* 2006), and species identities confirmed (Clare *et al.* 2007, Hebert *et al.* 2004; Moritz & Cicero 2004). Anthozoans (corals, sea anemones and their kin) present a challenge for barcoding because the 5′ segment of the mitochondrial cytochrome subunit I gene (COI), which is consensually applied for barcoding (Ward *et al.* 2005, Hajbabaei *et al.* 2006), has been found to be highly conserved (e.g., Flot *et al.* 2013) and a clear barcoding gap is absent in many genera due to low interspecific variability (Shearer *et al.* 2002). However, Keshavmurthy *et al.* (2013) were able to identify four deeply divergent clades (species) of the coral *Stylophora pistillata* within its range with COI. This emphasizes the value of barcoding in groups where taxonomically defining characteristics are variable and/or inconsistent with genetic units, such as is in the Anthozoa (e.g., Flot *et al.* 2008).

Research focusing on anthozoan barcoding has dealt almost exclusively with corals, taking little notice of other members of the group (Shearer *et al.* 2002, Hebert *et al.* 2003b, Shearer & Coffroth 2006, 2008, Oliverio *et al.* 2009). We here test the barcoding utility of a partial COI gene fragment in sea anemones and two additional markers that indicate potential for species identification: the highly polymorph nuclear Internal Transcribed Spacer II (Flot *et al.* 2013, Oliverio *et al.* 2009) and a Homing Endonuclease Gene (HEG) located within a self-splicing group I Intron within COI (Goddard & Burt 1999, Goddard *et al.* 2006). When present, this HEG is unique among metazoans and its invasion cycle may be sufficiently slow to provide potential for species delineation, without providing a host specific phylogenetic signal (Goddard *et al.* 2006). Sequences for three species of giant tropical sea anemones, *Heteractis magnifica* (Quoi & Gaimard 1833), *Heteractis crispa* (Ehrenberg 1834), and *Entacmaea*

quadricolor (Rüppel & Leuckart 1828) were generated and aligned with all available actiniarian GenBank sequences.

MATERIALS AND METHODS

All sea anemones for this study were collected and identified by M. Kochzius (one of the authors) and Janne Timm (Bremen University, Germany) from a total of nine locations in the Indo-Malay Archipelago, the South China Sea, and Okinawa. Samples from Japan, Borneo, and the Philippines where collected by J. Timm during workshops offered at local institutions and under their supervision and with their consent. Tentacle clippings were stored in 96 % EtOH. DNA was extracted using the CTAB extraction method, altered only by an additional Proteinase K digestion step for a minimum of 24 h at 55 °C. DNA fragments were amplified using primers and annealing temperatures listed in Table 5.11. PCR products were purified using Peqlab cycle pure spin columns (Peqlab, Erlangen) and subjected to a cycle PCR (Big Dye terminator Cycle Sequencing Kit (ver. 3.1; Applied Bioscience) with forward and reverse primers. The cycle PCR products were purified via ethanol precipitation. Sequencing was carried out on either an ABI Prism 310 or 3100 automated sequencer (Applied Biosystems, Weiterstadt).

The resulting forward and reverse sequences were aligned and edited in SeqMan (ver. 4.0.5, DNASTAR) and a total sequence alignment was achieved with the ClustalW algorithm (Thompson *et al.* 1994), as implemented in the software BioEdit (ver. 7.0.9.0) (Hall 1999) for COI and COI Intron sequences. ITS II sequences were aligned using MAFFT 7 (online version; Katoh and Standley 2013). Poorly aligned positions and divergent regions were removed with GBlocks 0.91b (Castresana 2000, Talavera & Castresana 2007) using the most relaxed criteria. Sequence divergences were calculated using the Kimura two-parameter (K2P) model of base substitution (Kimura 1980) for COI and simple pairwise differences for the COI Intron dataset. Maximum Parsimony (MP) and Neighbor-joining (NJ) (Saitou &

Nei 1987) trees including bootstrap analysis (1000 replications) (Nei & Kumar 2000) were performed using MEGA4 (Kumar *et al.* 2004), as were the calculations of intra- and inter-specific genetic divergence (K2P genetic distances). Maximum Likelihood (ML) (1000 bootstraps) was used to construct the ITS II tree applying a GTR model of evolution with a 0.04 fixed proportion of invariable sites, five substitution rate categories and a Gamma shape of 1.27 in PhyML (online version, Guindon *et al.* 2010). Model selection for the ML run in PhyML was determined with MEGA 6, as were inter- and intraspecific sequence divergence, using the ML algorithms and the complete deletion option for alignment gaps. Tajima's Relative Rate tests (Tajima 1993) was also carried out in MEGA 6, by comparing sequence pairs from all available species and using the *Zoanthus praelongus* sequence as an outgroup.

Table 5.11 PCR primers used to amplify COI, COI Intron, and ITS I-5.8S-ITS II.

Marker	T_a (C°)	Forward Primer	Reverse Primer
COI intron	62	5'-CTCGCTATATGCTGGAAARACCC-3'	5'-CAATAAGCGAAGCGTTTTCCA GCC-3'
COI	51	5'-GGT ATG ATA GGC ACA GCT-3'	5'-GAAAGTTGTATTAAARTTCCTATCTG-3'
ITS I&5.8S&ITS II	56	5'-GAG GAA GTA AAA GTC GTA AC-3'	5'-GGT CAA GAT GGA AAG ATA G-3'

Table 5.12 Family designations of species, number and source of sequences that were used for all three marker analyses, COI, COI Intron, and ITS II

Species	**Family**	**COI[1]**	**COI Intron[2]**	**ITS II[3]**	**Accession Number**
Actinia equina	Actiniidae	-	1	1	[2] DQ831335; [3] DQ831298
Actinia fragacea	Actiniidae	-	1	-	[2] DQ831334
Actinia bermudensis	Actiniidae	-	-	1	[3] JN118562
Anemonia sp.	Actiniidae	1	-	-	[1] AB441274
Anemonia viridis	Actiniidae	-	1	-	[2] DQ831333
Anthopleura balii	Actiniidae	-	-	1	[3] DQ831299
Anthopleura elegantissima	Actiniidae	2	-	-	[1] GU443180, AF480931
Aulactinia incubans	Actiniidae	-	-	3	[3] EF026587-EF026589
Aulactinia marplatensis	Actiniidae	-	-	6	[3] EF026592, EF026594, EF026595, EF026597, EF026601, EF026602
Aulactinia reynaudi	Actiniidae	-	-	5	[3] EF026593, EF026596, EF026598-EF026600
Aulactinia verrucosa	Actiniidae	-	-	2	[3] EF026590, EF026591
Aulactinia stella	Actiniidae	-	-	8	[3] JQ412857-JQ412860, JQ844113-JQ844116
Bunodosoma caissarum	Actiniidae	-	-	3	[3] JN118559, JN118560, JN118566
Bunodosoma cangicum	Actiniidae	-	-	4	[3] JN118561, [3] JN118567-JN118569
Bunodosoma granuliferum	Actiniidae	-	-	1	[3] JN118565
Bunodosoma sp.	Actiniidae	-	-	1	[3] JN118557, JN118563
Condylactis sp.	Actiniidae	-	-	1	[3] AB441419
Entacmaea quadricolor	Actiniidae	24	4	-	**[1] JQ839204-JQ839227; [2] JQ918745-JQ918748**
Phymactis papillosa	Actiniidae	-	-	1	[3] JN118564
Urticinopsis antarctica	Actiniidae	1	-	-	[1] AJ830011
Urticina columbiana	Actiniidae	1	-	-	[1] UCU91613
Urticina crassicornis	Actiniidae	1	-	1	[1] UCU91612, [3] JQ844117
Urticina lofotensis	Actiniidae	1	-	-	[1] U91614
Urticina felina	Actiniidae	1	-	-	[1] UFU91610
Aiptasia mutabilis	Aiptasiidae	-	-	1	[3] DQ831297
Aiptasia sp.	Aiptasiidae	-	1	-	[2] DQ831341
Megalactis sp.	Actino-dendronidae	-	1	-	[2] DQ831342
Edwardsiidae sp.	Edwardsiidae	-	-	8	[3] EU418268-EU418274, GQ464903

Table 5.12 *cont.*►

Table 5.12 *cont.*►

Nematostella vectensis	Edwardsiidae	2	-	-	[1] DQ538492, DQ538493
Adamsia carciniopados	Hormathiidae	-	1	1	[2] DQ831340; [3] DQ831304
Calliactis parasitica	Hormathiidae	-	1	6	[2] DQ831339, [3] DQ831303, FM161930,HQ156453-HQ156456
Calliactis polypus	Hormathiidae	-	-	165	[3] HQ156276-HQ156440
Calliactis tricolor	Hormathiidae	-	-	12	[3] HQ156441-HQ156452
Metridium senile	Metridiidae	3	3	1	[1] AF00023, U36783, NC000933; [2] NC000933, U36783, AF000023; [3] DQ831306
Actinothoe sphyrodeta	Sagartiidae	-	1	1	[2] DQ831338; [3] DQ831302
Cereus pedunculatus	Sagartiidae	-	1	1	[2] DQ831336; [3] DQ831300
Sagartia elegans	Sagartiidae	-	1	1	[2] DQ831337; [3] DQ831301
Sagartia troglodytes	Sagartiidae	-	-	1	[3] FM161931
Heteractis crispa	Sticho-dactylidae	27	3	-	**[1] JQ839177-JQ839203; [2] JQ918749-JQ918751**
Heteractis magnifica	Sticho-dactylidae	27	63	26	**[1] JQ839150-JQ839176; [2] JQ918688- JQ918744; [3] JQ918752 JQ918766**, AF050201-AF050211
Heterodactyla sp.	Thalassi-anthidae	-	-	1	[3] DQ831305

Sequences by the authors are in bold

RESULTS AND DISCUSSION

COI

The COI alignment (462 bp length) contained 91 sequences from 12 species, eight genera and four families (Table 5.12). The large majority of sequences stemmed from this study, since the number of actiniarian COI sequences in GenBank is extremely limited and multiple sequences to a species are rare. Species level resolution, i.e., adequate grouping of conspecies and divergence between congenerics, using the COI gene was partially unsuccessful in the Actiniidae, as species within the genus Urticina, as well as species of genera *Urticinopsis* and *Entacmaea* could not be delineated (Table S5.4, Fig. S5.2, supplementary materials). Overall, 16 % of all interspecific comparisons show no or minimal divergence (d=0.00-0.01), so that efforts to collect additional information on intraspecific

variability are unnecessary in the context of a single marker approach (Fig. 5.7). The ability of COI to delineate species is limited by a lack of inter-specific divergence, an obstacle that cannot be overcome by more comprehensive taxon sampling and has been found in other anthozoan orders (scleractinian corals, Shearer & Coffroth 2008). Contrary to patterns seen in higher metazoans, substitution rates in the mtDNA of cnidarians appeared to be much slower than in the nuclear DNA (Shearer *et al.* 2002). The slow mitochondrial sequence evolution found here corroborates findings from other anthozoan orders, and supports the hypothesis of an ancestral slow substitution state, rather than this being a secondarily acquired feature in the Anthozoa (Shearer *et al.* 2002, Huang *et al.* 2008).

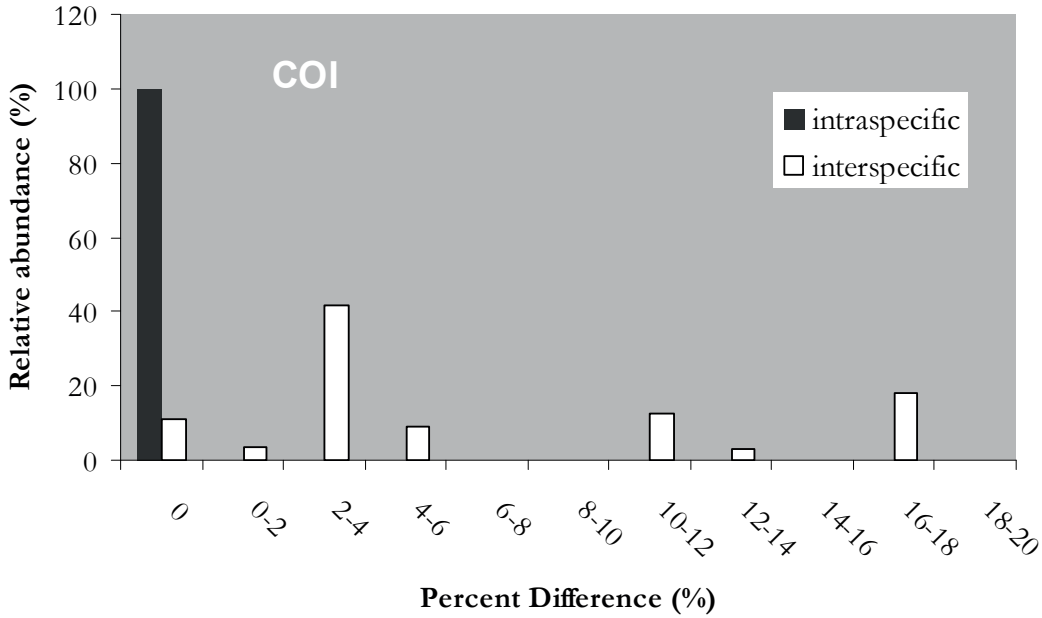


Figure 5.7 Bar chart showing the proportion of pair wise distance comparisons of the COI gene for each range of sequence divergence (K2P).

COI intron

The COI Intron alignment (590 bp length) contained 84 sequences from 14 species in 12 genera embedded in seven families (Table 5.12). The vast majority of sequences in this alignment stemmed from *H. magnifica*, since actiniarian GenBank sequences are limited, and multiple sequences/ species even less than for COI. The use of the COI Intron failed species delineation in at least three families: the Actiniidae, Hormathiidae, and Sagartiidae

(Table S5.5, Fig S5.3, supplementary materials). Where data were available, intraspecific variability was marginally higher (though still less than 1 %, Table S5.5), indicative of faster rates of evolution for the HEG fragment. Apart from the three instances of delineation failure, which were due to a lack of between species divergence, the COI intron produced a higher interspecific divergence than the previous marker (COI intron: max 38 %, mean=21 %), with two thirds of all comparisons falling above the maximum divergence seen with COI (COI: max. 18 %, mean= 7 %)(Fig. 5.8, Table S5.5). As seen in COI, the range of interspecific divergence (0.2 – 38 %) overlaps with the intraspecific variability (0 – 0.3 %) in a few instances, preventing a clear barcoding gap from forming (Fig. 5.8). Nevertheless, this unconventional marker may yet hold barcoding potential in some families, since intra-specific variation is very low in the species tested, while the interspecific divergence is markedly higher. The phylogenetic signal seen in the NJ-Tree (Fig. S2, supplementary materials) should not be interpreted as such, as it may well reflect the infection pathway of the HEG in Actiniaria and not relationships among sea anemones. Most problematic appears to be the Actiniidae, as here some species cannot be discriminated using either COI or the COI Intron.

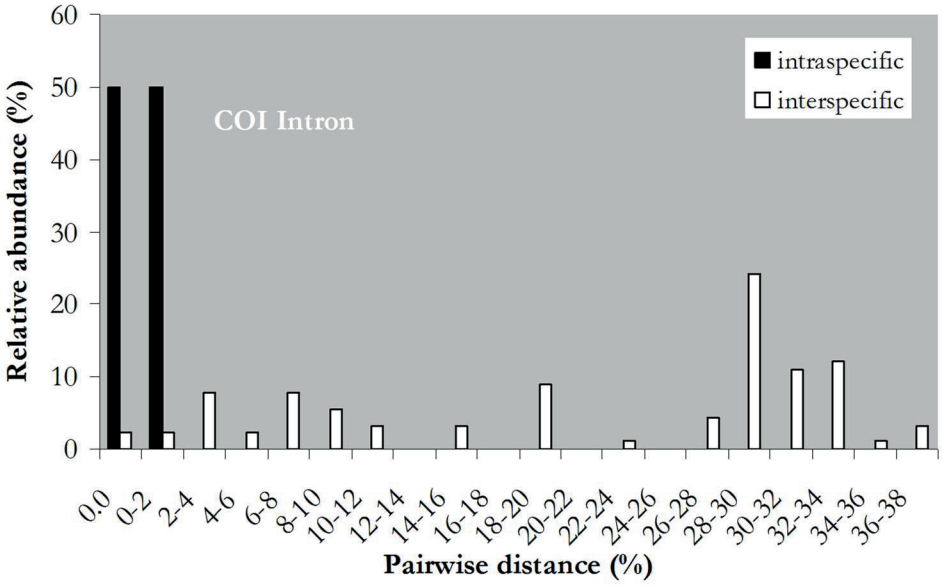


Figure 5.8 Bar chart showing the proportion of pair wise comparisons of the COI Intron for each range of sequence divergence (pairwise differences).

ITS II

ITS species divergence rates from 2–11 % have been reported in various coral genera (Medina *et al.* 1999, Diekmann *et al.* 2001, Hunter *et al.* 1997) and as high as 45 % in Zoantharia (Anthozoa: Hexacorallia) (Reimer *et al.* 2007). Based on these findings, the ITS II marker was assessed here as an actiniarian barcoding marker. The ITS II alignment contained 264 sequences (171 bp) from 28 species representing 17 genera from seven families (Table 5.12). The majority of sequences in this alignment stem from the public database and only sequences for *Heteractis magnifica* were contributed by the authors. Similar to the two previously discussed markers, the ability of ITS II to delineate species is limited by the lack of interspecific sequence divergence, which ranges between 0.00-65.5 %, overlapping in 13 instances with intraspecific sequence divergence (0.00-2.6 %) (Fig. 5.9, Table S5.5). These events of overlap were restricted to the family Actiniidae and included delineation failure within and between different genera. However, when ITS II was used,

Adamsia carcinopados and *Calliactis parasitica* (within the Hormathiidae), as well as *Cereus*

pedunculatus and *Actinothoe spyrodeta* (within the Sagartiidae) were well separated, which was not possible with the COI intron. A concatenated alignment of both markers, in addition to the information provided through the absence/presence of the intronic region may prove useful, though problems within the Actininiidae may still persist. Relative rate tests (Tajima 1993) with the available ITS II sequences indicated that members of the genus *Calliactis* may be evolving at a faster rate than other taxa tested here (significant $\chi 2 = 3.84$- 11.84, mean $\chi 2=5.61$). This indicates that there are differences in the genetic differentiation of congenerics that would have to be investigated thoroughly before this marker could be used with any reliability.

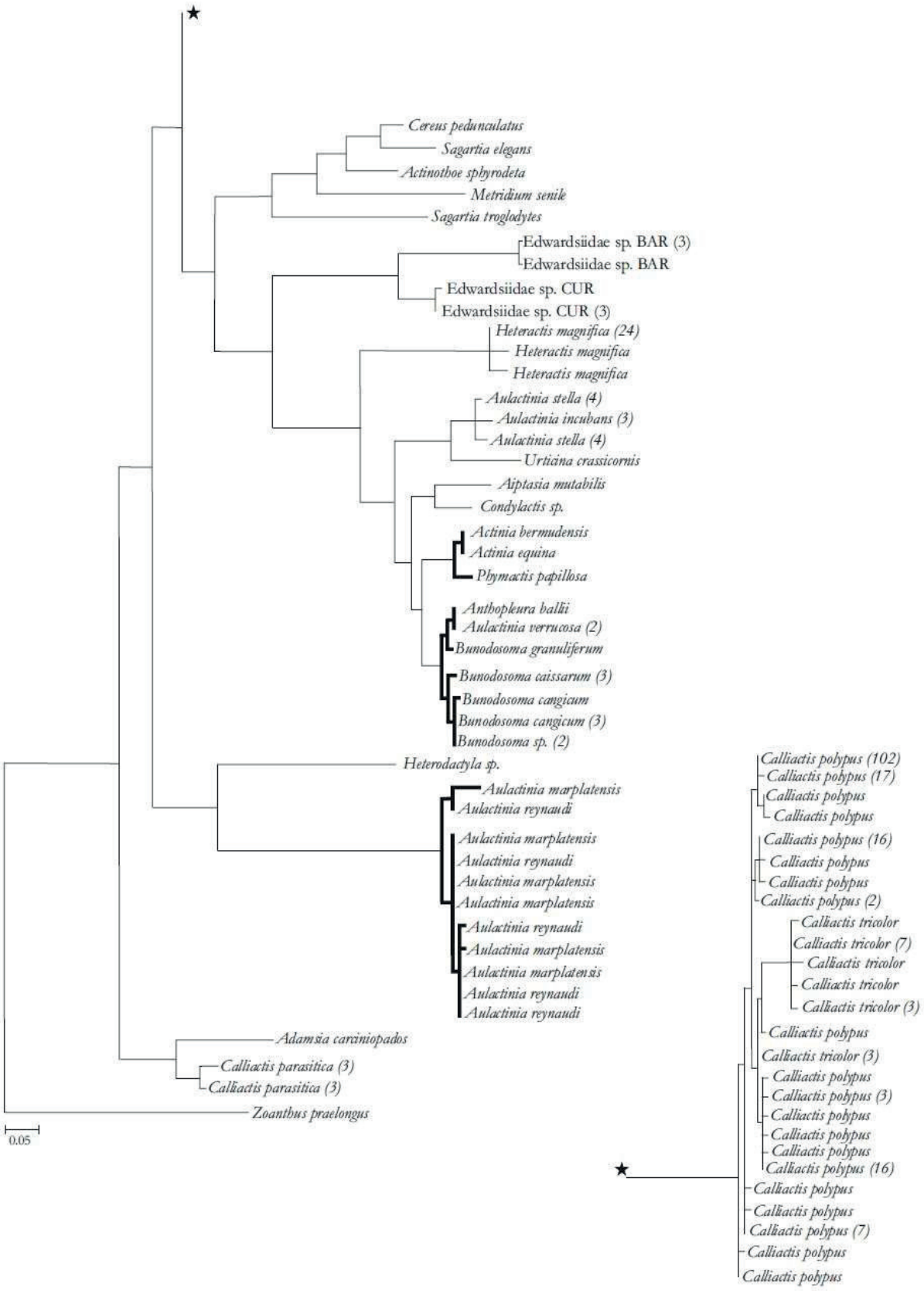


Figure 5.9 ML tree with all available actinian ITS II sequences, both from this study and the public databases (171 positions). Bootstrap values below 50 are not shown. Branches marked in bold denote nodes where species level resolution could not be achieved.

CONCLUSIONS

Though the data pool for this study is small, it represents the largest study on COI divergence in Actiniarians so far. Concatenation of COI Intron sequences and ITS II for an alignment based on both markers might prove useful, as the advantages of each might produce a useful marker system in some families. The absence of an intron can also serve as an informative character. Currently the dearth of data available for this taxon does not allow such a step. The Actiniaria is a taxonomically very challenging group, which would benefit immensely from a reliable barcoding system. In turn, the performance of the markers is gauged on how well they recover taxonomic categories, which may themselves be flawed or under discussion. The results from this study imply that the goal of finding a genetic marker applicable to the whole of the Actiniaria may prove futile, though for some families the mitochondrial and nuclear markers tested provide sufficient resolution. This should be further explored to include more comprehensive taxon sampling. For problematic groups, where the interspecific genetic variability clearly impedes species delineation, efforts should rather focus on exploring other markers or supplemental ID systems (Concepcion *et al.* 2008, Huang *et al.* 2008, Sinniger *et al.* 2008).

REFERENCES

Castresana, J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol. Biol. Evol. 17:540–552.

Clare, E.L., K.L. Burton, M.D. Engstrom, J.L. Eger, and P.D.N. Herbert. 2007. DNA barcoding of neotropical bats: species identification and discovery within Guyana. Mol. Ecol. Notes 7:184–190.

Concepcion, G.T., M.W. Crepeau, D. Wagner, S.E. Kahng, and R.J. Toonen. 2008. An alternative to ITS, a hypervariable, single-copy nuclear intron in corals, and its use in detecting cryptic species within the octocoral genus *Carijoa*. Coral Reefs 27:323–336.

Diekmann, O.E., R.P.M. Bak, W.T. Stam, and J.L. Olsen. 2001. Molecular genetic evidence for probably reticulate speciation on the coral genus *Madracis* from a Caribbean fringing reef slope. Mar. Biol. 139: 221–233.

Flot, J.-F., W.Y. Licuanan, Y. Nakano, C. Payri, C. Cruaud, and S. Tillier. 2008. Mitochondrial sequences of *Seriatopora* corals show little agreement with morphology and reveal the duplication of a tRNA gene near the control region. Coral Reefs 27:789–794.

Flot, J.-F., M. Dahl, and C. André. 2013. *Lophelia pertusa* corals from the Ionian and Barents seas share identical nuclear ITS2 and near-identical mitochondrial genome sequences. BMC Res. Notes 6:144.

Goddard, M.R., and A.Burt 1999. Recurrent invasion and extinction of a selfish gene. Proc. Natl. Acad. Sci. USA 96:13880–13885.

Goddard, M.R., J. Leigh, A.J. Roger, and A.J. Pemberton. 2006. Invasion and persistence of a selfish gene in the Cnidaria. PLoS One 1:e3.

Guindon, S., J.F. Dufayard, V. Lefort, M. Anisimova, W. Hordijk, and O. Gascuel. 2010. New algorithms and methods to estimate maximumlikelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. 59:307–321.

Hajbabaei, M., D.H. Janzen, J.M. Burns, W. Hallwachs, and P.D.N. Hebert. 2006. DNAbarcodes distinguish species of tropical *Lepidoptera*. Proc. Natl. Acad. Sci. USA 103:968–971.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acid Symp. Ser. 41:95–98.

Hebert, P.D.N., A. Cywinska, S.L. Ball, and J.R. deWaard. 2003a. Biological identifications through DNA barcodes. Proc. R. Soc. Lond. B 270: 313–322.

Hebert, P.D.N., S. Ratnasingham, and J.R. deWaard. 2003b. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proc. R. Soc. Lond. B 270:S96–S99.

Hebert, P.D.N., E.H. Penton, J.M. Burns, D.H. Janzen, and W. Hallwachs. 2004. Ten species in one: DNA barcoding reveals cryptic species in the Neotropical skipper butterfly *Astraptes fulgerator*. Proc. Natl. Acad. Sci. USA 101:14812–14817.

Huang, D., R. Meier, P.A. Todd, and M.C. Loke. 2008. Slow mitochondrial COI sequence evolution at the base of the metazoan tree and its implications for DNA barcoding. J. Mol. Evol. 66:167–174.

Hunter, C.L., C.W. Morden, and C.M. Smith. 1997. The utility of ITS sequences assessing relationships among zooxanthellae and corals. Proc. 8th Int. Coral Reef Symp. 2:1559–1602.

Katoh, K., and D.M. Standley. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30:772–780.

Keshavmurthy, S., S. Yang, A. Alamaru, Y. Chuang, M. Pichon, D. Obura, *et al.* 2013. DNA barcoding reveals the coral "laboratory-rat", *Stylophora pistillata*, encompasses multiple identities. Sci. Rep. 3:1520.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16:111–120.

Kumar, S., K. Tamura, and M. Nei. 2004. Mega3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform. 5:150–163.

Medina, M., E. Weil, and A.M. Szmant. 1999. Examination of the *Montastraea annularis* species complex (Cnidaria: Scleractinia) using ITS and COI sequences. Mar. Biotechnol. 1:89–97.

Moritz, C., and C. Cicero. 2004. DNA barcoding: promise and pitfalls. PloS Biol. 2:e354.

Nei, M., and S. Kumar. 2000. Molecular evolution and phylogenetics. Oxford University Press, New York.

Oliverio, M., A. Barco, M.V. Modica, A. Richter, and P. Mariottini. 2009. Ecological barcoding of corallivory by second internal transcribed spacer sequences: hosts of coralliophiline gastropods detected by the cnidarian DNA in their stomach. Mol. Ecol. Resour. 9:94–103.

Reimer, J.D., K. Takishita, S. Ono, J. Tsukahara, and T. Maruyama. 2007. Molecular evidence suggesting interspecific hybridization in *Zoanthus* spp. (Anthozoa: Hexacorallia). Zool. Sci. 24:346–359.

Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406–425.

Shearer, T.L., and M.A. Coffroth. 2006. Genetic identification of Caribbean scleractinian coral recruits at the Flower Garden Banks and the Florida Keys. Mar. Ecol. Prog. Ser. 306:133–142.

Shearer, T.L., and M.A. Coffroth. 2008. Barcoding corals: limited by interspecific divergence, not intraspecific variation. Mol. Ecol. Resour. 8:247–255.

Shearer, T.L., M.J.H. Van Oppen, S.L. Romano, and G. Wörheide. 2002. Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). Mol. Ecol. 11:2475–2487.

Sinniger, F., J.D. Reimer, and J. Pawlowski. 2008. Potential of DNA sequences to identify zoanthids (Cnidaria: Zoantharia). Zool. Sci. 25:1253–1260.

Tajima, F. 1993. Simple methods for testing molecular clock hypothesis. Genetics 135:599–607.

Talavera, G., and J. Castresana. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst. Biol. 56:564–577.

Thompson, J.D., D.G. Higgins, and T.J. Gibson. 1994. ClustalW: improving the sensitivity of progressive multiple sequence alignments through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673–4680.

Ward, R.D., T.S. Zemlak, B.H. Innes, P.R. Last, P.D.N. Hebert. 2005. DNA barcoding Australia's fish species. Philos. Trans. R. Soc. B Biol. Sci. 360:1847–1857.

SUPPLEMENTARY MATERIALS

Table S5.4 Intra- and interspecific distance calculations based on the pair wise analysis of the listed COI sequences. Standard error estimates are in italic and were obtained by a bootstrap procedure (1000 replicates). Analyses were conducted using the Kimura 2-parameter method in MEGA4. Numbers in brackets behind species names indicate the number of sequences used in the analysis. Bold values indicate lack of adequate interspecific divergence for species delineations. There were a total of 462 positions in the final dataset.

		Interspecific Distance										
Species	**Intraspecific Distance**	1	2	3	4	5	6	7	8	9	10	11
Urticinopsis antarctica (1)	na		*0.02*	*0.02*	*0.01*	*0.01*	*0.01*	*0.01*	*0.01*	*0.01*	*0.01*	*0.00*
Metridium senile (3)	0.00 (S.E± 0.00)	0.12		*0.02*	*0.02*	*0.02*	*0.02*	*0.02*	*0.02*	*0.02*	*0.02*	*0.02*
Nematostella vectensis (2)	0.00 (S.E± 0.00)	0.18	0.18		*0.02*	*0.02*	*0.02*	*0.02*	*0.02*	*0.02*	*0.02*	*0.02*
Urticina felina (1)	na	0.03	0.11	0.17		*0.00*	*0.00*	*0.00*	*0.01*	*0.01*	*0.01*	*0.01*
Urticina lofotensis (1)	na	0.03	0.11	0.17	**0.00**		*0.00*	*0.00*	*0.01*	*0.01*	*0.01*	*0.01*
Urticina crassicornis (1)	na	0.03	0.11	0.17	**0.00**	**0.00**		*0.00*	*0.01*	*0.01*	*0.01*	*0.01*
Urticina columbiana (1)	na	0.03	0.11	0.17	**0.00**	**0.00**	**0.00**		*0.01*	*0.01*	*0.01*	*0.01*
Anthopleura elegantissima (2)	0.00 (S.E± 0.00)	0.03	0.13	0.18	0.04	0.04	0.04	0.04		*0.01*	*0.01*	*0.01*
Heteractis magnifica (27)	0.00 (S.E± 0.00)	0.03	0.12	0.17	0.04	0.04	0.04	0.04	0.03		*0.01*	*0.01*
Hetractis crispa (27)	0.00 (S.E± 0.00)	0.03	0.12	0.17	0.05	0.05	0.05	0.05	0.02	0.04		*0.01*
Entacmaea quadricolor (24)	0.00 (S.E± 0.00)	**0.01**	0.13	0.18	0.04	0.04	0.04	0.04	0.04	0.04	0.05	

Table S5.5 Intra- and interspecific distance calculations based on the pair wise analysis of the listed COI Intron sequences. Standard error estimates are in italic and were obtained by a bootstrap procedure (1000 replicates). Analyses were conducted using the Kimura 2-parameter method in MEGA4. Numbers in brackets behind species names indicate the number of sequences used in the analysis. Boxed values indicate lack of adequate inter-specific divergence for species delineations. There were a total of 590 positions in the final dataset.

			Interspecific Distances													
	Species	**Intraspecific Distances**	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*
1	*Aiptasia sp.*	na		*0,023*	*0,017*	*0,029*	*0,026*	*0,031*	*0,016*	*0,028*	*0,028*	*0,016*	*0,026*	*0,031*	*0,031*	*0,029*
2	*Metridium senile (3)*	0,00 (S.E.±0,00)	0,239		*0,015*	*0,025*	*0,020*	*0,026*	*0,015*	*0,025*	*0,025*	*0,016*	*0,020*	*0,025*	*0,026*	*0,024*
3	*Sagartia elegans (1)*	na	0,157	0,117		*0,026*	*0,020*	*0,026*	*0,005*	*0,026*	*0,026*	*0,006*	*0,020*	*0,027*	*0,027*	*0,025*
4	*Anemonia viridis (1)*	na	0,336	0,274	0,282		*0,031*	*0,013*	*0,026*	*0,007*	*0,007*	*0,026*	*0,031*	*0,011*	*0,008*	*0,012*
5	*Calliactis parasitica (1)*	na	0,269	0,181	0,193	0,327		*0,029*	*0,020*	*0,031*	*0,031*	*0,019*	*0,000*	*0,030*	*0,031*	*0,028*
6	*Megalactis sp. (1)*	na	0,378	0,289	0,294	0,084	0,300		*0,027*	*0,011*	*0,011*	*0,026*	*0,029*	*0,010*	*0,013*	*0,007*
7	*Cereus pedunculatus*	na	0,157	0,115	0,019	0,282	0,194	0,294		*0,026*	*0,026*	*0,002*	*0,020*	*0,028*	*0,027*	*0,026*
8	*Actinia equina (1)*	na	0,338	0,282	0,290	0,028	0,335	0,066	0,284		*0,000*	*0,026*	*0,031*	*0,010*	*0,008*	*0,011*
9	*Actinia fragacea (1)*	na	0,338	0,282	0,290	0,028	0,335	0,066	0,284	**0,000**		*0,026*	*0,031*	*0,010*	*0,008*	*0,011*
10	*Actinothoe sphyrodeta (1)*	na	0,155	0,117	0,021	0,279	0,191	0,291	**0,002**	0,281	0,281		*0,019*	*0,028*	*0,027*	*0,025*
11	*Adamsia carciniopados (1)*	na	0,269	0,181	0,193	0,327	**0,000**	0,300	0,194	0,335	0,335	0,191		*0,030*	*0,031*	*0,028*
12	*Heteractis magnifica (63)*	0,00 (S.E.±0,00)	0,347	0,281	0,299	0,066	0,301	0,063	0,302	0,057	0,057	0,299	0,301		*0,012*	*0,012*
13	*Hetractis crispa (3)*	0,003 (S.E.±0,002)	0,377	0,293	0,304	0,038	0,335	0,087	0,307	0,040	0,040	0,304	0,335	0,081		*0,013*
14	*Entacmaea quadricolor (5)*	0,003 (S.E.±0,002)	0,364	0,287	0,286	0,086	0,301	0,033	0,286	0,068	0,068	0,283	0,301	0,076	0,094	

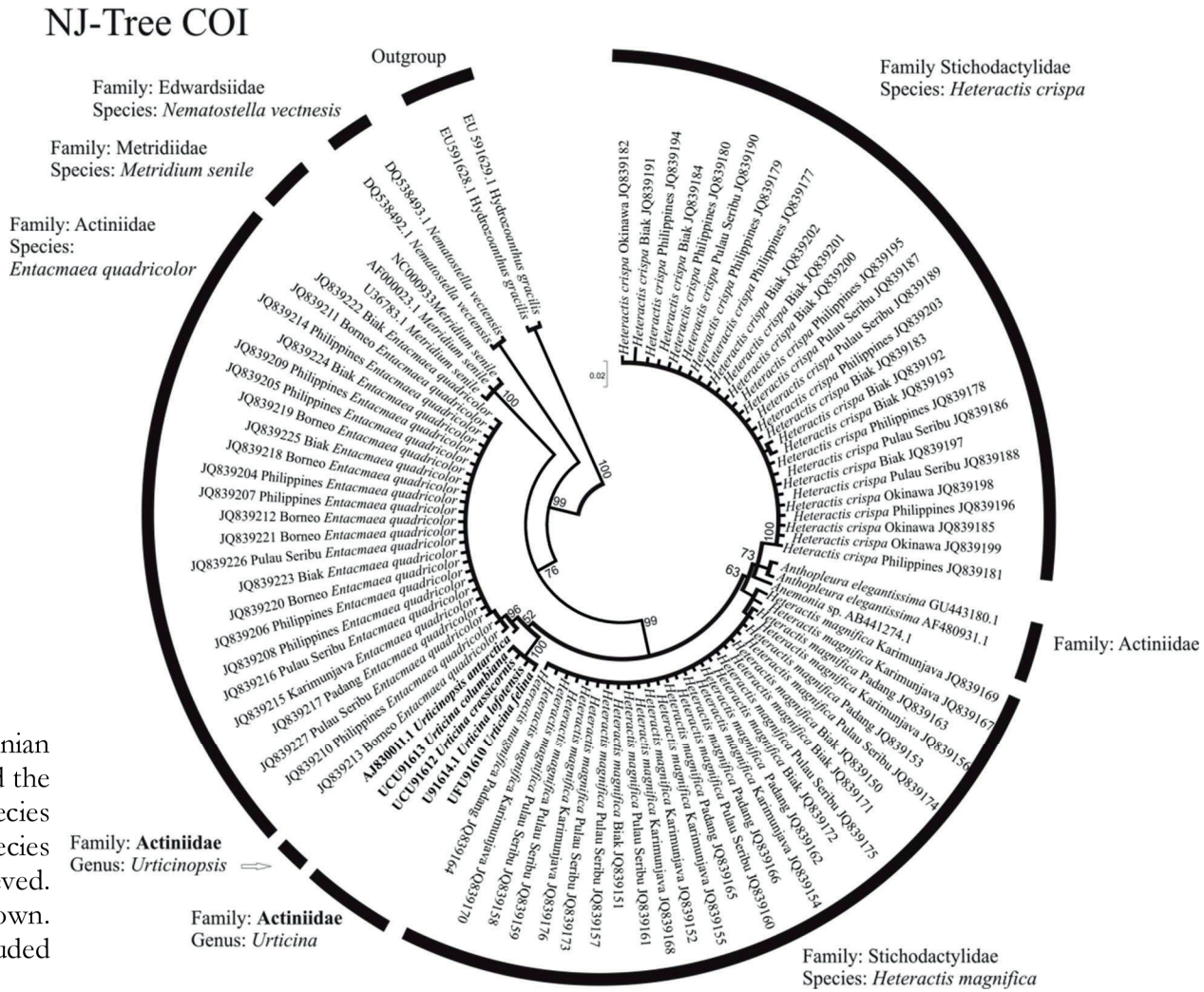


Figure S5.2 NJ-tree with all available actinian COI sequences, both from this study and the public databases (460 positions). Species marked in bold denote nodes where species level resolution could not be achieved. Bootstrap values below 50 are not shown. The Parazoanthus sequences were included as an outgroup.

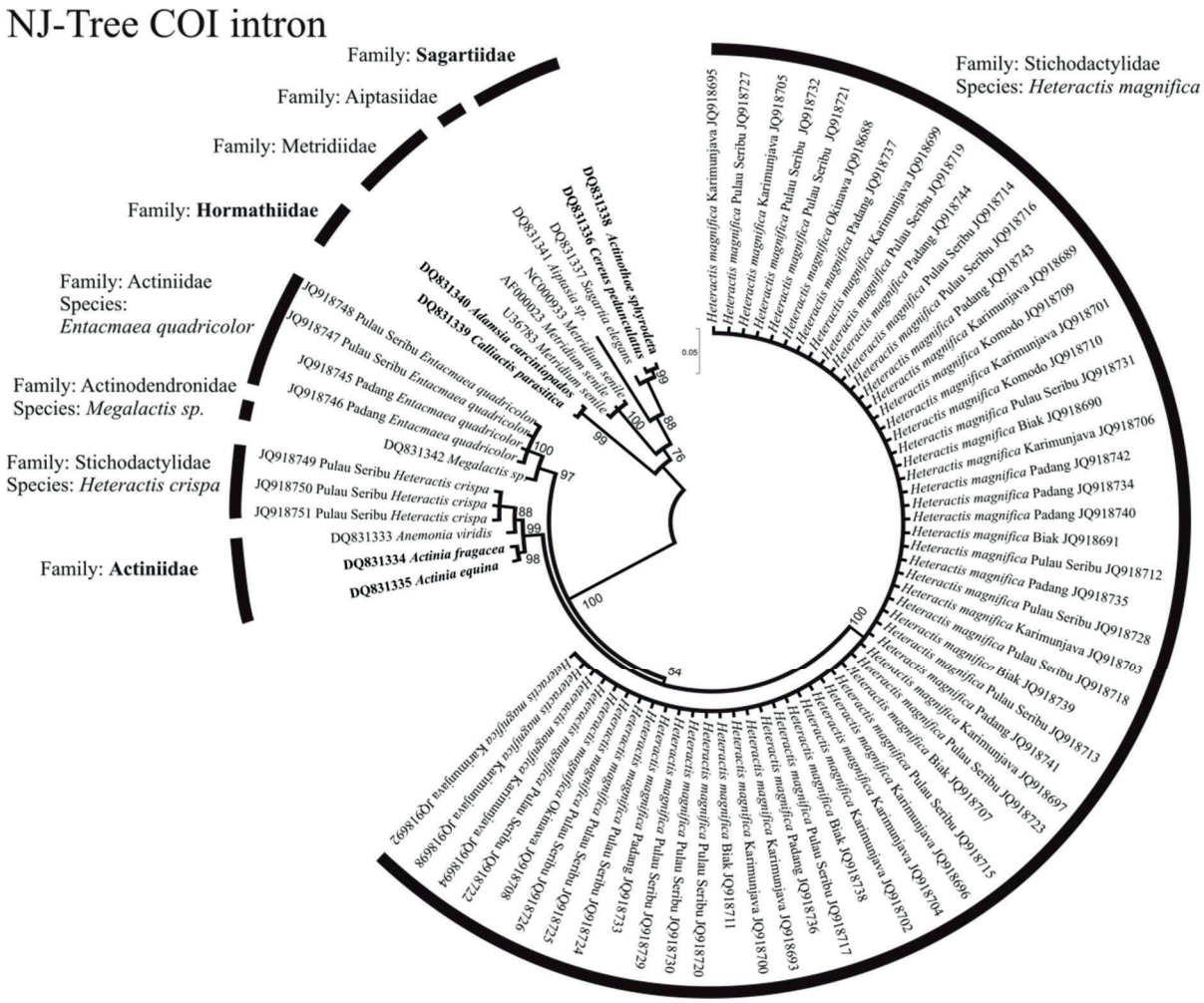


Figure S5.3 Neighbour-Joining tree with all available actinian COI Intron sequences, both from this study and the public databases (590 positions). Bootstrap values below 50 are not shown. Species marked in bold denote nodes where species level resolution could not be achieved.

Chapter IV

Development of polymorphic microsatellite loci for three species of giant tropical sea anemones (Cnidaria, Anthozoa, Actiniaria)

INTRODUCTION

Microsatellites (short tandem repeat units in the genomic DNA) were isolated for three species of giant tropical sea anemones (*Heteractis magnifica, Heteractis crispa, Entacmaea quadricolor*) and were to be used to investigate population structuring in the Indo-Malay Archipelago at three spatial scales and over time (see Levin 2006 for sampling design). As symbiont organisms to several obligate and semi-obligate fish and invertebrate species, these sea anemones fill an irreplaceable ecological niece that is impacted by their collection for the marine ornamentals trade. Microsatellites have shown to give the highest resolution in tracing population structuring for many species (Bossart and Prowell 1998). The planctonically dispersing planular larvae of these species are similar in form and motility to most larvae of reef building tropical corals. Assuming the passivity of these types of larvae, results indicating potential larval dispersal patterns for the area in sea anemones could also give indications for mechanisms acting on local reef building coral populations. The reproductive strategies of these species include cloning, binary fission, and sexual reproduction, presenting a challenge for the interpretation of population genetic statistics.

WORK SUMMARY

Following a protocol by Pinto *et al.* (1998) for zooxanthellae free DNA extraction from sea anemones, DNA from two sea anemone species, *Entacmaea quadricolor* and *Heteractis crispa,* was extracted and used in a procedure devised by Leese *et al.* (2008). The work on anemone microsatellite amplification was, however, halted, as it became apparent that the chance of finding amplifiable, polymorphic, single product loci, that did not also amplify in the symbiont algae, was very small, despite many efforts to overcome the obstacles that presented themselves. During the process of cutting a segment from the anemone foot muscle to produce DNA extracts for the construction of the microsatellite library, some zooxanthellae must have been transferred with the tissue. Even though the algae are said not to be present in areas where photosynthesis is not possible i.e. under the foot of the anemone, some algal cells must have been situated there. During the work process it became apparent hat many microsatellites from the library were also amplifiable in extracts from zooxanthellae cultures, provided by Wiebke Krämer from the Marine Botany Department at the University of Bremen.

Zooxanthellae cultures:

- CCMP2467- from Stylophora pistillata (Coral) red sea
- CCMP2433- from Pocillopora damicornis aus dem GBR (australia)
- CCMP2430- from Tridacna maxima aus GBR
- CCMP829 - from Tridacna crocea aus dem GBR
- Sym Tm and Sym Tm AW isolated from Tridacna maxima from the ZMT
- HI-0509 probably freeliving.

To avoid the publication of potential algal loci as anemone loci, all primers (Table 5.13 only fluorescently labeled primers) were tested on seven different zooxanthellae strains to check if product formed in the absence of anemone DNA. Most loci that had been selected so far were only amplifiable at low temperatures and high magnesium chloride concentrations, resulting in many zooxanthellae products at these conditions. However, each zooxanthellae culture produced a different product, making it impossible to predict which band would be amplified in the algae harboured in the animals studied here and emphasizing the large genetic differences assumed to exist between different clades of these algae. None of the extracts of algae were from sea anemones, amplifying the uncertainty when testing the primers. To remedy this problem several different strategies were pursued.

Density gradient centrifugation of macerated anemone tissue was used to try to separate zooxanthellae from the anemone tissue to obtain a sample of algal DNA: amplification with anemone specific primers showed that extracts were not pure. The next step was to isolate zooxanthellae from *Entacmaea quadricolor* and start cultures in an incubation chamber at optimal conditions. However, not all zooxanthellae strains can survive outside the host (as is the case with many strains harbored by corals) and was also the case with the algae isolated here. The zooxanthellae in all five cultures started dying off within a few days and did not recover even after several weeks of culture and treatment.

An alternative approach was to produce a completely zooxanthellae free anemone extract to check the primers. A live *Entacmaea quadricolor* was acquired and frozen to avoid zooxanthellae contamination during the cutting process, as must have occurred during the process leading to the construction of the microsatellite library. Small tissue segments were cut from various regions of the animal, always careful to avoid contamination with zooxanthellae that are assumed to only populate the outer membranes. Serial washings of the cut tissue prior to extraction were also performed to remove any freely floating algae cells. However, inspection of these tissue samples under the microscope always revealed the presence of some algal cells, irrespective of where the tissue was taken from (internal/external, foot/body). Subsequent extraction and amplification with zooxanthellae specific primers always showed amplification of the appropriate algal product.

Table 5.13 Listing and description of microsatellite loci currently under investigation. The total number of primer pairs that were tested is 31, but only those primer pairs yielding clear product bands on agarose gels were selected for fluorescence labeling.

Labeled Primer Name	Sequence	Motif Discription	Repetition motif	Description
FAM-MicroGA16_F	CCA ACC GTG GGT TAT TCA GT	GA_{16}	dimer	perfect
FAM-CT26micro_F	CGT AGT CTT TCT CCC CGA GT	CT_{26}	dimer	perfect
HEX-AG34micro_MH_F	CGG TTA CTA GCC TGA TGC AC	AG_3 AA AG_{30}	dimer	interrupted
FAM-CT34micro_F	GCG TAC CTT TCA CAT CTC CT	CT_4 TT CT_{31}	dimer	interrupted
HEX-CA34micro_F	CAT GCA ATC AGC ATC CG	CA_{16} TAGT CA_3 A CA_{17}	dimer	interrupted
FAM-GA26micro_F	GTT TCA CCT AAG AAG GGA TAT AGC	GA_{26}	dimer	perfect
HEX-MicroGTAT23_F	ACT CTA GCC GTC GTG TGG AT	GTAT GTTTAT $GTAT_{20}$	trimer	interrupted
HEX-MicroCTT27_F	TGC TTA GCT GGA ACA AAA GGA	CTT_{39} CTC CTT_5 CTC_2CTT_2	trimer	interrupted
FAM-MicroGT23G10_F	TGC AAA AGG CTA AAG GTT TTC	$GT_{23}G_{10}$	dimer-monomer	perfect combined
FAM-GTT28micro_R	CGC TAG TAT ATG ACT GGC TT	GTT_{28} $GTTTTT_2$	trimer	perfect
FAM-Micro152-15_F	CAC GGG AGA GCC AAT AAA AA	GTT	trimer	perfect
HEX-GAA48...GAA13micro_ F	GGA CGC CCT CTG GTG ATT G	GAA_{48}GCGAA$_{11}$ GAA_{13}	trimer-pentamer-trimer	combined
FAM-Micro 1+2 neu F	GGC GGC AAC CTA GTT TTG T	$CCCTAA_{15}$	hexamer	interrupted
FAM-MicroAG16GC8(2)_F	AGT CCA CGT CTT GTG GTG TG	$AG_{16}GC_8$	dimer-dimer	perfect combined
HEX-TD144-34_F_ MH	ACT TCT TAG CCA TCA ACC AGC	$GATA_7$ AATA GA_{26}	tetramer-dimer	combined
HEX-Micro152-67_F	TGC TTC TGC TCT CGA ACT CA	$GT_3GA_3GT_{14}$	dimer-dimer-dimer	interrupted

The method by Leese *et al.* (2008) employed here for the construction of the microsatellite library produced novel microsatellite motifs in other species for 98% of all sequenced inserts. In anemones, however, it was found that redundancies were very high, with up to 50% of returned sequences being identical. Therefore, sequencing of further plasmids without the ability to reliably check the source of the microsatellite did not seem feasible. Additional problems with multiple product amplification (see Fig. 5.10) slowed progress, since the fragment length analysis can only be carried out if the inadvertently amplified products lie outside of the allelic size range of the locus. Though primer design has a large influence on the amplification success and the specificity of the reaction, the fault is more likely to be found by the microsatellites themselves. It is suspected that some of the microsatellites are in fact satellites, so a chain of repetitions of the fragment in which the microsatellite is found. Therefore, primer binding sites are distributed throughout the satellite, giving rise to products of different lengths, that each contains a different number of repeats of the microsatellite fragment itself.

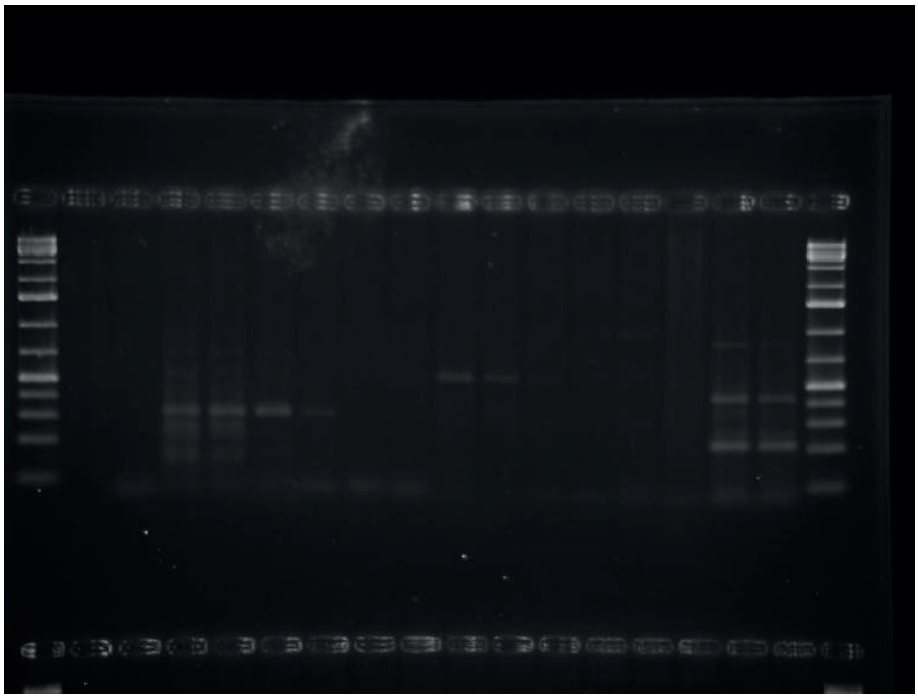


Figure 5.10 Gradient PCR for the amplification of microsatellite loci. Some Primers will amplify a single product, whereas most primers have amplified several products, despite large ranges of annealing temperature tested.

OUTLOOK

Without the ability to reliably check the source of microsatellites motifs, further efforts to test primers was not feasible. If a pure algal samples or a pure sea anemone sample from at least one of the species used here were available, work on the microsatellite library could continue. However, additional sequencing of plasmids would have to be carried out, since search for microsatellite motifs with suitable primers binding sites within the sequenced segment was exhaustive and new sequences would have to be generated. Most problematic is the presence of satellite DNA, producing long sequences containing repetitive motifs, interrupted by imperfect repetitive motifs of the same or a similar motif. The high frequency of repetitive sequences among runs also needs to be considered when applying the protocol by Leese *et al.* (2008) to these or other members of the Actiniaria.

REFERENCES

Bossart, J.L., and D.P Prowell. 1998. Genetic estimates of population structur and gene flow: limitations, lessons and new directions. TREE 13:202-206.

Leese, F., C. Mayer, and C. Held. 2008. Isolation of microsatellites from unknown genomes using known genomes as enrichment templates. Limnol. Oceanogr. Methods 6 :412-426.

Levin, L.A. 2006. Recent progress in understanding larval dispersal: new directions and digressions. Integr. Comp. Biol. 46:282-297.

Pinto, S,M, F.M.C. Fernandes-Matioli, and E. Schlenz. 2000. DNA extraction from sea anemone (Cnidaria: Actiniaria) tissues for molecular analyses. Genet. Mol. Biol. 23:601-604.

6.
Synoptic Discussion

6. Synoptic Discussion

Molecular methods have gained increasing application in efforts to understand the dynamics that impact and constrain marine populations and regional biodiversity, with special emphasis on biodiversity hotspots like the Indo-Malay. Generally large population sizes (Crandall *et al.* 2008) and logistical constraints in accessing the marine environment, highlight the practically of drawing inferences from molecular evidence for parameters which are difficult to measure or to observe directly. Directly measuring connectivity among marine populations would involve tracking larval fish cohorts in the huge expanse of water to determine where they go and at what frequency over time (Leis & Carson- Ewart 1997). The molecular approach measures how genetically distinct populations are under the assumption that population with little or no reproductive exchange will diverge over time, a process known as drift. Drift can also lead to the formation of new species, when two populations are isolated for long periods of time and are eventually so far diverged that reproductive exchange is no longer possible. This process of successive genetic population divergence is manifested within a species' genetic material and can be used to study population demographics, species histories and to understand species delineation and biodiversity in the sea.

The overall aim of this thesis was to develop, test and apply molecular tools to study different aspects of genetic and biological diversity of anemonefishes and their obligate symbiont sea anemone partners in the IMA and to add to the body of scientific evidence needed to support biodiversity conservation within the IMA. Specifically, the study was designed to increase our understanding of genetic variation found in anemonefish populations across the IMA through an interpretation of the structure found. By taking a comparative intergenomic (mitochondrial and nuclear) (Chapter I & II) and intrageneric (several species) (Chapter II) approach, the mechanisms shaping genetic diversity in natural

populations of anemonefishes were addressed and the variability inherent in the system was explored. In an effort to make results more accessible for application and implementation driven fields, multispecies geneflow barriers were identified and scaled (and thereby prioritized) using genetic evidence (Chapter II). To heed the close association between anemonefishes and their sea anemone partners, sequence variation in the Actiniaria and its implications for molecular species identification were explored (Chapter III) and the development of a set of polymorphic nuclear markers was attempted, to allow for population genetic and phylogeographic study of the host organisms (Chapter IV).

Benthic species reliant on larval dispersal for their exchange with proximate and distant populations (connectivity) are of special interest because genetic traces of population mixing can be ascribed exclusively to the movement of larvae among populations. This provides important information about the ability of populations to self seed or to act as larval sources for other regions, conveying a sense of local or regional population resilience to disturbance. With a burgeoning rural coastal population and an increasing number of anthropogenic and climate related disturbances, many regions within the IMA are marked by diminishing reef structures and (often unrecorded) loss of marine biodiversity (Brown 1997, Bruno & Selig 2007). Molecular methods can provide some of the information missing to achieve science based management of marine resources needed to protect and preserve the globally unmatched marine biodiversity in the region (von der Heyden et al. 2014).

Restrictions to geneflow in anemonefishes across the IMA

Convincing genetic evidence for strong restrictions to geneflow was presented here for three species of anemonefish (*A. perideraion*, *A. sandaracinos,* and *A. clarkii*) in much of their ranges (Chapter I & II). These results are supported by what has been shown for *A. ocellaris* (Timm & Kochzius 2008, Timm *et al.* 2012) and has been postulated for benthic breeders in general (Riginos *et al.* 2011). The intergenomic (mitochondrial and nuclear genomes sampled) and

intrageneric (multispecies congeneric sampling) sampling approach taken here further strengthened the drawn conclusions, since substantial concordance among the detected patterns of regional subpopulation structure could be shown (Chapter II). Populations of anemonefishes are characterized by extensive regional substructure across the IMA, with a pronounced divergence of populations in the Java Sea and on the eastern coast of New Guinea. All four anemonefishes followed this pattern (where data was available), although connectivity between eastern and western New Guinea subpopulations was species specific, emphasizing the need to study the diversity of patterns in highly similar species and to use markers which allow for a direct comparison of the results, like the mitochondrial control region applied here.

Diversity gradients and the multispecies approach

Given the shared biology and very similar life history of the fish species studied here, the results from the single species studies of *A. sandaracinos* and *A. perideraion* are not fully surprising. However, differences in the extent of the population structure and diverging results for regional diversity gradients among the fishes, in addition to conflicting results in other studies focusing on highly similar congenerics (Barber *et al.* 2011; DiBattista *et al.* 2012) highlight the need to further explore this diversity. Diversity indices can potentially alert marine resource managers to vulnerable populations or regions, which rely predominantly on self seeding, leading to an eventual decrease in genetic diversity. Under strong fishing pressure these populations may be slow to recover from exploitation, lacking exchange with other larval sources. Chapter II could show that nucleotide diversity was generally lower in northern, than in southern populations, indicating that there is a need to further explore the source/sink dynamics in the north of the Archipelago. Findings of a shared geneflow barrier across the Celebes Sea by three of the anemonefishes, further supported by significant pairwise distances (Chapter I) and evidence for reduced genetic exchange at the Philippine site for *A. perideraion,*

all add to the body evidence suggesting reduced connectivity of the Philippine Archipelago with regions further south. This is an important finding as exploitation of anemonefishes and their hosts for the marine ornamental trade is suggested to be at unsustainable levels there (Shuman *et al.* 2005). Reduced genetic diversity of *A. perideraion* in Karimunjava and the failure to find any fish of this species in more western locations may also indicate a vulnerable population status (Chapter I). Since results are drawn from a small population sample, other methods (e.g. underwater survey) could be applied to confirm or refute these suspicions. This is a good example of how genetic data can provide information to help focus additional research efforts on regions or populations needing additional attention, for an optimal use of the resources available for research.

Regions with a large number of endemics or high levels of genetic diversity should be given special attention because these areas may be most vulnerable to change. The coexistence of high species numbers, as is common in coral reefs, always entails a heterogeneity and complexity of habitat to provide adequate niche space and number. The more species share a given area of seascape the more likely extinction become, because niche space is narrow, so that small shifts in habitat composition or environmental parameters can eliminate vital habitat and species may not subsist under direct competition (Munday 2002, 2004). New Guinea has been implicated as a region of high diversity (Allen 2008), which is supported by results from this research. With the exception of *A. ocellaris*, all other anemonefish species studied there have high nucleotide diversities in haplotypes from the Guinean coast. The *A. sandaracinos* population sample from East New Guinea contained highly divergent haplotypes, which were genetically more similar to a sample from the Solomon Islands than to other population samples from the Archipelago. Similar findings were presented by Timm *et al.* (2008) for *A. percula*, where a highly divergent haplotype (82bp) was found in East New Guinea, which clustered with the Solomon Islands and New Britain in a phylogeny. The degree of divergence

found in A. *sandaracinos* and *A. percula* is similar to what is found to delineate anemonefish species with this marker. East New Guinea may well present a region of overlap between highly divergent gene pools, thereby contributing to the high diversity found in the IMA.

Imprint of Host Specialization and PLD on Population Structure

There is an active and ongoing debate about the impact of different life history and physiological traits on the realized dispersal of pelagic larvae (Bradbury et al. 2008, Kelly & Palumbi 2010, Selkoe & Toonen 2011, Faurby & Barber 2012, Riginos *et al.* 2014). The four species studied here differ in their host specialization and the length of their PLD, which allowed a tentative look at the resultant relationship to the genetic structure found in each species. Despite the inability to demonstrate a causal relationship with the present data, it can, however, be said, that the ability of the host generalist *A. clarkii* to recruit to far more hosts than its congeners did not reduce population structure, but rather increased far beyond that found in its more specialized congeners. *A. clarkii* is also the fish with the shortest projected PLD, which could provide an explanation for the lack of imprint of host specialization. It has been suggested that host specialist anemonefishes, like *A. sandaracinos*, compensate this restriction by tolerating a larger range of environmental gradients, whereas host specialists, such as *A. clarkii*, have restrictive tolerance levels. This may hold true for regions where vertical or horizontal stratification of anemonefish assemblages were found, but this is not always the case. Where data was available, an increasing PLD was associated with decreasing levels of genetic structure. As yet, the PLD of anemonefishes may be a good predictor for the relative extent of population structure to be expected, but this relationship needs to be statistically explored and PLD data for *A. sandaracinos* acquired.

Chapters I and II present strong additional evidence for the restricted dispersal ability of anemonefish larvae, identifying multispecies geneflow barriers and diversity gradients which can be used for conservation purposes. While a multispecies approach is valuable in meeting resource management needs (to find a 'common denominator'), single species studies are valuable in providing a higher resolution of the detected structure and identifying areas of conservation priority or additional research needs. Both goals were met by this study, adding to the knowledge base needed to assess, protect and manage these and other benthic guarding reef fishes under the threat of changing climate regimes, reef demise and projected increase in fishing pressure in Indonesia waters and other Asian nations.

Molecular species identification

Chapter III of this study presented research towards the molecular species identification of sea anemones by testing different markers with potential to delineate species. The classical COI barcoding marker has been shown to fail to delineate members of the Anthozoa (corals, sea anemones and their kin). However, results were generalized for the whole of the group without further evidence from members of the Actiniaria (Cnidaria, Anthozoa, Actiniaria) which comprises 1040 species from all aquatic environments. While most of the analyses in this research (Chapters I, II, and III) rely on the theorem that genetically isolated populations will diverge over time, this mechanism appears to be controlled by other dynamics in members of the Anthozoa. This can result in identical sequences being found in species from different families, questioning the ability to study sea anemones with this type of approach (Shearer *et al.* 2002, 2008). However, research evaluating COI or other molecular markers for species identification in sea anemones is scarce and it was therefore explored here.

The analysis of the COI fragment in Actiniaria confirmed low interspecific genetic divergence limiting the ability of conspecific to be delineated. Overall, 16% of all interspecific comparisons showed no or only minimal divergence. This confirms for the Actiniaria what has been shown for other Anthozoan orders (Shearer *et al.* 2002) and has been suggested for all lower metazoans (Schröder et al 2003; Wörheide 2006; Erpenbeck *et al.* 2005). The dataset for the study was very small due to the low number of Actiniarian sequences in the database. However, the conclusion about the unsuitability of COI in Actiniarian barcoding stands firm, because additional data cannot remedy the lack of interspecific divergence. Findings of slow mitochondrial sequence evolution presented here further supported the conclusion that the slow rate describes the condition of the ancestral metazoans, rather than being a secondarily acquired feature (Huang et al. 2008, Schearer et al. 2002).

Testing the Homing Endonuclease Gene (HEG) located within a self-splicing COI intron (Goddard & Burt 1999; Goddard et al. 2006) for its barcoding application produced a slightly higher intraspecific variability, but maintained the lack of interspecific divergence needed for a clear barcoding gap. Again interspecific divergence was too low and overlapped with intraspecific variability. The phylogenetic signal derived from this marker does not describe relationships among anemones, but possible infection pathways of this HEG in Actiniaria.

The ITS II marker was similarly limited by interspecific divergence, though failure to delineate within or among different families was not fully identical to the two previous markers. Sequence alignments contain large gaps, presenting a challenge when aligning sequences from distantly related species and would discourage the use of this marker for automated sequence similarity analysis, like it has been implemented for COI.

In conclusion, all three markers tested could not reliably delineate species or genera in all parts of the phylogenies, showing that these markers can not be expected to reliably detect

new species or identify known ones based on sequence similarity alone. Analysis with COI and the COI Intron show limitations through low interspecific divergence and not high intraspecific variability. Therefore, additional data cannot remedy problems with the resolution of these markers. The goals of barcoding could not be met and the search for more suitable markers continues, though the possibility of concatenating sequence information from two or more of the markers studied here, may prove to be a useful approach. Little overlap in species specific sequence information between markers does did not allow such a step at this point, but could be considered in future research. Concepcion *et al.* (2008) proposed the use of a short nuclear intronic region encoding the 54 kDa subunit of the signal recognition particle because it was found to resolve relationships between closely related coral species. Currently, only one sea anemone sequence (*Nematostella vectnesis*) is available, so its use in the Actiniaria as a barcoding marker needs to be assessed in future research. Preliminary efforts to amplify this marker in *H. crispa*, *H. magnifica,* and *Entacmaea quadricolor* with universal primers proposd by Jarman *et al.* (2002), yielded marker unspecific product, which appeared to be of bacterial origin when submitted to a blast search.

Microsatellite development in sea anemones

The development of a set of polymorphic microsatellite loci for population genetic and phylogeographic analysis of sea anemones was attempted in this research. Unfortunately, this research objective could not be realized and the work was halted. Much effort went into working around the numerous problems arising from the presence of dinoflagellate endosymbiont DNA in anemone tissue and DNA extracts. Others, who have been successful in developing microsatellite loci for corals, where able to capture larvae prior to endosymbionts acquisition or reportet microsatellites fom the holobiont (Concepcion *et al.* 2010). Endosymbiont transfer in sea anemones takes place prior to the release of eggs,

as is also the case in many coral species, eliminating the possibility of acquiring symbiont free organisms. Chapter IV summarizes the steps that were taken to ensure inadvertent inclusion and amplification of endosymbionts DNA and the testing of microsatellite loci amplification and characterization before the work was halted to pursue other research objectives (Chapter I, II, III).

REFERENCES

Allen, G. R. 2008. Conservation hotspots of biodiversity and endemism for Indo-Pacific coral reef fishes. Aquatic Conserv: Mar. Freshw. Ecosyst., 18: 541-556

Bradbury, I.R., B. Laurel, P.V.R. Snelgrove, P. Bentzen, and S.E. Campana. 2008. Global patterns in marine dispersal estimates: the influence of geography, taxonomic category and life history. Proc. R. Soc. Lond. 275:1803-1809.

Brown, B. E. 1997. Coral bleaching: causes and consequences. Coral Reefs 16:129-138.

Bruno, J.F., and E.R. Selig. 2007. Regional decline of coral cover in the Indo-Pacific: timing, extent, and subregional comparisons. PLoS One 2:e711.

Concepcion, G.T., N.T. Polato, I.B. Baums, and R.J. Toonen. Development of microsatellite markers from four Hawaiian corals: *Acropora cytherea*, *Fungia scutaria*, *Montipora capitata* and *Porites lobata.* Cons. Genet. Res. 2:11-15.

Crandall, E.D., M.A. Frey, R.K. Grosberg, and P.H. Barber. 2008. Contrasting demographic history and phylogeographical patterns in two Indo-Pacific gastropods. Mol. Ecol. 17:611-626.

Erpenbeck, D., J.N.A. Hooper, and G. Wörheide. 2005. CO1 phylogenies in diploblasts and the 'Barcoding of Life' - are we sequencing a suboptimal partition? Mol. Ecol. Notes 6:550–553.

Faurby, S., and P.H. Barber. 2012. Theoretical limits to the correlation between pelagic larval duration and population genetic structure. Mol. Ecol. 21:3419-3432.

Gaither, M.R., B.W. Bowen, T.-R. Bordenave, L.A. Rocha, S.J. Newman, J.A. Gomez, et al. 2011. Phylogeography of the reef fish Cephalopholis argus (Epinephelidae) indicates Pleistocene isolation across the Indo-Pacific Barrier with contemporary overlap in The Coral Triangle. BMC Evol. Biol. 11:189.

Hellberg, M.E. 2009. Gene Flow and Isolation among Populations of Marine Animals. Annu. Rev. Ecol. Evol. S. 40:291-310.

Huang, D., R. Meier, P.A. Todd, M.C. Loke. 2008. Slow Mitochondrial COI Sequence Evolution at the Base of the Metazoan Tree and Its Implications for DNA Barcoding. J. Mol. Evol. 66:167-174.

Jarman, S.N. R.D. Ward, and N.G. Elliot. 2002. Oliegonucleotide Primers for the Amplification of Coelomate Introns. Mar. Biotechnol 4:347-355.

Kelly, R.P. and S.R. Palumbi. 2010. Genetic Structure Among 50 Species of the Northeastern Pacific Rocky Intertidal Community. PLoS One 5: e8594.

Leis, J.M. and B.M. Carson-Ewart. 1997. In situ swimming speeds of the late larvae of some Indo-Pacific coral reef fishes. Mar. Ecol. Prog. Ser. 159 :165-174.

Munday, P.L. 2002. Does habitat availability determine geographical-scale abundances of coral-dwelling fishes? Coral Reefs 21:105-116.

Munday, P.L. 2004. Habitat loss, resource specialization, and extinction on coral reefs. Glob. Change Biol. 10: 1642-1647.

Riginos, C., Y. M. Buckley, S. P. Blomberg, and E.A. Treml. 2014. Dispersal Capacity Predicts Both Population Genetic Structure and Species Richness in Reef Fishes. The American Naturalist 184:52-64.

Riginos, C., K.E. Douglas, Y. Jin, D.F. Shanahan, and E.A. Treml. 2011. Effects of geography and life history traits on genetic differentiation in benthic marine fishes. Ecography 34:566-575.

Schröder, H.C., S.M. Efremova, V.B. Itskovich. 2003. Molecular phylogeny of the freshwater sponges in Lake Baikal. J Zool. Syst. Evol. Res. 41:80–86.

Selkoe, K.A., and R.J. Toonen. 2011. Marine connectivity: an new look at pelagic larval duration and genetic metrics of dispersal. Mar. Ecol. Prog. Ser. 436: 291-305.

Shearer, T.L., and M.A. Coffroth. 2008. Barcoding corals: limited by interspecific divergence, not intraspecific variation. Mol. Ecol. Resour. 8:247–255.

Shearer, T.L., M.J.H. Van Oppen, S.L. Romano, and G. Wörheide. 2002. Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). Mol. Ecol. 11:2475–2487.

Shuman, C., Hodgson, G., Ambrose, R., 2005. Population impacts of collecting sea anemones and anemone fish for the marine aquarium trade in the Philippines. Coral Reefs 24:564-573.

Timm, J., Figiel, M., and M. Kochzius. 2008. Contrasting patterns in species boundaries and evolution of anemonefishes (Amphiprioninae, Pomacentridae) in the centre of marine biodiversity. Mol. Phylogenet. Evol. 49:268-276.

Timm, J., S. Planes, and M. Kochzius. 2012. High similarity of genetic population structure in the false clown anemonefish (Amphiprion ocellaris) found in microsatellite and mitochondrial control region analysis. Conserv. Genet. 13:693–706.

von der Heyden, S., M. Beger, R.J. Toonen, L. van Herwerden, M.A. Juinio-Meñez, R. Ravago-Gotanco, et al. 2014. The application of genetics to marine management and conservation: examples from the Indo-Pacific. Bull. Mar. Sci. 90:123-158.

Wörheide, G. 2006. Low variation in partial cytochrome oxidase subunit I (COI) mitochondrial sequences in the coralline demosponge Astrosclera willeyana across the Indo-Pacific. Mar. Biol. 148:907–912.

ACKNOWLEDGEMENTS

I would like to thank Prof. Dr. Marc Kochzius for giving me the opportunity to write this thesis and for being my thesis advisor. The research for this thesis saw more setbacks than successes, but you still tried to provide whatever funding and logistical support was necessary for me to explore new avenues and come up with this thesis in the end. Thank you for your support.

Thank you to Prof. Dr. Blohm for hosting me at his lab and providing the best work environment possible under the circumstances. I know you had to do a lot of juggling after the formal dissolution of the Work Group Biotechnology and Molecular Genetics to accommodate the remaining students and I am grateful for your efforts and your loyalty to us.

I am also grateful to Christoph Held for letting me do the microsatellite isolation at his lab and encouraging me to join their discussion rounds. Without a Semester-ticket Bremerhaven ended up being too far, but I still regret I did not have more opportunity to learn from you.

I hope Dr. Janne Timm already knows how grateful I am to her for the endless support, encouragement and friendship through the years and her enduring enthusiasm for this research. You never grew tired of discussing the thesis work or consoling me when the work did not work. I also want to thank you for all the manuscript revisions and helpful discussions. Absolutely couldn't have done it without you. Thank you.

A huge thank you goes to Dr. Wiebke Krämer, who basically showed me the ropes in the lab while she was working on her Diploma Thesis. I would have been totally lost without you and your skills, being such a rooky when I first arrived. You are also such a dear friend to me and I will always miss our time together at the UFT and here in Bremen.

There are so many little and big things I want to thank Reinhard Zelm for, I will not list them here. I am most grateful for your willingness to help with all matters, from simple computer problems to trying any weird new protocol with me that I showed up with. Your natural curiosity and optimism was always contagious and made life at UFT much more fun. Sorry, that our various reward systems to encourage writing of our thesis texts, worked so badly, it could have saved us some years. Then again, we would have had less time together, so all is well.

I would also like to thank Min Hui for the great time together at our lab and for being a good friend to me. You were fun to work with and I hope you will come back to Europe in the future so we can meet again.

My friends and family have been the greatest support for me in completing this thesis and I hope they will eventually learn to forgive me for dragging it out for so long.

Triedel und Michael: ich werde das Doktorandenasyl sehr vermissen! Vielen Dank

I would like to thank my mother, Dagmar zu Dohna, for helping me with absolutely everything whenever things got tight and spending so much time with Phillip and Laura

when I could not, thank you so much. This thesis could not have been finished without you and you loving support!

My father, Dr. Rudolf Wagner, spent a lot of time and effort with proofing my texts and with creating optimal conditions for 'closure' on the thesis. Apart from all the work I got done while staying with you, I also enjoyed the time we got to spend together. A rare treat for a grown daughter, but much enjoyed and treasured. Cathy, cannot thank you enough for always encouraging me and showing your love in so many ways. Thank you both very much.

To Gerold, Phillip and Laura I owe both apologies and my deepest gratitude for letting me finish this thesis with all possible support from you and for following me down this rocky path. Your belief in me was my driving force and my daily happiness. Thank you for being there for me through it all. Thank you with all my heart.

APPENDED MICROSATELLITE LIBRARY

Isolation source: H. crispa
Length variation: yes
Number of sequences obtained: 4
Primers:
>TD152-41
ACAAAAAAGCCCATAAAAATAGTAAATGTTCGTCTCGGAGTAATACAATTTGATGCACATACATCAGAAAGACTATCTA
GAGCACCATTTGAGTATAAGAAGTGAAAACAGCCGGGAGGTAGGAAAGTGTAATATGGAGTCTCAGCGCGTTCGATT
ACTTACTCGAGCCTAAAGCGTTCGTTTACTTGATAAGGTCGCAAGTCGCGCTTACTCGAGACTAACATATGATAGTGAA
TACATAATAATGCAAATATGGATGAAGGCCCGCTCTGTGCTATTTTTATTTCTTGTTTATTGATGTTTTATTCCATGAAA
AGCGTGATGTCCTTGCCCCCCCCCC~~~~~ACACACACACACAC~~TTTTAGCAGTGGTACGTATCCTACGCGCCT

>'HC142_F+R_micro' (1,541)
ACAAAAAAGCCCATAAAAATAATAAATGTTCGTCTCGGAGTAATACGATTTGATGCACATACACCAGAAAGACTATCTA
GAGCACCATTTGAGTATAAGAAGTGAAAACAGCCGGGAGGTAGGAAAGTGTAATATGGAGTCTCAGCGCGTTCGATT
ACTTACTCGAGCCTAAAGCGTTCGTTTACTTGATAAGGTCGCAAGTCGCGCTTACTCGAGACTAACATATGATAGTGAA
TACGTAATAATGCAAATATGGATGAAGGCCCGCTCTGTGCTATTTTTATTTCTTGTTTATTGATGTTTTATTCCATGAAA
AGCGTGATGTCCTTGCCCCCCCCCCCC~~~ACACACACACACAC~~TTTTAGCAGTGGTACGTATCCTACGCGCC

>'HC112_F+R_micro' (1,567)
ACATAAAAGCCCATAAAAATAACAAATGTTCGTCTCGGAGTAATACAATTTGATGCACATACATCAGAAAGACTATCTA
GAGCACCATTTGAGTATAAGAAGTGAAAACAGCCGGGAGGTAGGAAAGTGTAATATGGAGTCTCAGCGCGTTCGATT
ACTTACTCGAGCCTAAAGCGTTCGTTTACTTGATAAGGTCGCAAGTCGCGCTTACTCGAGACTAACATATGATAGTGAA
TACATAATAATGCAACTATGGATGAAGGCCCGCTCTGTGCTATTTTTATTTCTTGTTTATTGATGTTTTATTCCATGAAA
AGCGTGATGTCCTTGCCCCCCCCCCCCCC~ACACACACACACACACTTTTAGCCGTGGTACGTATCCTACGCGCT

>'HC131_R_micro' (1,599)
ACAAAAAAGCCCATAAAAATAATAAATGTTCGTCTCGGAGTAATACAATTTGATGCACATACATCAGAAAGACTATCTA
GAGCACCATTTGAGTATAAGAAGTGAAAACAACCGGGAGGTAGGAAAGTGTAATATGGGGTCTCAGCGCGTTCGATT
ACTTACTCGAGCCTAAAGCGTTCGTTTACTTGATAAGGTCGCAAGTCGCGCTTACTCGAGACTAACATATGATAGTGAA
TACATAATAATGCAACTATGGATGAAGGCCCGCTCTGTGCTATTTTTATTTCTTGTTTATTGATGTTTTATTCCATGAAA
AGCGTGATGTCCTTGCCCCCCCCCCCCCCCCACACACACACACACACATTTTTAGCAGGGGTACTTATCCAACCCCC

Isolation source: H. crispa and ?
Number of sequences obtained: 4
Length variation: yes
Primers:
>Fertig (AG16CG8 micro) 'TD144-18_F+R_micro' (1,564)
TACATTTCATCTTCAAATCCAGCTTGGCTTTACAGTGGAAACGCGCGCGCGCGCGCG~~~~CTCTCTCTCTCTCTCTCTC
TCTCTCTCTCTCTGGTCTATCCATCGTATCTGTCTGGTTTGCGAACTAGTTATAAAGTTATAATATTGTCGATGTTGATG
ACTATTATAATGATGCTGAGTTAGTACGTTGCTCCCCCCCTAACAGCCATAAACACCAATACAATAACTACCCACACCAC
AAGACGTGGACTAATGCCTGTTGTGCCGAACAGACGTCAAACTCTATTTCAACATCAGCATCGTCATCTGGTTTCTAAA
CGTTTTATTGTTCACAAATTGATCATGTCATCGTTAGTGTGCATACCGAATAATTCTGCACATGTATAAACACCCGTGC
>Fertig (AG16CG8 micro) Micro 06 (1,621)
CTACATTTCACTCTTCAAATCCAGCTTGGCTTTACAGTGGAAACGCGCGCGCGCGCGCG~~~~CTCTCTCTCTCTCTCTC
TCTCTCTCTCTCTStGGTCTATCCATCGtATCTGTSTGGTKTGCGAACTAKWTATAAAGWTATAATAKTGTCGATGTTGAT
GACTATTATARTGATGCTGAGTTAGTACGTTGCTCCCCCCCTAACAGCCATAAACACCAATACAATAACTACCCACACCA
CAAGACGTGGACTAATGCCTGTTGTGCCGAACAGDCGTCAAACTCTATTTCAACATCAGCATCGTCATCTGGTTTCTAA
ACGTTTTATTGTTCACAAATTGATCATGTCATCGTTAGTGTGCATACCGAATAATTCTGCACATGTATAAACACCCGTGC
>Fertig (AG16CG8 micro) 'TD_144-27_F+R_micro' (1,563)
TACATTTCATTTTCAAATCCAGCTTGGCTTTACAGTGGAAACGCGCGCGCGCGCGCG~~CTCTCTCTCTCTCTCTCTC
TCTCTCTCTCTCTGGTCTATCCATCGTATCTGTCTGGTTTGCGAACTAGTTATAAAGTTATAATATTGTCGATGTTGATG
ACTATTATAATGATGCTGAGTTAGTACGTTGCTCCCCCCCTAACAGCCATAAACACCAATACAATAACTACCCACACCAC
AAGACGTGGACTAGTGCCTGTTGTGCCGAACAGACGTCAAACTCTATTTCAACATCAGCATCGTCATCTGGTTTCTAAA
CGTTTTATTGTTCACAAATTGATCATGTCATCGTTAGTGTGCATACCGAATAATTCTGCACATGTATAAACACCCGTGC
>'HC108_F&R_micro' (1,552)
ACTACATTTCATTTTCAAATCCAGCTTGGCTTTACAGTGGAAACGCGCGTGCGCGCG~~CTCTCTCTCTCTCTCTCTC
TCTCTCTCTCTCTCTGGTCTATCCATCGTATCTGTCTGGTTTGCGAACTAGTTATAAAGTTATAATATTGTCGATGTTGA
TGACTATTATAATGATGCTGAGTTAGTACGTTGCTCCCCCCCTAACAGCCATAAACACCAATACAATAACTACCCACACC
ACAAGACGTGGACTAATGCCTGTTGTGCCGAACAGACGTCAAACTCTATTTCGACATCAGCATCGTCATCTGGTTTCTA
AACGTTTTATTGTTCACAAATTGATCATGTCATCGTTAGTGTGCATACCGAATAATTCTGCACATGTATAAACACCCGTG
C

Isolation source: ?
Number of sequences obtained: 1
Length variation: na
Primers:
>TD152-25
ACTTACAATACCCACAAACACACCACACACACACATACACACACACACACACACACACACACACACACACACACACACA
CACACACACACACACACACACACACACACACTCAATGTGTTACAGTGGGAGAGGGCTCTCCTGAAGTAGGCGCCGATGGTGG
TATCTAGGTATTCTCTGGGGTCACTCACTTTGTCCGTTCAGGCAGATCCACATATTTGATGGTTTCACATACATCCTGAT
GTTGATGCTGTCGGTATCTTTCTTGAGTAGGCCGTTCTCCT

Isolation source: ?

Number of sequences obtained: 1
Length variation: na
Primers:
>Fertig TD152-15 (micro 152-15)
CATAAATCGATTCATTTCAATATCATCAGAACAGGAAGAAAGTCTACGTAAATGCGATCTGATGACGAAAAACAACAAC
AAAAAGACACAGACAACAACAACAACAACAAAAACAACAACAACAACAACAACAACAACAACAACAACGACAACAA
CAACAACAACATCAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACA
ACAACAACAACAGCGACAACAACAACAGCAACAACAACAATAATAATAATTAGAAACCCAATGGCGAGTTTTTATTGGC
TCTCCCGTGTACTGACCTCACCCCCC

Isolation source: *H. crispa and E. quadricolor*
Number of sequences obtained: 6
Length variation: no
Primers:
>Fertig (micro neu 1+2) 'TD144-30_F+R_micro' (1,550)
~AACGAAACCAGACTTATCTCTGGACCTGCGCCAGAACTTTCGGGGGCGGCAACCTAGTTTTGTGCCCCCGTTCCGTAAA
CCTAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACCCTAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACC
CTAACCCTAACCCTAATAACCCTAACCAATTTACCATGTGACCGAGTTACTGTGACCAAATTTCCGGTGACCAAATTTCC
TGTGACCTAATTACCGCGCCCCAATTTTCTATGTGGAAAAGCGCATTTTGATCCATTTTGTCGGCACGAACCCAAGAAT
TTCTTGTTGTTGGTAAGTGTTTCCAC
>TD152-27
AAACGAAACCAGACTTATCTCTGAACCTGCGCCAAAACTTTCGGGGCGGCAACCTAGTTTTGTGCCCCCGTCCCGTAAA
CCTAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACC
CTAACCCTAACCCTAATAACCCTAACCAATTTACCATGTGACCGAGTTACTGTGACCAAATTTCCGGTGACCAAATTTCC
TGTGACCTAATTACCGCGCCCCAATTTTCTATGTGGAAAAGCGCATTTTGATCCATTTTGTCGGCACGAACCCAAGAAT
TTCTTGTTGCTGGTAAGTGTTTCCAC
>TD152-39
AAACGAAACCAGACTTATCTCTGAACCTGCGCCAGAACTTTCGGGGCGGCAACCTAGTTTTGTGCCCCCGTCCCGTAAA
CCTAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACC
CTAACCCTAACCCTAATAACCCTAACCAATTTACCATGTGACCGAATTACTGTGACCAAATTTCCGGTGACCAAATTTCC
TGTGACCTAATTACCGCGCCCCAATTTTCTATGTGGAAAAGCGCATTTTGATCCATTTTGTCGGCACGAACCCAAGAAT
TTCTTGTTGTTGGTAAGTGTTTCCAC
>TD152-26
AAACGAAACCAGACTTATCTCTGAACCTGCGCCAAAACTTTCGGGGCGGCAACCTAGTTTTGTGCCCCCGTCCCGTAAA
CCTAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACC
CTAACCCTAACCCTAATAACCCTAACCAATTTACCATGTGACCGAGTTACTGTGACCAAATTTCCGGTGACCAAATTTCC
TGTGACCTAATTACCGCGCCCCAATTTTCTATGTGGAAAAGCGCATTTTGATCCATTTTGTCGGCACGAACCCAAGAAT
TTCTTGTTGCTGGTAAGTGTTTCCAC
>TD152-58
AAACGAAACCAGACTTATCTCTGAACCTGCGCCAGAACTTTCGGGGCGGTAACCTAGTTTTGTGCCCCCGTCCCGTAAA
CCTAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACC
CTAACCCTAACCCTAATAACCCTAACCAATTTACCATGTGACCGAATTACTGTGACCAAATTTCCGGTGACCAAATTTCC
TGTGACCTAATTACCGCGCCCCAATTTTCTATGTGGAAAAGCGCATTTTGATCCATTTTGTCGGCACGAACCCAAGAAT
TTCTTGTTGTTGGTAAGTGTTTCCAC
>TD152-05
~AACGAAACCAGACTTATCTCTGAACCTGCGCCAGAACTTTCGGGGCGGCAACCTAGTTTTGTGCCCCCGTCCCGTAAA
CCTAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACC
CTAACCCTAACCCTAATAACCCTAACCAATTTACCATGTGACCGAATTACTGTGACCAAATTTCCGGTGACCAAATTTCC
TGTGACCTAATTACCGCGCCCCAATTTTCTATGTGGAAAAGCGCATTTTGATCCATTTTGTCGGCACGAACCCAAGAAT
TTCTTGTTGTTAGTAAGTGTTTCCAC

Isolation source: *E. quadricolor and ?*
Number of sequences obtained: 2
Length variation: no
Primers:
>'TD144-21_F+R_micro' (1,621)
ACACAAACGCATATATAAACAgGACACAATTGCACACAAACACAAGCACACACTCATGCGCACAGCACACACACACACAC
AAACACATTAATAAACAGCGGCAATTGCACACAAACACACGCACGCACTTACACGCACACATGCACACACACACACACA
CACACACACAAACACCTGAGACCACATGCCTGCCAGCAGGGATCAGGGTTACCAACGTTGCCGGGTGTTTTTGTTTTG
TTTTTGTGGAGAGGAATTTTCATTATAATGGATGGTGTTGTTGGTTGCCTGACTGACTGACTACCTATCTCTTGCGTCC
CCGTCTCTTGAGATATATAGTTGTGTTCGCTGTATTTATCTACTCTATTTGTCTTTTTTATTTGATTAGAAAGCCAGCCA
TATACTGACGCGGGTACATGGCCT
>Micro 08 (1,646)
ACACAAACGCATATATAAACAGGACACAATTGCACACAAACACAAGCACACACTCATGCGCACAGCACACACACACACA
CAAACACATTAATAAACAGCGGCAATTGCACACAAACACACGCACGCACTTACACGCACACATGCACACACACACACAC
ACACACACACAAACACSTGAGACCACATGCCTGCCAGCAGGGATCAGGGTTACCAACGTTGCCGGGTGTTTTTGTTTTG
TTTTTGTGGAGAGGAATTTTCATTATAATGGATGGTGTTGTTGGTTGCCTGACTGACTGACTACCTATCTCTTGCGTCC
CcGTCTCTTGAGATATATAGTTGTGTTCGCTGTATTTATCTACTCTATTTGTCTTTTTTATTTGATTAGAAAGCCAGCCAT
ATACTGACGCGGGTACATGGCCT

Isolation source: *E. quadricolor and ?*
Number of sequences obtained: 1
Length variation: na
Primers:
>'TD144-34_F+R_Micro' (1,540)
AACAAAGGTCCCTCGGTTTTAGCCACACTCAACTTCCCCTCCTCCCTCTCATCATTGTTTTCCCTTTCACCATCCATTGAA
TTCCCCCTCACCACTTCCAACTTCCGACCACACCTCTTAGATCATCTACAGTATATTCCATTCTCTTCCCCTCACACTYTCA
CCCCAYWCATTTCCCTTTACCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTATTTATCTA

TCTATCTATCTATCTATCTATCCTTCATATGTCATTCAGCGTTCTTTATTGTTTGCTTTGTTTGTTTGTGATTTCCTGTTAC
AGCTGGTTGATGGCTAAGAAGTTTTGTAATGCATGTTAGGGTCC

Isolation source:*?*
Number of sequences obtained: 1
Length variation: na
>Contig_1 (1,546)
Primers:
CTCCGGGCTCCCACTNAGTAACGGGCCGCCAGTGGTGGMCTGGAATTCGCCCTTGATGAGTCCTGAAGTAAACATCAC
TTTTTGCTGTCAAATGTGTTATAATCCCTTTTCAATCAATAAAATCAAAAATATCGCAAATACAATCTTTAGTTtGAAGTG
AGAATACGTCAGGAAATCATAGAAAATAAGACTTTATCATCTCCATATTTTGTTAGTATAACTAATGTACGTACTATTGA
ACACACACACACCCACACACAACCACCCACACACCCACACACACATGTATATGTGAATCTTTTGCCTTCACACAATAACG
KYMGGGAMMWWWAYMMMACWYTCYACATWKAAACTATTGATGGATGACAATACAACGATCTCGCATTCACTGCTCT
GCCTCC

Isolation source:*?*
Number of sequences obtained: 1
Length variation: na
>Contig_1 (1,646)
Primers:
GGCCATGTACCCGCGTCAGTATATGGCTGGCTTTCTAATCAAATAAAAAAGACAAATAGAGTAGATAAATACAGCGAA
CACAACTATATATCTCAAGAGACgGGGACGCAAGAGATAGGTAGTCAGTCAGTCAGGCAACCAACAACACCATCCATTA
TAATGAAAATTCCTCTCCACAAAAACAAAACAAAAACACCCGGCAACGTTGGTAACCCTGATCCCTGCTGGCAGGCATG
TGGTCTCASGTGTTTGTGTGTGTGTGTGTGTGTGTGTGTGCATGTGTGCGTGTAAGTGCGTGCGTGTGTTTGTGTGC
AATTGCCGCTGTTTATTAATGTGTTTGTGTGTGTGTGTGTGCTGTGCGCATGAGTGTGTGCTTGTGTTTGTGTGCAAT
TGTGTCCTGTTTATATATGCGTTTGTGTT

Isolation source:*?*
Number of sequences obtained: 1
Length variation: na
>Contig_1 (1,535)
Primers:
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTAGTCTCAACCTCTGATGGGTGTTACAAAGCCATA
ACCACACATGAAGACGTCTAGGGTTTTCTTAGATCCATTTTGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGCG
TGATCTTTTGACGACAGWTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAMTCGCTCTCTCTCTCTCTCTCTCTCTCT
CTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCCT

Isolation source:*?*
Number of sequences obtained: 1
Length variation: na
>Contig_1 (1,570)
Primers:
AACGAAACCAGACTTATCTCTGAACCTGCGCCAGAACTTTCGGGGCGGCAACCTAGTTTTGTGCCCCCGTCCCGTAAAC
CTAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACCC
TAACCCTAACCCTAATAACCCTAACCAATTCACCATGTGACCGAATTACTGTGACCAAATTTCCGGTGACCAAATTTCCT
GTGACCTAATTACCGCGCCCCAaGTTTCTATGTGGAAAAGCGCATTTTGATCCATTTTGTCGGCACGAACCCAAGAATTT
CTTGTTGTTGGTAAGTGTTTCCACT

Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
>'EQ109_F&R_micro' (1,538)
Primers:
AATTCACTGGCCGTCGTTTTACATCACAACCATAACCCTAACTACAACCACAACCACAGTCACAAGCACAACCATAACCA
CAACCACAACTACAACAACAACAACAATAACAACAGCAACAACAACAACAACAACAACAACAACAACAACAACAACAACA
ACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACCACAATTACAACCACAACCACAACCAA
AACCATAACTACAACCACCACAACCACAACCACCACAACCATAACCACAACCACAACCACAACCATAACCATAACT

Isolation source: *?*
Number of sequences obtained: 1
Length variation: na
>Contig_1 (1,621)
Primers:
GCACGGGTGTTTATACATGTGCAGAATTATTCGGTATGCACACTAACGATGACATGATCAATTTGTGAACAATAAAAC
GTTTAGAAACCAGATGACGATGCTGATGTTGAAATAGAGTTTGACGHCTGTTCGGCACAACAGGCATTAGTCCACGTC
TTGTGGTGTGGGTAGTTATTGTATTGGTGTTTATGGCTGTTAGGGGGGGAGCAACGTACTAACTCAGCATCAYTATAA
TAGTCATCAACATCGACAMTATTATAWCTTTATAWMTAGTTCGCAMACCASACAGATaCGATGGATAGACCaSAGAGAG
AGAGAGAGAGAGAGAGAGAGAGAGCGCGCGCGCGCGCGTTTCCACTGTAAAGCCAAGCTGGATTTGAAGAGTGA
AATGTAGTT

Isolation source: *E. quadricolor and ?*
Number of sequences obtained: 1
Length variation: na
Primers:
>'TD144-15_F+R_micro' (1,523)
ACTAAAACTGACTTGTAAGGTAAAAATAGTGGACAAGATTACTGTAAGTATTAGGCCTTACATATGTGTTTTTTTTTATT
CATGCTTATTTTACTATTATACTTATTATTATTCAGTATTTATACCGTATGTTTTTTTATGTAAATTTTTATATTTATCTAC
ATTATTTATAAGCTAATATTTATCATTTATACAACCACCCACTTCTCCCCAAACACCCACTAAACAACCAGACACCAACAC

ACGTGCGCGCTCACACACGCACACACACGCACACACACGCGCGCACGCTATACATGTATTTTTTTATATCTAATATAAAA
ACTATAAATGCC

Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
Primers:
>'TD144-46_F+R_Micro' (1,432)
ACAGTAGGTACATCCAGGCGCTAAATAATAACTCTTCCCAACTCCCACACACACACACACACACACACACACACACAC
ACACACACACACACACACACACACACACACACACAYATACACACACACACACACACACACACACACACACACCAACACACCCACACA
CATACACACACACACACGCCCATACACACACACACTCACACACGCGCGCGCGCAGAATTGTAAATGTGGATATCGGTCG
TGATTCGACGCTGGTCC

Isolation source: *E. quadricolor and ?*
Number of sequences obtained: 3
Length variation: no
Primers:
>Contig_1 (1,575)
AACGAAACCAGTACTTATCTCTGAACCTGCGCCAGAACTTTCGGGGGCGGCAACCTAGTTTTGTGCCCCCGTCCCGTAAA
CCTAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACC
CTAACCCTAACCCTAATAACCCTAACCAATTTACCATGTGACCGAATTACTGTGACCAAATTTCCGGTGACCAAATTTCC
TGTGACCTAATTACCGCGCCCCAATTTTCTATGTGGAAAAGCGCATTTTGATCCATTTTGTCGGCACGAACCCAAGAAT
TTCTTGTTGTTGGTAAGTGTTTCCAC

>'TD_144-22_F+R_micro' (1,537)
ACGAAACCAGACTTATCTCTGAACCTGCGCCAGAACTTTCGGGGGCGGCAACCTAGTTTTGTGCCCCCGTCCCGTAAACC
TAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACCCT
AACCCTAACCCTAATAACCCTAACCAATTTACCATGTGACCGAATTACTGTGACCAAATTTCCGGTGACCAAATTTCCTG
TGACCTAATTACCGCGCCCCAATTTTCTATGTGGAAAAGCGCATTTTGATCCATTTTGTCGGCACGAACCCAAGAATTTC
TTGTTGTTGGTAAGTGTTTCCAC

>'TD144-04_F+R_micro' (1,509)
AACGAAACCAGACTTATCTCTGAACCTGCGCCAGAACTTTCGGGGGCGGCAACCTAGTTTTGTGCCCCCGTCCCGTAAAC
CTAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACCC
TAACCCTAACCCTAATAACCCTAACCAATTTACCATGTGACCGAATTACTGTGACCAAATTTCCGGTGACCAAATTTCCT
GTGACCTAATTACCGCGCCCCAATTTTCTATGTGGAAAAGCGCATTTTGATCCATTTTGTCGGCACGAACCCAAGAATT
TCTTGTTGTTGGTAAGTGTTTCCAC
>

Isolation source: *H. crispa*
Number of sequences obtained: 1
Length variation: na
Primers:

>TD152-71
ACACCTAAACGAAAAGGTCAAACTAGACGCACACGCACACACACACACACACACACACATACATACATACATACATACATAC
ACAGTATACACACAGCACACGTATACACGCAGGCGAATCACTATCACCAAGGATTCATTCATAGCTAGATAATGACTTC
AACTGATTACTGCAAATAGAAACAAAATGAATTACTGGATCGTGCGTTCGAATCCTGGTGACTTTGCAACGAATTTTTT
ATGCCTGAAATCAATACTCAAGCATCCGCCCACCTGTCAGTTTGACCTGGTATCCGTTGTTAGTCGAATGTAATGGTCC
CACCTAGCAGAAGTTCTTGTCTTTGTATTGATCT

Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
Primers:

>'EQ67_F&R_micro'
ACCCTAAAGGTGTTTATTTTGATGTCTCTAATTGTCGTCGATGGACGAAGTGCGCACGCAAGATTGCACGACTTCCAAA
AATAATTGTTTTTTCTCAAAATATTATGGATCTTCGAGAACAAGAGGGCTTTGAAAATACACCACGATGTGTTGAGGTG
TAGAGATAAGGAAAACATACAGAGAAATATAAAAATATGCACACTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCAT
GGCCCAGGCCTCTCTTTCACAGTCGTAATATAAATGTATGTAATAGTTTTCTGCATAATATTATTGTAGATATCTACCAG
GTATACTGAATAACCCACGGTTGGTGATGAAAGATGCGTTGATATGTTGAAAATAGAACCTCGACTAATCTCGAGTAC
CCAATATCATCTGATTTTGTCATCAGGGCGTACGGTGCTACACACTATCGAGTTGCACCAT

Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
Primers:

>'EQ68_F&R_micro'
AGAGTTACAGCTGCGGCGTGCCTTGTAACGATGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCCAAGGATA
AAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATCAGTCAGTC
AGCCCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTAC
GTGGTATAAGATTGTATAGAACATATTATTTATCTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAACC
TATAAATTGTTGTATGTCAAAATATGTCACATGAGCTGAATAATGTATGAATGTTTGAGGAGGTTGT

Isolation source: *E. quadricolor*
Number of sequences obtained: 1

Length variation: na
Primers:

>'EQ72_F&R_micro'
GAACAGACTGGAAGCTGGAAGCAGTTTCAGTCGGTATTTCCACCGGAATTGTTTACATTGTGGTGAGCAGGTGCATTA
TCATATATGAAAACCACACGGGCATTAGGAGCAAGGAACGGGTTTGAAGCAAGAAATCTTAGAATCTCTGGGCAGTCA
TGCTTCCTATTTCTGCAGATTGATGCACAAGGCCAGTCCTAGTTGAGATGGCCATCACAAGGGTCACATTTCTTCCATG
TTGTCCACACACACACACACACACACACACACACACACACACACCGGTCTGTGCCCCGTTTCTCACTGCTCC

Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
Primers:
>'EQ73_F&R_micro'
AGCTCAACGGCCGCACCCCTCCCTTGCATGCCACATATTCACGCCTATCAAGACACAAGACCCAAAAGCAAGAAGGCTC
TCTCACTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTTCTCAATAATTCCATGTGGCAATAATTCT
ATGTCGCAATAATTTCATGTCGCAATAATTATATGTTTTACAAACATGCACAACGCACAGAACATAATGTCATAGAAGA
AATGTTTTCATATGGGCTCAGCATGCAATACGTGTGCATCTATATGTCAGTATGCCGCCTGAATCGATGAAATATAAGA
CTAGGAAAATTCCTTGAAC

Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
Primers:
>'EQ77_F&R_micro'
ATATAACACAAATATAAACAGCAACTTTACCCACCGGAGTCTCTACAAGTTGAAAATTACAAGAGGAATCATGATTTGT
TGTTTTGTCCTCTCTGTGCTACACGTATTTYTACAATAAACGTAATAATAACAAATACTTTCCATCGGGCCCATGTAGAA
CGGGTAGCTCAAGGCCATTTGTCAGCTACATTAGAGAGAGAGAGAGAGAGAGATAGAGAGAGAGCGAGAGAGAGA
GAGAGGGGATTGAGATAATGTCC

Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
Primers:
>'EQ80_F&R_micro'
GGGACTGAATTTGTATGACTTCAAATCCATCGAACACAATCAACCAAAATAAACAAAATATCCAATTTCAGAGATAATG
ATTGTGAAATTTAGAACACAAATATATTCCAAGAAATCAACTTACCATATACCAGGTTCTAGTCGTTCACTGACTACATC
GGATTCTCCAAACACGTGACCYGGAAAAAACAACAACAAATTTTTATTTAGAAMTGATMGACATACGGGARGGAGAGA
GAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAAACAAAATGTGAAGGAGATGTGAAAGGTACGCAAAAAATGATTCA
TTGAAGATGAAATAGCGT

Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
Primers:
>'EQ81_F&R_micro'
GGTTTCTTTCACATAAACTGGTACTGTGTATCCAACCTGTAAGTTTGCTTCAACACGTTACATCCAATACACCTTTTACCT
TCCCAATCAATGGGCTGTGAAATATTCTTATGGATATTGCCTGATAACGACAAGATAACACTGAACTGAACTAGGTATT
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTTCTCTATCTCTCTTTCTCTCCCTGCCTGACTCTATCT
CTCTCATGTATAAAGCCACCACTGGATATGCCTAAGAGTTTCCCCAATTATTGCGACAACCTTTATATCTATACTTATCTA
AAACGAATACATTGATACGATATCAAACATACATGTATTTGAAATACGCTGATGAGGGTGCATCAGGCTAGTAACCGG
TGT

Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
Primers:
>'EQ89_F&R_micro'
AACACTAAARCTGACTTGTAAGGAAAAAATAGTGGACAAGATTACTGTAAGTATTAGGCCTTACATATGTGTTTTTTTT
ATTCATGCTTATTTTACTATTATACTTATTATTATTCAGTATTTATACCGTATGTTTTTTTATGTAAATTTTTATATTTATC
TACATTATTTATAAGCTAATATTTATCATTTATACAACCACCCACTTCTCCCCAAACACCCACTAAACAACCAGACACCAA
CACACGTGCGCGCTCACACACGCACACACACGCACACACACGCGCGCACGCTATACATGTATTTTTTTATATCTAATATA
AAAACTATAAATGCC

Isolation source: *H. crispa*
Number of sequences obtained: 1
Length variation: na
Primers:
>'HC129_F+R_micro' (1,527)
AACTATACCTAATTGGGGGCTTCTGTCGACGCCGCTATAGGTAGTATAGACCGAGGACGTCCTCGGTTGTATCATGGT
CCTCGATTAGAAGGTGTGTATTTACATATGTGTCCTCGATGTACACCTTATACAAACGCAGAACACACCCAGACACACA
CACACACACACACACACACACACCCAGTAAACACGTCCCTGGTACGGGGTAAAACAATCAAAGCTGTTATAAAAAATCTTG
TATCATTACCTTTTCGTAACAAACTGTTTTACGGTACGGTAGTTATATTATGTCTGTATCACAGACTTTCACGCAGAATC
ACATTGAAGCTTTCCCACTTTCCACACCATGCTCATTGGTAGAAGTGAAGGCAACC

Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na

Primers:

>'EQ91_F&R_micro'
AAATTAACAGATACACTGTTTTACTTCTGCATGTCACCCTACCAAATAAATTAGTTATGACAACCGAATTCTCACACTTCC
GATATCTCCGAAAATGTACTGAGTGCATTGATATGTCCCCCTCACACACACACACACCCACACACACACACACTTTGTCT
CTGTCTCTGTCTCTCTTTGTATACATAATGCTCCACTGTTTGGCACTGTAAGACCAATTATCTAATTTAGTAGTCTAATAC
ATGTAGGTCAATCCTGAAACATATTGCATAGTATAACATACCTCTCCATGGTACTTGTAGATCAATGTATTCTCCCTTGA
CATGCGTAGGAAATCTCAAACAACTGTAGAAAATAAGGCGACGCGAAGGGCGACGTCGGGGGTAAAAATGCGACGTCG
GGTTAGCTCCCCATACY

Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
Primers:

>'EQ95_F&R_micro'
AAACTAAACGGGACTTATCTCTGAACCTGCGCCAGAACTTTCGGGGCGGCAACCTAGTTTTGTGCCCCCGTCCCGTAAA
CCTAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACC
CTAACCCTAACCCTAATAACCCTAACCAATTTACCATGTGACCGAATTACTGTGACCAAATTTCCGGTGACCAAGTTTCC
TGTGACCTAATTACCGCGCCCCAATTTTCTATGTGGAAAAGCGCATTTTGATCCATTTTGTCGGCACGAACCCAAGAAT
TTCTTGTTGTTGGTAAGTGTTTCCAC

Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
Primers:
>'EQ96_F&R_micro'
AAGACTGGGAAATTTTGTCGATAGCCCCGAACATGCAGACTCAAAAACACTGTAAAATCTGAGTTTGTTAGCTAGAGA
AACAACTAGCATAAGTAAATCGGTCAAATGTTCGTGGCACATACATTTCAGTACTAACTCTCGTGAGTAAAGTAATTTA
GTTTGTAGTCTGAGTCTGAGCTTGAGATTATTTGTTTGTTGCTGATCCCCAGGCCAGATCATAGAATTCAAGAGCAAA
GTAAACTTCAATATATAGTACGGTACCATAAGAGACACGCACACACACACCCACACACACAGCCTAAATGTTCGTTTGT
TGAGGATTTGC

Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
Primers:
>'EQ98_F&R_micro'
AACGCTATTTCATCTTCAATGAAWCATTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTTCTCTCTCTCTCTCTC
TCTCTCTCTCTCTCTCCTTCCCGTATGTCGATCAGTTCTAAATAAAAATTTGTTGTTGTTTTTTCCAGGTCACGTGTTT
GGGGAATCCGATGTAGTCAGTGAACGACTAGAACCTGGTATATGGTAAGTTGATTTCTTGGAATATATTTGTGTTCTA
AATTTCACAATCATTATCTCTGAAATTGGATATTCTGTTTATTTTGGTTGATTGTGTTCGATGGATTTGAAGTCATACAA
ATTCAGTCCC

Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
Primers:
>'EQ105_F&R_micro' (1,414)
GACATTATCTCAATCCCCTCTCTCTCTCTCTCGCTCTCTCTCTATCTCTCTCTCTCTCTCTCTAATGTAGCTGACAAATGGC
CTTGAGCTACCCGTTCTACATGGGCCCGATGGAAAGTATTTGTTATTATTACGTTTATTGTAGAAATACGTGTAGCACA
GAGAGGACAAAACAACAAATCATGATTCCTCTTGTAATTTTCAACTTGTAGAGACTCCGGTGGGTAAAGTTGGCTGTTT
ATATTTGTGTTATAT

Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
Primers:
>'EQ112_F&R_micro' (1,704)
ACACACACAAGCAGACGTACACACAGAGGTACACGCACAAACACGCACACACACTCACACACACACACACACACGCACA
CGCACTCTTTCACACACAACTCACACCACACACTTGCTCTCTCGATCCCTCACTCAACCCAATCCGGTCAAAAATTTACCG
TATAGAAGGCACGCTTCGCAGCTTCTCAAAACCGGTTGATGGGCCAGAACTGCTTTGCTGTAAAGATAGGCAAAAAAA
TTTCAATCGCCAGAGTATCCAGCGGAAGCACTAACGGTACAAAATTATCCATAATTATGAAATAGAAATAGGAACTGCT
CCTCCATTCTCCCTAATCCTGTTCATCCCGAGACTTTACCCTCAGCTCCCGGAATGGATGTAGGTGCTCCCTCCCTAACC
AAGTCCTTCACCACAGTCCTCCCCTAATCCCTCGAAACTCCTCCTTCGCCCTCAGAGCCATTACC

Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
Primers:
>'EQ502_F&R_micro' (1,535)
GTGGAAACACTTACCAACGACAAGAAATTCCTGGGTTCGTGCCGACAAAATGGATCAAAATGCGCTTTTCCACATAGA
AAATTGGGGCGCGGTAATTAGGTCACAGGAAATTTGGTCACCGGAAATTTGGTCACAGTAATTCGGTCACATGGTAAA
TTGGTTAGGGTTATTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTTAGGTTTACGGTTAGGGTTTTGGTTAGGGTTA
GGGTTTAGGTTTACGGTTAGGGTTTTGGTTAGGTTTACGGGACGGGGGCACAAAACTAGGTTGCCGCCCCGAAAGTT
CTGGCGCAGGTTCAGAGATAAGTCTGGTTTCGTT

Isolation source: *E. quadricolor*

Number of sequences obtained: 1
Length variation: na
Primers:

>'EQ503_F&R_micro' (1,513)
ACAGCAATAGCGGTCCTACCAGTAAATGATACCTTTATACATACATAAATACATCTACATACATACATACATACATACAT
ACACATACATACATACATACATACATACATACATACATACATACATACATACATACATACATACATAAACATACTTTTTCA
GTGGCAGGATCACCGAAACGAAGAAGGAGCTGTCTCTGGATTGGGATCCACACGACGGCTAGAGTATTTTTATTCCTG
GAAGCAACTATTACAGCTGACTAACACTGGTTCCTAAATTGGCTACTTAGGTACACACGAGCTGTTTTACGTCATAGGT
ACGCTCTCACGAGCGGAGACC

Isolation source: *H. crispa*
Number of sequences obtained: 1
Length variation: na
Primers:
>TD152-46
AACCTACGAATGTGTTCTAATGTATTACATTGAATTTGCCTCTCACTCACCCACTCACTCACTCACTCACTCACTCACTCA
CTCACTCACTCACTCACTCAATCACTCACTCACTCACTCACTCACTCACTCTCTATCTCTTGTTCTCCTCTCTCTATGTCTCT
CTCTTTCCTCTCCCTCTGTTTCTCTCTCTCTCTTTCTCTCACTCTCTATCTGTCTGTCTGTCTGTCTGTCTGTCGGTCTATC
TCCCTCTCTCTCTCTCTCTCTCTCCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTC

Isolation source: *H. crispa*
Number of sequences obtained: 1
Length variation: na
Primers:
>'HC124_F+R_micro' (1,531)
ACWYMAYTGRSYKYYGTWTYACAAAATAAACACACACACACATACATACATACATACATACATACATACATACATA
CATACATACATACATACATACATACACGCACACACACACACGCACACACACACACACACACATATTTGACACATATGCCA
GGTTTCTACGGCTGGGGAGGACACAACATCTGTACATATGTGCGTGTTTGCAGGACGCACAAACTCAAAACAACAGAT
ACCGCTAACTTTAGATTGTTTGGCAGACTGAAATAGTTTGTCCTACATAGAAAACGGCGCAATCATTGTGAGCCGCTTA
GTCTGATTCAATCAACCTGAGTATTTCCTGCTATGAGTAAGCGTTGAACATCTTCT

Isolation source: *H. crispa*
Number of sequences obtained: 1
Length variation: na
Primers:
>'HC104_F&R_micro' (1,563)
GTGTGCTTACAAACATGTACACTACATACCCCATACATTACTTGAGCAATAACAAATCCCCCCCCCCCCACACACACACAC
ACACACACACACACACCAGCTCTCCTCTGATATATGAAGCTTTATACTTTGTATAATGGGTAACTTTCGCTACCAAAAAA
ATGTTTTATACGTCTTTTTAGAAAACCTTTAGCCTTTTGCATAGAATATTGTTCCTGCTTTTTTCAATAAGCATGAACATA
AATGTTCCTACTGCACTGCCTTGCAGGGAACAATTTGAAACCCCCAGTCAAACACGACTGGAGAAGTTGGGATTTTCTT
CACTCCCACAGCTGGATAACAATTATGTTTGCGGGGTCTTCACTTGCATGTACCCTAGGCACCTGGCAGAACTGGGGT
TGT

Isolation source: *H. crispa*
Number of sequences obtained: 1
Length variation: na
Primers:
>'HC106_F&R_micro' (1,558)
AGAAAAGAGAAGCAAACCTCTCTAGACAGGTTTCTCATGAGACAGCAAGCACAGTCTGTATGAGAGGAAGCAGCACCT
GCAGCAGGGTCTAGTGGGTAGCCTACATAAGGCAGAGGATGGAATCAACACCTGAAATGAATATCGTGCCTGAAGTC
TTTATGGAGGGGGCTCCCCTTCCCAACAATAACTTCTCTTCTTCTCCTCCTCCTTTTCCACCTTCTCCACCTTCTCCTCCTC
GTCTTCCTCCTCCTCCTCCTCCTCCTTCTCCTCCTCCTTATCCTTCTCCTCCTCTTCATTTCCTACTCCTCCTTTTCCTCCTTC
TCCTCATCCTCCTTATACTCCTCGTAGTTGTCATCGTCGTCGTCATCGTCGTCGTCGTACTCCTCCTCCTCCTCCTCCTCCT
CCTCCTCC

Isolation source: *H. crispa*
Number of sequences obtained: 1
Length variation: na
Primers:
>'HC108_F&R_micro' (1,552)
ACTACATTTCATTTTCAAATCCAGCTTGGCTTTACAGTGGAAACGCGCGTGCGCGCGCTCTCTCTCTCTCTCTCTCTC
TCTCTCTCTCTCTGGTCTATCCATCGTATCTGTCTGGTTTGCGAACTAGTTATAAAGTTATAATATTGTCGATGTTGATG
ACTATTATAATGATGCTGAGTTAGTACGTTGCTCCCCCCCCTAACAGCCATAAACACCAATACAATAACTACCCACACCAC
AAGACGTGGACTAATGCCTGTTGTGCCGAACAGACGTCAAACTCTATTTCGACATCAGCATCGTCATCTGGTTTCTAAA
CGTTTTATTGTTCACAAATTGATCATGTCATCGTTAGTGTGCATACCGAATAATTCTGCACATGTATAAACACCCGTGC

Isolation source: *H. crispa*
Number of sequences obtained: 1
Length variation: na
Primers:
>'HC120_F+R_micro' (1,519)
AGAACATATTACAAGCTATGTATAACTTGTTTCCAACGCACAAGGGAAAAACAAATCAAATGTTGGCGGCCATTTTGAA
AAATCGCTCCGCACCGACCTCAAAATTGTTTTTCACTCAAAATTCCTACATTTTCATGATTTCCATCAAAAATGGCCTCTA
AATCCACCATGCATTCATTTTCGACCCCCATTGTCCCAACCCTATAATTATAAGGTGCAAACATATAATTGATTCCAACA
GGAACTACTTTGTGCGTGATGTGTGTGTGTGTGTGTGTGTGAGTCAGTCCAGTCGGCACTTCGCGCGTGTACTTCACA
CAATACCCCAGACGTGAAGAGAGGGCTACCATTTTGGCAATCAACCATCATTTACTCGCAGTAACC

Isolation source: *H. crispa*
Number of sequences obtained: 1
Length variation: na
Primers:
>'HC130_F+R_micro' (1,555)
ATGAAGAGATACACTGCAAGGAATTTGCATGTCACCCTACCAAATAAATTAGTTATGACAACCGAATTCTCACACTTCC
GATATCTCTGAAAATGTACTGAGTGCATTGATATGTCCCCCTCACACACACACACACACACCCCCACACACACACTTTGT
CTCTGTCTCTGTCTCTCTTGTATACATAATGCTCCACTGTTTGGCACTGTAAGACCAATTATCTAATTTAGTCTAATACAT
GTAGGTCAATCCTGAAACATATTGCATAGTATAACATACCTCTCCATGGTACTTGTAGATCAATGTATTCTCCCTTGACA
TGCGTAGGAAATCTCAAACAACTGTAGAAAATAAGGCGACGCGAAGGGCGACGTCGGGGTAAAAAATGCGACGTCG
GGTTAGCTCCCCATACC

Isolation source: *H. crispa*
Number of sequences obtained: 1
Length variation: na
Primers:
>'HC125_F+R_micro' (1,415)
TCCCCCCCCCCCCCCCCAACATACACACACACACGCACACCTACAACATGTCCGCAATTGGCCACAAACTTTTTATATTATA
ATATTTGAATAGTAACAGTTTGCTCACCTAAAATTGATAACCTATCATCTACAATCAACCCTAAGGCCCCCTTTACACAG
AGCGCGCGATTGCTGAGTGTTCGCTGAGAGACCAAAAAATGGCCAGATCTCTGAAAGATCTGTCAATGATCGCCCAGC
GGTCTGCAAGATCTCTCCCTGATCTTCAATGATCTTTCCTTGATCTCCATGGTCGCCACGGTCATCTGGATGT

Isolation source: *H. crispa*
Number of sequences obtained: 1
Length variation: na
Primers:
>'HC154_F_micro_schlecht' (1,478)
GGTGTCAGCAACAACAAGAGCAACCAGAACAAAAAACAAAACAACAACAACAACAATAACAACAAATACCACAACAACA
ACAACAACAACAACAACAAAAACAACAACAACAAAGACAACAACAACAACAAAAACAACAACAACAACAACAACAACAA
CAACAACAAAAACAACAACAACAACAACAAAAACAACAACAAAAACAACAACAACAACAAAAAAAACAAAACAACAACA
ACAAATAAAAACAACAACAACAACAGCATACTGAAGTGGTATGCATGAATTTATAATTCAACCGCAGGTTCCATTCCCC
CTAGACACTCGGCTGT

Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
Primers
>'EQ71_F&R_micro'
AACGGTCGTCGTTCTCATCCACTAGTAACGGCCGCCAGTGTGCTGGAATTCGCCCTATAGTGAGTCGTATTACAATTCA
CTGGCCGTCGTTTTACTCCTGTAGCGACATGGTATCCCATGATCGCTACAGCTAAAAAAAAGAACCAACGGCTCAGTTT
TTCCCTCACCCCCCAACCCCCACCACAAGGTAATAAATAGGATTACCTGACCTGACCTCTTTCTCACTTCTTCTTTTCACT
AAGTCCAGTCAACAGCCTCTCTCTCTCTTTCTCTCTCTCTYTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTC
TCTCTCTCTCTCTCTCTCTCTCTCTCCGTACAGCAGTAACCCCGCCAAACGTCTTATCACCTACAGCTGCAGCACCCACGT
TGTCTAT

Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
Primers
>'EQ103_F&R_micro' (1,463)
ACACTCCGGCACTGTTGTACTCTCGCACTCTCGCGCTCACACTCTTGCTCTCTCTATCTCTATGATTCTCTCTCTATATCG
TTCTCACACTCATTTATCGAACTCTCTCCCTCTCTTTCTCATTTCCTTCTCTCTCTCYYYYYYYYYtCTCTCTCTCTCTCTCTC
TCTCTCTCTCTCTCTCATAAACTTCATACTTTTTACTCTGCATCATCGACTATTCAAGTGGATTGTGTTACTAACAGTACT
TTGGTATCCCATTTATCGTGGCATTGTCGGTTTATGGAAGGTAAAGAGGCGACCAATGAATTGTAATCCGGCCCCCTC
AAGGGCGAATTCCAGCACACTGGCGGCCGTTACTAGTGGAT-----

Isolation source: *E. quadricolor*
Number of sequences obtained: 3
Length variation: no
Primers
>'EQ84_F&R_micro'
AACATATCAGTGGTAAGGTGGAACGATATAGGAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCCAT
AACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGC
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCCCTCTCTCTCTCTCTCTCTCTCTCT
CTCTCTCTCCCTCTTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGG
CCACAGCC

>'EQ88_F&R_micro'
AACATATCACTGGGAAGGTGGAAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCAA
TAACCACACACGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCGTTACAATACAATAAGGACTTC
GCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCGCTCTCTCTCTCTCTTTTCTCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAG
GCCACAGCC

>'EQ69_F&R_micro'

TCTCAACCTCTGCAGGGTGCTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGAATTACTATGATCAA
ATAACTTCGTTACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAA

GCAATCCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCCTCTCTCTCTTCTCTCTCTCTCCCTCCAACCGAATTGTTCAC
TTGGCTTTCGCCCCCGCGCTA----------

Isolation source: *H. crispa*
Number of sequences obtained: 2
Length variation: no
Primers:
>'HC133_F+R_micro_' (1,522)
AATTCACTGGCCGTCGATAACCTGAGGGATCATCGGTTCCTGACCTTTTGGAATCCACGACTTCATGAGCCCCTAATTC
AGGTACATACTTTGAACTCTCCGTCGCCGGTTTATTCAGAGCAATATTTACACCACCTAAATATAGACATAAAAGTCCTT
CAATGAGACAAAACCCTTTATAACAAACATAGTATTAGAAAACACACAAATTATTCAACGACTCTCTCTCTCTCTCTCT
CTCTCTCTCTCTCTCTCTCTCTCACTCTGTTTGTCAAACTTTTGAACATACATAAATTATATCAGCAAGTGCTGCCACTTA
CCTGGTAATTTGTAACATTTTGACGGATGACAAAGATTTCCATCAGTGCTAGGTGAATCTACAGCAT

>'HC140_F+R_micro' (1,544)
-
GGTCTAAGCAGTATGATAACCAGGGGGATCATCGGTTCCCGACCTTTTGGAATCCACGACTTCATGAGCCCCTAATTCA
GGTACATACTTTGAACTCTCCGTCGCCGGTTTATTCAGAGCAATATTTACACCACCTAAATATAGACATAAAAGTTCTTC
AATGAGACAAAACCCTTTATAACAAACATAGTATTAGAAAACACACAAATTATTCAACGACTCTCTCTCTCTCTCTCTC
TCTCTCTCTCTCTCTCTCTCTCACTCTGTTTGTCAAACTTTTGAACATACATAAATTATATCAGCAAGTGCTGCCACTTAC
CTGGTAATTTGTAACATTTTGACGGATGACAAAGATTTCCATCAGTGCTAGGTGAAACTACAACAT
>
Isolation source: *E. quadricolor*
Number of sequences obtained: 2
Length variation: yes
Primers
>'EQ101_F&R_micro'
AGGTACGCTCGGGTGCCTTGAACATGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCCAAGGATAAAGCGCC
TGGACGGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATTTAAAAACTATCAAAAATCAGTCAGTCAGCCTCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCT-
~ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTA
TCTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACAT
GAGCTGAATAATGTATGAATGTTTGAGGAGGTTGT

>'EQ85_F&R_micro'
AGGTACGCTCGGGTGCCTTGAAG~TGCACGGTTCAGTACACGATCCAGGCATACTCGGATATCCAAGGATAAAGCGCC
TGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATCAGTCAGTCAGCCTCTC
TCTCTCTCTCTCTCTCTCTCTCTCTCTCT----
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGT

Isolation source: *E. quadricolor* and *H. crispa*
Number of sequences obtained: 5
Length variation: yes
Primers

>'EQ501_F&R_micro' (1,551)
-
GGTACGCTCGGGTGCCTTGAAG~TGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCCAAGGATAAAGCGCCT
GGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATCAGTCAGTCAGCCTCTCT
CTCTCTCTCTCTCTCTCTCTCTCTCT------
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTATGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGT

>'EQ108_F&R_micro' (1,526)
GGTACGCTCGGGTGCCTTGAAG~TGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCCAAGGATAAAGCGCCT
GGACAGTGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATCAGTCAGTCAGCCTCTCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCT----
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGTC

'HC143_F+R_micro' (1,511)
GGTACGCTCGGGTGCCTTGAAG~TGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCCAAGGATAAAGCGCCT
GGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGCGATCTAAAAACTATCAAAAATCAGTCAGTCAGCCTCTCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCT—
ACATGAATGCACACTTACATATATATGCAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAACGTATGAATGTTTGAGGAGGTTGT

'HC110_F&R_micro' (1,525)
GGTACGCTCGGGTGCCTTGAAA~TGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCCAAGGATAAAGCGCCT
GGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATCAGTCAGTCAGCCTCTCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCT----
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT

CTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGT

'HC113_F+R_micro' (1,533)
GGTACGCTCGGGTGCCTTGAACATGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCCAAGGATAAAGCGCCT
GGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAA~CTATCAAAAATCAGTCAGTCAGCCTCTCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTG
GTATAAGATTGTATAGAACATATTATTTATCTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAACCTAT
AAATTGTTGTATGTCAAAATATGTCACATGAGCTGAATAATGTATGAATGTTTGAGGAGGTTGT

'HC123_F+R_micro' (1,533)
GGT~CGCTGGGGTGCCTTGAACATGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCCAAGGATAAAGCGCCT
GGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATCAGTCAGTCAGCCTCTCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCT—
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGT

Isolation source: *H. crispa*
Number of sequences obtained: 1
Length variation: na
Primers:

'HC126_F&R_micro' (1,844)
AGTACGCTCGGGTGCCTTGAAG~TGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCCAAGGATAAAGCGCCT
GAACAGCGTAGTAGTTCTATGATATCACGCAAGTGAAAGTGGACTAAAAACTCCCAAAAGTCAGTCAGTCAGTCAGTG
TCATTTTGCTCAGCGTTAGCTGGCAAGTTTCAATTGGACATCAACGAAAATTCTAGAAAACTCACCGACAGGCGGTAAA
ACTTGTTGCTGAGCAAAATGACACGAATGACATTTTCCTCAGCATTTTCCTCACTGTGTGCGGTATGTTGTTGTTGTTG
TTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGT
CGTTGTYGTYGTYGTTGTYGTYGTTGTCGTTGTCGTTGTCGTTGTCGTTGTCGTTGTCGTTGTCGTTGTCGTTGTT
GTCGTTGTCGTTGTCGTTGTCGTTATTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTTTTGTTGTTGTTATGG
GTTGTACTGTGACCGATCTTACACCCAATAGTGTAAAACRACGGCCAGTGAATTGTAATMCGACTCACTMTAGGGCGA
ATTCCAGCACACTGGCGGCCGTTACTAGKGATTTAGAACGACGACCAGANCCNNTAATCAT---
>
Isolation source: *H. crispa* and *E. quadricolor*
Number of sequences obtained: 4
Length variation: yes
Primers:
>'HC137_F+R_micro' (1,494)

ACTTCACTGGACGTCGTTTTAAACCTGCGCCAGAACTTTCGGGGCGGCAACCTAGTTGTGT~GCCCCCGTCCCGTAAAC
CTAAACCCTAACCCTAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAA----------
CCCTAACCCTAACCCTAATAACCCTAACCAATTTACCATGTGACCGAATTACTGTGACCAAATTTCCGGTGACCAAATTT
CCTGTGACCTAATTACCGCGCCCCAATTTTCTATGTGGAAAAGCGCATTTTGATCCATTTTGTCGGCACGAACCCAAGA
ATTTCTTGTCGTTGGTAAGTGTTTCCAT

>'HC127_F_micro' (1,435)
GATCCTGAGTAAAACGAAACCAGAC~TATCNCTGAACCTNCGCCAGAACTTTCGGGGCGGCAACCTAGTTTTGTTGCC
CCCGTCCCGTAAACCT----------
AACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACCCTA
ACCCTAACCCTAATAACCCTAACCAATTTACCATGTGGCCGAATTACTGTGACCAAATTTCCGGTGACCAAATTTCCTGT
GACCTAATTACCGCGCCCCAATTTTCTATGTGGAAAAGCGCATTTTGATCCATTTTGTCGGCACGAACCCAAGAATTTCT
TGTTGTTGGTAAGTGTTTCCAC

>'HC117_F+R_micro' (1,480)

AACTAAACCAGACTTATCTCTGAACCTGCGCCAGAACTTTCGGGGCGGCAACCTAGTTTTGT~GCCCCCGTCCCGTAAA
CCT---
AACCAAAACCCTAACCGTAAGCCTAAACCCTAACCCTAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACCCTA
ACCCTAACCCTAATAACCCTAACCAATTTACCATGTGACCGAATTACTGTGACCAAATTTCCGGTGACCAAATTTCCTGT
GACCTAATTACCGCGCCCCAATTTTCTATGTGGAAAAGCGCATTTTGATCCATTTTGTCGGCACGAACCCAAGAATTTCT
TGTTGTTGGTAAGTGTTTCCAC

>'EQ107_F&R_micro' (1,514)

AACTAAACGGGACTTATCTCTGAACCTGCGCCAGAACTTTCGGGGCGGCAACCTAGTTTTGT~GCCCCCGTCCCGTAAA
CCT---
AACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACCCTA
ACCCTAACCCTAATAACCCTAACCAATTTACCATGTGACTGAATTACTGTGACCAAATTTCCGGTGACCAAATTTCCTGT
GACCTAATTACCGCGCCCCAATTTTCTATGTGGAAAAGCGCATTTTGATCCATTTTGTCGGCACGAACCCAAAAATTTCT
TGTTGTTGGTAAGTGTTTCCAC
>
Isolation source: *H. crispa*
Number of sequences obtained: 2
Length variation: yes
Primers:
>'HC136_F+R_micro' (1,524)

GGAATCATTGCCAAGCACATTCTGACACAAAACACACTGACCCTTCACAACACCTTTATCAGAGAGGGAAGTAAAACCA
TATTGTATA--
GTCCTCACAGAAGGTTCTTTTAGTCTTACTCGTATCTGCCATCATACTCGTGAATGTGGGAGCCCTGAAGAGATAGAAG
TGAACAACATTCAGCAAACAATACTGCACACATCACACACACACTCACACACAATCACACACACA~~~~~~~~~~~~~~~
~~~~~~~~~~~~~~~TCAAATTTTCTTTAGGTGAGTTCGAGAGCAGAAGCATTGCAGACTACAACCACAGCAGCTTTAG
TCAGTTGATATCTTGGTGATGAGATATCACTGATAGACCCCACCGCTGAGAGAGCTAAATGTGAGTTAGTTGTTCCAA
AGCTCAGTGATGTGG

>'HC146_F+R_micro' (1,584)
GAATCATTCCCAAGCACATTCTGACACAAAATACACTGACCCTTCACAACACATTTATCAGAGAGGGAAGTAAAACCAT
ATTGTATATGGTCCTCACAGAAGGTTCTTTT------------------------
CTCGTAAATGTGGGAGCCCTGAAGAGATAGAAGTGAAAAATATTCAGCACTGTACTACACACATCACACACACACACTA
ACACACACACACACACACACACACACACACTCACTCTCTCACACACACATCAAATTTTCTTTACGTGAGTTCAAGAGCGG
AAGCATTGAAGACTACGACCACGGCAGCTTTAGTCGGTTGATATCTTGGTGATGAGATATCACTGATGGACCCCACCG
CTAAGAGAGCTAAATTTGAGTTATTTGTAAAAGGACGCCCAGAGAAGGGTAATCCGACTCCATCTAGGGCGAATTCCA
GCACACTGGCGGCCGTTACTAGTGATTCGAGCTACGACCCANGNNGN-----------
>

Isolation source: *E. quadricolor* and *H. crispa*
Number of sequences obtained: 3
Length variation: yes
Primers
>'EQ97_F&R_micro'
AGGACTGAATTTGTATGACTTCAAATCCATCGAACACAATCAACCAAAATAAACAAAATATCCAATTTCAGAGATAATG
ATTGTGAAATTTAGAACACAAATATATTCCAAGAAATCAACTTACCATATACCAGGTTCTAGTCGTTCACTGACTACATC
GGATTCTCCAAACACGTGACCTGGAAAAAACAACAACAAATTTTTATTTAGAACTGATCGACATACGGGAAGGAGAGA
GAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGGGAGAGAGAGAGAA
ACAAAATGTGAAGGAGATGTGAAAGGTACGTAAAAAATGTGTAAAACGACGGCCAGTGAATTGTAATACGACTCACT
ATAGGGCGAATTCCAGCACACTGGCGGCCGTTACTAGTGGA-------------------------------

>'HC144_F+R_micro' (1,504)
GGGACTGAATTTGTATGACTTCAAATCCATCGAACACAATCAACCAAAATAAACAAAATATCCAATTTCAGAGATAATG
ATTGTGAAATTTAGAACACAAATATATTCCAAGAAATCAACTTACCATATACCAGGTTCTAGTTGTTCACTGACTACATC
GGATTCTCCAAACACGTGACCTGGAAAAAACAACAACAAATTTTTATTTAGAACTGATCGACATACGGGAAGGAGAGA
GAGAGAGAGAGAGAGAGAGAGAG~~~~~~AAACAA
AATGTGAAGGAGATGTGAAAGGTACGCAAAAAATGATTCATTGAAGATGAAATAGCGT

>'HC153_F+R_micro_' (1,477)
GGGACTGAATTTGTATGACTTCAAATCCATCGAACACAATCAACCAAAATAAACAAAATATCCAATTTCAGAGATAATG
ATTGTGAAATTTAGAACACAAATATATTCCAAGAAATCAACTTACCATATACCAGGTTCTAGTCGTTCACTGACTACATC
GGATTCTCCAAACACGTGACCTGGAAAAAACAACAACAAATTTTTATTCAGAACTGATCGACATACGGGAAGGGGAGA
GAGAGAGAGAGAGAGAGAGAGAGAGAGAG~~~~~~AAACAA
AATGTGAAGGAGATGTGAAAGGTACGCAAAAAATGATTCATTGAAGATGAAATAGCGT
>
Isolation source: *H. crispa*
Number of sequences obtained: 1
Length variation: na
Primers
>TD152-04
AGGCTTCTTTCACATAAACTGGTACTGTGTATCCAACCTGTAAGTTTGCTTCAACACGTTACATCCAATACACCTTTTAC
CTTCCCAATCAATGGGCTGTGAAATATTCTTATGGATATTGCCTGATAACGACAAGATAACACTGAACTGAACTAGGTA
TTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTTCTCTATCTCTCTTTCTCTCCCTGCCTGACTCTATCT
CTCTCATGTATAAAGCCACCACTGGATATGCCTAAGAGTTTCCCCAATTATTGCGACAACCTTTATATCTATACTTATCTA
AAACGAATACATTGATACGTTATCAAACATATGCTGTTTTGAAATACACTGATGAGGGTGCATCAAGCTAGTAACCGGT
>
Isolation source: *H. crispa*
Number of sequences obtained: 14
Length variation: yes
Primers

>TD152-66
ACGCTATTTCATCTTCAATGAATCATTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTT---------------------
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCTTCCCGTATGTCGATCAGTTCTAAATAAAAATTTGTTGTTGTTTTTTC
CAGGTCACGTGTTTGGAGAATCCGATGTAGTCAGTGAACGACTAGAACCTGGTATATGGTAAGTTGATTTCTTGGAAT
ATATTTGTGTTCTAAATTTCACAATCATTATCTCTGAAATTGGATATTTTGTTTGTTTTGGTTGATTGTGTTCGATGGAT
TTGAAGTCATACAAATTCAGTCCCT

>TD152-72
ACGCTATTTCATCTTCAATGAATCATTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTT-------------------
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCTTCCCGTATGTCGATCAGTTCTAAATAAAAATTTGTTGTTGTTTTT
TCCAGGTCACGTGTTTGGAGAATCCGATGTAGTCAGTGAACGACTAGAACCTGGTATATGGTAAGTTGATTTCTTGGA
ATATATTTGTGTTCTAAATTTCACAATCATTATCTCTGAAATTGGATATTTTGTTTATTTTGGTTGATTGTGTTCGATGG
ATTTGAAGTCATACAAATTCAGTCCCT

>'HC139_F+R_micro' (1,499)
ACGCTATTTCATCTTCAATGAATCATTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTT~~~~~~~~~~~~~~~~~
~~~CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCTTTCCGTATGTCGATCAGTTCTAAATAAAAATTTGTTGTTGTT

TTTTCCAGGTCACGTGTTTGGAGAATCCGATGTAGTCAGTGAACGACTAGAACCTGGTATATGGTAAGTTGATTTCTT
GGAATATATTTGTGTTCTAAATTTCACAATCATTATCTCTGAAATTGGATATTTTGTTTATTTTGGTTGATTGTGTTCGA
TGGATTTGAAGTCATACAAATTCAGTCCC

>TD152-85
ACGCTATTTCATCTTCAATGAATCATTTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTT----------
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCTTCCCGTATGTCGATCAGTTCTAAATAAAAATTTGTTGTTGTTTTT
TCCAGGTCACGTGTTTGGAGAATCCGATGTAGTCAGTGAACGACTAGAACCTGGTATATGGTAAGTTGATTTCTTGGA
ATATATTTGTGTTCTAAATTTCACAATCATTATCTCTGAAATTGGATATTTTGTTTATTTTGGTTGATTGTGTTCGATGG
ATTTGAAGTCATACAAATTCAGTCCCT

>TD152-83
ACGCTATTTCATCTTCAATGAATCATTTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTT----------
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCTTCCCGTATGTCGATCAGCTCTAAATAAAAATTTGTTGTTGTTTTT
TCCAGGTCACGTGTTTGGAGAATCCGATGTAGTCAGTGAACGACTAGAACCTGGTATATGGTAAGTTGATTTCTTGGA
ATATATTTGTGTTCTAAATTTCACAATCATTATCTCTGAAATTGGATATTTTGTTTATTTTGGTTGATTGTGTTCGATGG
ATTTGAAGTCATACAAATTCAGTCCCT

>'HC135_F+R_micro' (1,489)
ACGCTATTTCATCTTCAATGAAACATTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTT~~~~~~~~~~~~~~~~~~
~CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCTTCCCGTATGTCGATCAGTTCTAAATAAAAATTTGTTGTTGTT
TTTTCCAGGTCACGTGTTTGGAGAATCCGATGTAGCCAGTGAACGACTAGAACCTGGTATATGGTAAGTTGATTTCTT
GGAATATATTTGTGTTCTAAATTTCACAATCATTATCTCTGAAATTGGATATTTTGTTTATTTTGGTTGATTGTGTTCGA
TGGATTTGAGGTCATACAAATTCAGTCCC

>'HC151_F_micro_kurz' (1,195)
ACGCTATTTCATCTTCAATGAATCATTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTTCTCTCTCTCTCTCTCTCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCAC

>'HC151_F_micro_kurz' (1,195)
ACGCTATTTCATCTTCAATGAATCATTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTTCTCTCTCTCTCTCTCTCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCAC

>TD152-70
ACGCTATTTCATCTTCAATGAATCATTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTTCTCTCTCTCTCTCTCTCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCTTCCCGTATGTCGATCAGTTCTAAATAAAAATTTGTTGTTGTTT
TTTCCAGGTCACGTGTTTGGAGAATCCGATGTAGTCAGTGAACGACTAGAACCTGGTATATGGTAAGTTGATTTCTTG
GAATATATTTGTGTTCTAAATTTCACAATCATTATCTCTGAAATTGGATATTTTGTTTATTTTGGTTGATTGTGTTCGAT
GGATTTGAAGTCATACAAATTCAGTCCCT
>
>TD152-85
ACGCTATTTCATCTTCAATGAATCATTTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTT----------
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCTTCCCGTATGTCGATCAGTTCTAAATAAAAATTTGTTGTTGTTTTT
TCCAGGTCACGTGTTTGGAGAATCCGATGTAGTCAGTGAACGACTAGAACCTGGTATATGGTAAGTTGATTTCTTGGA
ATATATTTGTGTTCTAAATTTCACAATCATTATCTCTGAAATTGGATATTTTGTTTATTTTGGTTGATTGTGTTCGATGG
ATTTGAAGTCATACAAATTCAGTCCCT

>TD152-83
ACGCTATTTCATCTTCAATGAATCATTTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTT----------
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCTTCCCGTATGTCGATCAGCTCTAAATAAAAATTTGTTGTTGTTTTT
TCCAGGTCACGTGTTTGGAGAATCCGATGTAGTCAGTGAACGACTAGAACCTGGTATATGGTAAGTTGATTTCTTGGA
ATATATTTGTGTTCTAAATTTCACAATCATTATCTCTGAAATTGGATATTTTGTTTATTTTGGTTGATTGTGTTCGATGG
ATTTGAAGTCATACAAATTCAGTCCCT

>'HC151_F_micro_kurz' (1,195)
ACGCTATTTCATCTTCAATGAATCATTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTTCTCTCTCTCTCTCTCTCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCAC

>TD152-70
ACGCTATTTCATCTTCAATGAATCATTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTTCTCTCTCTCTCTCTCTCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCTTCCCGTATGTCGATCAGTTCTAAATAAAAATTTGTTGTTGTTT
TTTCCAGGTCACGTGTTTGGAGAATCCGATGTAGTCAGTGAACGACTAGAACCTGGTATATGGTAAGTTGATTTCTTG
GAATATATTTGTGTTCTAAATTTCACAATCATTATCTCTGAAATTGGATATTTTGTTTATTTTGGTTGATTGTGTTCGAT
GGATTTGAAGTCATACAAATTCAGTCCCT

>TD152-66
ACGCTATTTCATCTTCAATGAATCATTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTT----------
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCTTCCCGTATGTCGATCAGTTCTAAATAAAAATTTGTTGTTGTTTTTTC
CAGGTCACGTGTTTGGAGAATCCGATGTAGTCAGTGAACGACTAGAACCTGGTATATGGTAAGTTGATTTCTTGGAAT
ATATTTGTGTTCTAAATTTCACAATCATTATCTCTGAAATTGGATATTTTGTTTGTTTTGGTTGATTGTGTTCGATGGAT
TTGAAGTCATACAAATTCAGTCCCT

Isolation source: *H. crispa*
Number of sequences obtained: 1
Length variation: na
Primers
>TD152-95

ATAGATGAAAATTCTTGAAAAACTAATATAATCGCTCTCTTCCTCTCTCTTTTATTCGCTTTATCCCCTTCTCGTTTAGTC
TCTGACTCTCTTTCTCTCTCTCTCTAACTCACTCACTCTCTCTCTTTGTCTCTCTCTCTGTCTCTCTCTCTGTCTCTCTCT
ATCGCTCTCTCTCTCGATCCTCCTTCGATCTGGTAGCAATCCTGATCAGGTCATGGATTCTATGACGTCATTCGCCTGCT
>
Isolation source: *E. quadricolor*, *H. Crispa* and ?
Number of sequences obtained: 21
Length variation: yes
Primers

>'EQ501_F&R_micro' (1,551)
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAGTGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCC
AAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATCA
GTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT------
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTATGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGT

>'EQ108_F&R_micro' (1,526)
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAGTGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCC
AAGGATAAAGCGCCTGGACAGTGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATCA
GTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGTC

>'HC113_F+R_micro' (1,533)
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAACATGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCC
AAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAACTATCAAAAATCAG
TCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTACATGAATGCACACTTACATATATATGTAAGACTG
TGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTATCTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGT
AAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATGAGCTGAATAATGTATGAATGTTTGAGGAGGTTGT

>'EQ101_F&R_micro'
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAACATGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCC
AAGGATAAAGCGCCTGGACGGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATTTAAAAACTATCAAAAATCA
GTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT-
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGT

>'HC110_F&R_micro' (1,525)
~~~~~~~~~~~~~~~~~GG-
ACGCTCGGGTGCCTTGAAATGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCCAAGGATAAAGCGCCTGGAC
AGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATCAGTCAGTCAGCCTCTCTCTCT
CTCTCTCTCTCTCTCTCTCTCTCT----
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGT

>'EQ85_F&R_micro'
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAGTGCACGGTTCAGTACACGATCCAGGCATACTCGGATATCC
AAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATCA
GTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGT

>TD152-02
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAGTGCACAGTTCAGTACACGATCCAAGCATATTCGGATATCC
AAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATCA
GTCAGTCAGCCTCTCTCTCTCCCTCTCTCTCTCTCTCTCTCT------
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGTT

>TD152-90
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAGTGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCC
AAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATCA
GTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT------
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCGGGCTTCAAGCTTCAGTCAATGTAAGTGTCAACCTATAAATTGTCGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGGGGAGGTTGTT

>TD152-60
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAGTGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCC
AAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATCA
GTCAGTCAGCCTCTCTCTCTCTCTCCCTCTCTCTCTCTCTCTCT—
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
~~~~~~~~~~~~~~~~~

CTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGTT

>TD152-37
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAGTGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCC
AAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACAATCAAAAATCA
GTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----
CCATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTGCGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGTT

>TD152-30
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAGTGCACAGTTCAGTACACGTTCCAAGCATACTCGGATATCC
AAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATCA
GTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCCCTCTCTCTCTCT—
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAATCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGTT

>TD152-76
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAGTGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCC
AAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATCA
GTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT—
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCCTCAAACTTCCGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGCCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGTT

>TD152-48
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAGTGCACAGTTCAGTACACGATCCAAGCATACTCGGATATTC
AAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAGGTGATCTAAAAACTATCAAAAATCA
GTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCAAACTCCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGTT

>TD152-24
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAGTGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCC
AGGGATAAAGCGCCTGGATAGCGCAGTAGGTCTATAATATCTCGTAAGTGAAAATGATCTAAAAACTATCAAAAATCA
GTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT—
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCGAACTTCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGTT

>'HC143_F+R_micro' (1,511)
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAGTGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCC
AAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGCGATCTAAAAACTATCAAAAATCA
GTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT—
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAACGTATGAATGTTTGAGGAGGTTGT

>Fertig (AG18 micro) 'TD144-35_F+R_Micro' (1,541)
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAGTGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCC
AAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATCA
GTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTACATGAATGCACACTTACATATATATGTAAGACT
GTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTATCTAACGCTGTGCAGGCTTCAAACTTCAGTCAATG
TAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATGAGCTGAATAATGTATGAATGTTTGAGGAGGTTGT

>Fertig (AG18 micro) Contig_1 (1,583)
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAGTGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCC
AAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATCA
GTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT—
ACAAGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGT

>Fertig (AG18 micro) 'TD144-12_F+R_micro' (1,578)
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAGTGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCC
AAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATCA
GTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTACAAGAATGCACACTTACATATATATGTAAGACT
GTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTATCTAACGCTGTGCAGGCTTCAAACTTCAGTCAATG
TAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATGAGCTGAATAATGTATGAATGTTTGAGGAGGTTGT

>'HC126_F&R_micro' (1,844)
AGGGCTCTGACGGCATAGTACGCTCGGGTGCCTTGAAGTGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCC
AAGGATAAAGCGCCTGAACAGCGTAGTAGTTCTATGATATCACGCAAGTGAAAGTGGACTAAAAACTCCCAAAAGTCA
GTCAGTCAGTCAGTGTCATTTTGCTCAGCGTTAGCTGGCAAGTTTCAATTGGACATCAACGAAAATTCTAGAAAACTCA
CCGACAGGCGGTAAAACTTGTTGCTGAGCAAAATGACACGAATGACATTTTCCTCAGCATTTTCCTCACTGTGTGCGGT
~~~~~~~~~~~~~~~~~

ATGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTCGTTGTCGTTGTCGT
TGTCGTTGTCGTTGTCGTTGTYGTYGTYGTTGTYGTYGTTGTCGTTGTCGTTGTCGTTGTCGTTGTCGTTGTC
GTTGTCGTTGTCGTTGTCGTTGTCGTTGTCGTTGTCGTTATTGTTGTTGTTGTTGTTGTTGTTGTTGTTTT
TTGTTGTTGTTATGGGTTGTACTGTGACCGATCTTACACCCAATAGTGTAAAACRACGGCCAGTGAATTGTAATMCGAC
TCACTMTAGGGCGAATTCCAGCACACTGGCGGCCGTTACTAGKGATTTAGAACGACGACCAGANCCNNTAATCAT

>Contig_1 (1,583)
~~~~~~~~~~~~~~~~AGGTACGCTCGGGTGCCTTGAAGTGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCC
AAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATCA
GTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTAACAAGAATGCACACTTACATATATATGTAAGACT
GTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTATCTAACGCTGTGCAGGCTTCAAACTTCAGTCAATG
TAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATGAGCTGAATAATGTATGAATGTTTGAGGAGGTTGT
TTACTCAGGACTCATCAAGGGCGAATTCTGCAGATATCCATCACACTGGCGGCCGCTCGAGCATGCAT-
TAGAGGGCCCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGKACTGAAACCG—

>Contig_1 (1,583)
~~~~~~~~~~~~~~~~AGGTACGCTCGGGTGCCTTGAAGTGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCC
AAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATCA
GTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTAACAAGAATGCACACTTACATATATATGTAAGACT
GTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTATCTAACGCTGTGCAGGCTTCAAACTTCAGTCAATG
TAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATGAGCTGAATAATGTATGAATGTTTGAGGAGGTTGT
TTACTCAGGACTCATCAAGGGCGAATTCTGCAGATATCCATCACACTGGCGGCCGCTCGAGCATGCAT-
TAGAGGGCCCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGKACTGAAACCG--
>

Isolation source: *E. quadricolor*, *H. Crispa*
Number of sequences obtained: 2
Length variation: yes
Primers
>'EQ73_F&R_micro'

AGCTCAACGGCCGCACCCCTCCCTTGCATGCCACATATTCACGCCTATCAAGACACAAGACCCAAAAGCAAGAAGGCTC
TCTCACTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTTCT----
CAATAATTCCATGTGGCAATAATTCTATGTCGCAATAATTTCATGTCGCAATAATTATATGTTTTACAAACATGCACAAC
GCACAGAACATAATGTCATAGAAGAAATGTTTTCATATGGGCTCAGCATGCAATACGTGTGCATCTATATGTCAGTATG
CCGCCTGAATCGATGAAATATAAGACTAGGAAAATTCCTTGAAC

>TD152-49
TTGTTTTACCCCGTACCAGGGACGTGTTTACTGGGTGTGTGTGTGTGTGTGTGTGTGTGTCTGGGTGTGTTCTGC
GTTTGTATAAGGTGTACATCGAGGACACAAGCGAAACCAAAGCACCCCTCTCTTGCATGCCACATATTCACGCCTATCA
AGACACAAGACCCAAAAGCAAGAAGGCTCTCTCTCTCCCTCTTTCTCTCTCTCTCTCTCTCTCTCTCTCTTTCT
CTCTCTCTCAATAATTCCATGTGGCAATAATTCCATGTCGCAATAATTTCATGTCGCAATAATTATATGTTTTACAAACAT
GCACAACGCACAGAACATAATGTCATAGAAGAAATGTTTTCATATGGGCTCAGCATGCAATACGTATGCATCTATATGT
CAGTATGCCGCCTGAATCGATGAAATATAAGACTAGGAAAAGTCCTTGAACT
>
Isolation source: *H. Crispa*
Number of sequences obtained: 1
Length variation: na
Primers

>TD152-96
AGGACATTATCTCAATCCTCTCTCTCTCTCTCGCTCTCTCTCTATCTCTCTCTCTCTCTCTCTCTAATGTAGCTGACAAATG
GCCTTGAGCTACCCGTTCTACATGGGCCCGATGGAAAGTATTTGTTATTATTACGTTTATTGTAGAAATACGTGTAGCA
CAGAGAGGACAAAACAACAAATCATGATTCCTCTTGTAATTTTCAACTTGTAGAGACTCCGGTGGGTAAAGTTGGCTG
TTTATATTTGTGTTATAT

Isolation source: *E. quadricolor*, *H. Crispa* and ?
Number of sequences obtained: 76
Length variation: yes
Primers

>TD152-32
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAA~CGATATAGAAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCG
TTACAATACCAAAAGAACTTCTCGTAGTCTTTCCCCCCGAGTTCTTCTCCTGGCTCTCTCTCTCTCTCTCTCTCTCTCTC
TCTCTCTCTCTTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>'HC149_F+R_micro' (1,487)
----------ACTTATCTGTGGGAAGGTGGAA-
CAATATAGGAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGG
GTTTTCATAGATTTATTATGATCAAATAACTTCGTTACGATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATT
GGGTCGGCTCCGGTGTTCGGCGAAGCAATCCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTC
TCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCT

>TD152-45
~~~~~~~~~~~~~~~~~~~~~~~~

~~~~~~~~~~----------
~AACATATCACTGGTAAGGTGGAA~CGATATAGGAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCC
ATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGTTACAATACCAAAAGAACTTC
GCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGSGAAGCAATCCCTCTCTCTCTCTCTCTCTCTCT
CTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCT------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>micro 04 (1,520)
GCCCTTGATGAAGTCCTGAGTAAACATATCACTGGTAAGGTGGAAACGATATAGAAAGTCCGAAAGTTCGTCTCAACC
TCTGCAGGGTGTTACAGAGCAATAACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTC
GTTACAATACAATAAGGACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCG
CTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGC
GCTACCTTCGTGCACAG
GCCACAGCC

>Contig_1 (1,520)
~~~~~~~~~~~~~~~~~~~~~~~~ACATATCACTGGTAAGGTGGAAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAGAGCAATAACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCG
TTACAATACAATAAGGACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGC
TCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGC
GCTACCTTCGTGCACAGGCCACAGCCT

>Contig_2 (1,362)
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAA~CGATATAGAAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAAAGCAATAACCACACATGAAGACGTCTAGGGTTTTCATAGATGTATTATGATCAAATAACTTCT
CGTAGTCTTTCTCCCCGAGTTCTTCTCCTGGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTNN
AAC

>'EQ100_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~~ACATATCACTGGTAAGGTGGAA~CGATATAGAAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAAAGCAATAACCACACATGAAGACGTCTAGGGTTTTCATAGATGTATTATGATCAAATAACTTCT
CGTAGTCTTTCTCCCCGAGTTCTTCTCCTGGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTMTWCTCTCTCT
CTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCCT

>'EQ86_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~~ACATATCACTGGTAAGGTGGAA~CGATATAGAAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAAAGCAATAACCACACACGAAGACGTCTAGGGTTTTCATAGATGTATTATGATCAAATAACTTCT
CGTAGTCTTTCTCCCCGAGTTCTTCTCCTSKCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTTCTCTCTCTCTC
TCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCCT

>'EQ87_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~~ACATATCACTGGTAAGGTGGAA~CGATATAGGAAGTCCGAAAGTTCGTCTCAACC
TCTGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGACTTATTATGATCAAATAACTTC
GTTACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCC
CTCTCTCTCTCTCCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCCAACCGAGTT
GTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCCT

>'HC147_F+R_micro' (1,489)
~~~~~~~~~~~~~~~~~~~~~~~~ACATATCTGTGGTAAGGTGGAA~CGATATAGAAAGTCCGAAAGTTCGTCTCAACC
TCTGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTC
GTTACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCG
CTCGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCC
GCGCTACCTTCGTGCACAGGCCACAGCC

>micro 04 (1,520)
~~~~~~~~~~~AGTCCTGAGTAAACATATCACTGGTAAGGTGGAAACGATATAGAAAGTCCGAAAGTTCGTCTCAACC
TCTGCAGGGTGTTACAGAGCAATAACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTC
GTTACAATACAATAAGGACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCG
CTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGC
GCTACCTTCGTGCACAGGCCACAGCC

>'EQ83_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~~ACATATCACTGGTAAGGTGGAA~CGATATAGAAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAGAGCAATAACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCG
TTACAATACAATAAGGACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGC
TCTCTCTCTCTCTCTCTTCTCTCTCTCTCCCTCTCTCTCTCTCTCTCTCGCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCC
GCGCTACCTTCGTGCACAGGCCACAGCCT

>'EQ74_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~~ACATATCACTGGTAAGGTGGAA~CGATATAGAAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAGAGCAATAACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCG
TTACAATACAATAAGGACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGC
TCTCTCTCTCTCTCWCTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCAACCGAGTTGTCCACTTGGCTTTCGCCC
CCGCGCTACCTTCGTGCACAGGCCACAGCC-

>'EQ76_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~~ACATATCACTGGTAAGGTGGAA~CGATATAGAAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGATACAGAGCAATAACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATARCTTCG
~~~~~~~~~~~~~~~~~~~~~~~~

TTACAATACAATAAGGACTTCGCGTGGTCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTCCGGCGAAGCAATCKC
TCTCTCTCTCTCTCTCTTYTCTCTCTCTATCTCTCTCTCTCTCTCTCTCTCCAACCGAGTCGTTCACTTGGCTTTCGCCCC
CGCGCTACCTTCGTGCACAGGCCACAGC—

>'EQ106_F&R_micro' (1,528)
~~~~~~~~~~~~~~~~~~~~~~~ACATATCACTGGTAAGGTGGAA~CGATATAGGAAGTCCGAAAGTTCGTCTCAACC
TCTGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTC
GTTACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCC
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCCCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCT
WYCWTMGTGCACAGGCCACAGCC-

>'HC114_F+R_micro' (1,473)
~~~~~~~~~~~~~~~~~~~~~~~ACATATCACTGGTAAGGTGGAA~CGATATAGAAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCG
TTACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGC
TCGCTCTCTCTCTCTCTCTCTCTCTCTCTTTTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTA
CCTTCGTGCACAGGCCACAGCC-

>'EQ110_F&R_micro' (1,475)
~~~~~~~~~~~~~~~~~~~~~~~ACATATCAGTGGTAAGGTGGAAA~GATATAGAAAGTCCGAAAGTTCGTCTCAACC
TCTGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTC
GTTACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCC
CCGCGCTACCTTCGTGCACAGGCCACAGCC

>'EQ66_F&R'
~~~~~~~~~~~~~~~~~~~~~~~ACATATCACTGGTAAGGTGGAA~CGATATAGAAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAGAGCAATAACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCG
TTACAATACAATAAGGACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGC
TCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCTCTCTCTCCCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCC
CCCGCGCTACCATAGTGCACAGGCAGTGAGCC

>'EQ111_F&R_micro' (1,461)
~~~~~~~~~~~~~~~~~~~~~~~ACATATCACTGGTAAGGTGGAA~CGATATAGAAAGTCCGAAAGTTCGTCTCAACCT
CTGATGGGTGTTACAAAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCG
TTACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTCATTGGGTCGGCTCCGGTGTTCGGCGAAGCGATCGC
TCGCGCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCC
GCGCTATCCTTCGTGCACAGGCCACAGCC

>'HC115_F+R_micro' (1,452)
~~~~~~~~~~~~~~~~~~~~~~~ACATATCACTGGTAAGGTGGAA~CGATATAGAAAGTCCGAAAGTTAGTCTCAACCT
CTGATGGGTGTTACAAAGCCATAACCACACATGAAGACGTCTAGGGTTTTCTTAGATCCATTTTGATCAAATAACTTCG
TTACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGC
TCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACA
GGCCACAGCC-

>'EQ93_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~ACATATCACTGGTAAGGTGGAA~CGATATAGAAAGTCCGAAAGTTAGTCTCAACCT
CTGATGGGTGTTACAAAGCCATAACCACACATGAAGACGTCTAGGGTTTTCACAGATTTATTATGATCAAATAACTTCG
TTACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGC
TCTCTCTCTCTCTCTTCTCTCTCTCTCTTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCT
ACCTTCGTACACAGGCCACAGCCT

>'EQ88_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGGAAGGTGGAAACGATATAGAAAGTCCGAAAGTTCGTCTCAACC
TCTGCAGGGTGTTACAGAGCAATAACCACACACGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTC
GTTACAATACAATAAGGACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCG
CGCTCTCTCTCTCTCTTTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCC
CCGCGCTACCTTCGTGCACAGGCCACAGCC

>'EQ69_F&R_micro'
~~~~~~TCTCAACCTC
TGCAGGGTGCTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGAATTACTATGATCAAATAACTTCGT
TACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCCCTC
TCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCCTCTCTCTCTTCTCTCTCTCTCCCTCCAACCGAATTGTTCACTTGGCTTTCG
CCCCCGCGCTA---------------------

>TD152-23
~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAAAGCAATAACCACACATGAAGACGTCTAGGGTTTTCATAGATGTATTATGATCAAATAACTT------
----
CTCGTAGTCTTTCTCCCCGAGTTCTTCTCCTGGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTC
TCTCTCT--------------------------------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-38
~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAAAGCAATAACCACACATGAAGACGTCTAGGGTTTTCATAGATGTATTATGATCAAATAACTT------
~~~~~~~~~~~~~~~~~~~~~

CTCGTAGTCTTTCTCCCCCGAGTTCTTCTCCTGGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTC
TCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGACTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-47
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAAAGCAATAACCACACATGAAGACGTCTAGGGTTTTCATAGATGTATTATGATCAAATAACTT------
----
CTCGTAGTCTTTCTCCCCGAGTTCTTCTCCTGGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTC
TCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-61
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAAAGCAATAACCACACATGAAGACGTCTAGGGTTTTCATAGATGTATTATGATCAAATAACTT------
----
CTCGTAGTCTTTCTCCCCGAGTTCTTCTCCTGGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTC
TCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCCT

>'EQ86_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAAAGCAATA
ACCACACACGAAGACGTCTAGGGTTTTCATAGATGTATTATGATCAAATAACTT----------
CTCGTAGTCTTTCTCCCCGAGTTCTTCTCCTSKCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTTCTCTCTC
TCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCCT

>'EQ100_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAAAGCAATA
ACCACACATGAAGACGTCTAGGGTTTTCATAGATGTATTATGATCAAATAACTT----------
CTCGTAGTCTTTCTCCCCGAGTTCTTCTCCTGGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTMTWCTCTCT
CTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCCT

>TD152-13
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAAAGCAATAACCACACATGAAGACGTCTAGGGTTTTCATAGATGTATTATGATCAAATAACTT------
----
CTCGTAGTCTTTCTCCCCGAGTTCTTCTCCTGGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTC
TCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCCT

>'EQ504_F&R_micro' (1,444)
~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAAAGCAATA
ACCACACATGAAGACGTCTAGGGTTTTCATAGATGTATTATGATCAAATAACTT----------
CTCGTAGTCTTTCCCCCCGAGTTCTTCTCCTGGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----------
----------CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>'HC122_F+R_micro' (1,455)
~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAAAGCAATA
ACCACACATGAAGACGTCTAGGGTTTTCATAGATGTATTATGATCAAATAACTT----------
CTCGTAGTCTTTCTCCCCGAGTTCTTCTCCTGGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTC
TCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCATAGCGCCGGCCAGTGAGCCTTAATCAGGCTCCATCAAG
GGCGAATTCCAGCACACTGGCGGCCGTTACTAGGGATGCGAGCGACGNCCAGTNCNN----------

>TD152-59
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGGAAGTCCGAAAGTTAGTCTCAACCT
CTGATGGGTGTTACAAAGCCATAACCACACATGAAGACGTCTAGGGTTTTCTTAGATCCATTTTGATCAAATAACTTCG
TTACAATACCAAAGGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGC
TCTCTCTCTCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCCT

>TD152-55
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAATGTGGAACGATATAGAAAGTCCGAAGGTTCGTCTCAACCTC
TGCAGGGTGTTACAGGGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGT
TACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCT
CTCTCTCTCTCTCTCTTCTCTCTCTCTTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCCT

>TD152-16
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTAGTCTCAACCTC
TGATGGGTGTTACAAAGCCATAACCACACATGAAGACGTCTAGGGTTTTCTTAGATCCATTTTGATCAAATAACTTCGT

TACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCT
CTCTCTCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-33
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAAAGCAATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGT
TACAATACCGAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCCCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTG
GCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-93
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCATTGGTAATGTGGAACGATATAGAAAGTCCGAAGGTTCGTCTCAACCTC
TGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGT
TACAATACCAAAAGAACTTCGCGTGACCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCT
CTCTCTCTCTCTCTCTTTCTCTCTCTCTTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-62
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGGAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCG
TTACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCCCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-74
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAGAGCAATAACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCGT
TACAATACAATAAGGACTTCGCGTTATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCT
CTCTCTCTCGCCTCTCTCTCTCTCTCTCTCTCTCTCTTTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>Contig_1 (1,520)
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAGAGCAATAACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCGT
TACAATACAATAAGGACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCT
CTCTCTCTCTCTCTCTTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-91
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGGAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCG
TTACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCCCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCACTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-80
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGGAAGTCCGAAAGTTCGTCTCAGCCT
CTGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCTAATAACTTCG
TTACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCCCC
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCT----
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-53
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGT
TACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCT
CGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-87
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAGAGCAATAACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTACCAAATAACTTCGT
TACAATACAATAAGGACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCT
CTCTCTCTCTCTCTCTTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGC

>TD152-57
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGT
TACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCT
CGCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-11
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAGAGCAATAACCACACATGAAGACGTCTAGGGATCTCATAGATGTATTATTATCAAATAACTTCGT
TACAATACAATAAGGACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCT
~~~~~~~~~~~~~~~~~~~~~~~~

CTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-08
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGGAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCG
TTACAGTACCAAAAGAACTTCGCGTGATCTTCTGACGACAGWTATTGGGTCGGCTCCGGTGTTCGGSGAAGCAATCCC
TCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCC----
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>'EQ65_-F_&_R_micro'
~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTAGTCTCAACCTCTGATGGGTGTTACAAAGCCATA
ACCACACATGAAGACGTCTAGGGTTTTCTTAGATCCATTTTGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGCG
TGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCTCTCTCTCTCTCTCTCTCTCTC
TCTCTCT------------------------------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>Micro 05 (1,535)
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTAGTCTCAACCTC
TGATGGGTGTTACAAAGCCATAACCACACATGAAGACGTCTAGGGTTTTCTTAGATCCATTTTGATCAAATAACTTCGT
TACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGWTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAMTCGCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCT------------------------------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>'HC156_F+R_micro_' (1,476)
~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCCATA
ACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGC
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCGCTCTCTCTCTCTCTCTCTC
TCTCTCTCTCTTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGC

>Micro 05 (1,535)
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTAGTCTCAACCTC
TGATGGGTGTTACAAAGCCATAACCACACATGAAGACGTCTAGGGTTTTCTTAGATCCATTTTGATCAAATAACTTCGT
TACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGWTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAMTCGCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCT------------------------------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>'HC138_F+R_micro' (1,462)
~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCCATA
ACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGT
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCGCTCTCTCTCTCTCTCTCTC
TCTCTCTCTCTCTTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>'HC107_F&R_micro' (1,463)
~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGCGTTACAGAGCAATA
ACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCGTTACAATACAATAAGGACTTCGC
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCTCTCTCTCTCTCTCCTCTCTC
TCTCTCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>'EQ102_micro' (1,491)
~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGGAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCCATA
ACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGC
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCCCTCTCTCTCTCTCTCTCTCTCTCT
CTCTCCCTCTCTCTCTCTTCTCTCTCTCTCT------
CCAACCGAGTTATTCACTTGGCTTTCGCCCCCGCGCTAGTATAGTGCGCAGGCAGTGAGCCTTAATCAGGCCCCATCAA
GGGCGAATTCCAGCACACTGGCGGCCGTTACTAGGGATGCGAACTACGT----------

>'EQ84_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~AACATATCAGTGGTAAGGTGGAACGATATAGGAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCG
TTACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCCCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCCTCTTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-42
~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTAGTCTCAACCTC
TGATGGGTGTTACAAAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGT
TACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTGTTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCT
CGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-43
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCATTGGTAATGTGGAACGATATAGAAAGTCCGAAGGTTCGTCTCAACCTC
TGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGT
TACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCT
CTCTCTCTCTCTCTTCTCTCTCTCTTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACGGCC

>TD152-50
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGGAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCG
TTACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCCCC
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCT------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-52
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGCGTTACAAAGCAATAACCACACATGAAGACGTCTAGGGTTTTCATAGATGTATTATGATCAAATAACTTCGT
TACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCT
CGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC
>TD152-19
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAGAGCAATAACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCGT
TACAATACAATAAGGACTTCGCGTGATCTTTTGACGACAGTTATTGGGTTGGCTCCGGTGTTCGGSGAAGCAATCGCT
CTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCGCAGGCCACAGC

>TD152-78
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATCTATTATGATCAAGTAACTTCGT
TACAATACCAAATGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCT
CTCTCTCTCCCTCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTCGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGC

>TD152-35
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAGAGCAATAACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCGT
TACAATACAATAAGGACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGSGAAGCAATCGCT
CTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGC

>TD152-20
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAGAGCCATAACCACGCATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGT
TACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCT
CGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCT----------
CCAGCCGAGTTGTTCGCTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC
>TD152-29
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAGAGCAATAACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCGT
TACAATACAATAAGGACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGSGAAGCAATCGCT
CTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCCTCGTGCACAGGCCACAGCC

>TD152-44
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGGAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCG
TTACAATACCGAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGC
TCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>'HC147_F+R_micro' (1,489)
~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCTGTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCCATA
ACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGC
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCGCTCTCTCTCTCTCTCTCTC
TCTCTCTCTCTTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>'EQ87_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGGAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCCATA
ACCACACATGAAGACGTCTAGGGTTTTCATAGACTTATTATGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGC
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCCCTCTCTCTCTCTCCCTCTCTCTCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCG
CTACCTTCGTGCACAGGCCACAGCCT
~~~~~~~~~~~~~~~~~~~~~~~~

>'EQ83_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCAATA
ACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCGTTACAATACAATAAGGACTTCGC
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCTCTCTCTCTCTCTTCTCTCTCTC
TCCCTCTCTCTCTCTCTCTCGCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCCT

>'EQ74_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCAATA
ACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCGTTACAATACAATAAGGACTTCGC
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCTCTCTCTCTCTCTCWCTTCTCTCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTCCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC-

>'EQ76_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGATACAGAGCAATA
ACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATARCTTCGTTACAATACAATAAGGACTTCGC
GTGGTCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTCCGGCGAAGCAATCKCTCTCTCTCTCTCTCTCTTYTCTCT
CTCTATCTCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTCGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGC—

>'EQ106_F&R_micro' (1,528)
~~~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGGAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCCATA
ACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGC
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCCCTCTCTCTCTCTCTCTCTCTCTCT
CTCTCTTCTCTCTCTCCCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTWYCWTMGTGCACAGGCCACAGCC-

>'HC114_F+R_micro' (1,473)
~~~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCCATA
ACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGC
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCGCTCTCTCTCTCTCTCTCTC
TCTCTTTTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC-

>'HC115_F+R_micro' (1,452)
~~~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTAGTCTCAACCTCTGATGGGTGTTACAAAGCCATA
ACCACACATGAAGACGTCTAGGGTTTTCTTAGATCCATTTTGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGCG
TGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCTCTCTCTCTCTCTCTCTCTC
TCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC-

>'EQ93_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTAGTCTCAACCTCTGATGGGTGTTACAAAGCCATA
ACCACACATGAAGACGTCTAGGGTTTTCACAGATTTATTATGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGC
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCTCTCTCTCTCTCTTCTCTCTC
TCTCTTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTACACAGGCCACAGCCT

>'EQ88_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGGAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAGAGCAATAACCACACACGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCG
TTACAATACAATAAGGACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGC
GCTCTCTCTCTCTCTCTTTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

Isolation source, *H. Crispa*
Number of sequences obtained: 1
Length variation: na
Primers

>
~~~~~~~~~~~~~~~~~~~~~~
>TD152-56
GGTCCCTCAGTTCCTGTCGTACGCCTTCAGTAAGGGCTCTGACGGCATGCTTCGTTGCACAGTAGAAATGGAGACCTG
CACCTCCTGTGACTTGGTGGCCGACAATACTGTAAGAAACAATAAGGTGTTTCTGAGTACACACACACACACGTACACA
CACACGTACATGCATGTGCACACGCACACGCACACGCACACGCACACACACGCACACACACACACACACCTCTGTAAAT
ATATATATACACATATATACACATATATATATATACACATACAGTGAAACCTCACTTTTCGTACAGTCCCCTTTTCGTACGCTTCACTT
TTCGTTCACTTTTTTCGTCCTATTTTTGTCTCACTTTTCGTACATTGCCTCATATTTCGTACTTTTTTTTGTT
>
~~~~~~~~~~~~~~~~~~~~~~

Isolation source, *H. Crispa*
Number of sequences obtained: 1
Length variation: na
Primers
>TD152-34
ACACAAAATACAAAACTAGATTAGATTTGTTCCGATCCTGGAACTCATGAATAAATTTGTACTATTGGTAGGGGGAGG
CTGGATGGCCTATGCTGTTTCTTGCAGTCAGAGCTCATCCATAGACAACATGCACACCCGAATGTACATGCACACACAA
ACACACACACACACACACACAAACACACGAACACACACACACACAATTGCGGACATACACACAAAACAGTACACACAAAT
ACGTATAAAGACATGTGTACACACATATGCAATCAAACACTAACAAACAAACACATACAAACACAAACATGTAAACACA
CATACACACGTTTTTCTTTCCT
>
Isolation source, *H. Crispa*
Number of sequences obtained: 1
Length variation: na
Primers
>TD152-03
ACTACTTCTTCTCAAAAACATCTTCTCAAAAACACACACACACACACACACACACACACACATATATAGGTTCTTCATATA
TTATCCAGCAATTCCTTCTAATACCAAAAACCACTAGGCCTATCTCAAATGACAAGCTAATAAATGAAAACAGTGTTTAC
TGTATCACAAAATGGTCCAAATTGCATAAGGCATTGGTTTATCAAGCAATAAATAGAATCAAATAACAATTCATAAGAT
TATTTTTTGAAATTCCAAAATATCATTTTTAGTGCCACATGGTACTTTCTGGAGAAATGTGTGGTCCT
>
Isolation source, *H. Crispa*
Number of sequences obtained: 2
Length variation: yes
Primers

>'HC109_F&R_micro' (1,430)
GAATTCGCCCTTGATGAGTCCTGAGTAAACACACCCCTTTAGTCCTCCCCCACCTCCACACACACACACACGCACACACA
CACACACACATCTGGCCACACCCACACATGTACATACATACCCCTACACACCCGGCCACAATAGAGGGAGGTTGAGGGT
TAGCTTTAGGATTTTTCTGGTCTTCCATTTCTACTCTACATTTCATTTTCATTTACTGTGGTGATATTCTCACGATTCCTT
TCTCTTCCTTTTCTTTTCTTATTACCGATGCATTGAATGTGATAGGTAACATATAGGTCGGTACTATTTCATAAACAAAC
AAACAAACACACACACACATTCATCTGTCC

>TD152-77
----------ACACACCCCTTTAGTCCTCCCCCACCTCCACACACACACACACACGCACACACACACACA--
TCTGGCCACACCCACACATGTACATACATACCCCTACACACCCAGCCACAATAGAAGGAGGTTGAGGGTTAGCTTTAGG
ATTTTTCTGGTCTTCCATTTCTACCCTACATTTCATTTTCATTTACTGTGGTGATATTCTCACGATTCCTTTCTCTTCCTTT
TCTTTTCTTATTACCGATGCATTGAATGTGATAGATGACATATAGGTCGGTACTATTTCATAAACAAACAAACAAACACA
CACACACATTCATCTGTCCT

Isolation source, *H. Crispa*
Number of sequences obtained: 1
Length variation: na
Primers >

>TD152-79
GGCAGATTACTCATGCTCGTAAGGATAAGCGTTTTATTCCATTGTTCTCGTTGGTGCCTAACAAGACTCTCCAATATAC
AGTAATCACACAAACACGTGCAGGCATAGACTCACACACACACACACACACACACACACACACACACACACACACACA
CGCCCGCTTTCTAGCTCTCTGTATTTTACTCGCTCTCTTTTGCTCTCTCCCTCTCTCTCTTGCTCACTCTCTCATTTGTTGT
CTTT
>
Isolation source, *H. Crispa*
Number of sequences obtained: 1
Length variation: na
Primers
>TD152-84
AGGCTAGGATACAATTGCTGCTGGGAAATTATAAACCCTGCAAACAAAAACTGTAATCACTGTCAAACAAACACAGAG
CAGCCTCTCTTACACTATTTACTGGAATGCCACATAACAGAGCAACTCAGAGAAAACTTCAATGAAAGCTGCAATATAA
CTAGTCTCTATGCAAGAAAAACAGCTTCTCTCTTGGTTAGACACATTTTCTTCCAAATGGACAGACTCCAAAATATCCTC
CAAACCTTCCTGCCTCCAAGATAACTATAAAAAGAACCATCCTTACCCTTACTCTTATCCTAACCCAACTCCTTATCTTCTC
CTATCAACACACATACACACTTATGTACATGCGTAACACACACACACACACACACACACACACACACACACACACACA
CCACATGCACATAAGTGCACCCACACCTAAATCATATTTCACACAATTCATTCTATCCTGATGCCCTAGGGCCTAGGGGC
>
Isolation source, *H. Crispa*
Number of sequences obtained: 1
Length variation: na
Primers
>TD152-86
AGGTACTCAAACTATTCTGTTTTCAGATTTATTTTATTTCTGTCCGGATCGTCCAGACAATTATTTTTGACAGTAGGTAC
CTGGGTCACCAATACACACGCTCACCCACACACACACACACACACACACACACACACAACTCAAAAACACACACAACTAAAACA
CACACACAACTCAAACACACACACACGCACACAACTAAAACACACACACACAACTCAAACACACACACACACAAATCACA
TACACACACAACTCAAAACTGGAGATCCAGATCATCCCGAGCAGATAGAAGTTTTGTATAACCGTCAACAACTACGATT
TATTGTATTCTAACGATCCATACATGCGGGTCGTCTAAGGCCATGTACCGGCGCCAGTATATGACTGGC
>

> Isolation source, *H. Crispa*
Number of sequences obtained: 3
Length variation: yes

Primers

>'HC104_F&R_micro' (1,563)
GTGTGCTTACAAACATGTACACTACATACCCCATACATTACTTGAGCAATAACAAATCCCCCCCCCCCCACACACACACAC
ACACACACACACACACCA----------
GCTCTCCTCTGATATATGAAGCTTTATACTTTGTATAATGGGTAACTTTCGCTACCAAAAAAA-
TGTTTTATACGTCTTTTTAGAAAACCTTTAGCCTTTTGCATAGAATATTGTTCCTGCTTTTTTCAATAAGCATGAACATAA
ATGTTCCTACTGCACTGCCTTGCAGGGAACAATTTGAAACCCCCAGTCAAACACGACTGGAGAAGTTGGGATTTTCTTC
ACTCCCACAGCTGGATAACAATTATGTTTGCGGGGTCTTCACTTGCATGTACCCTAGGCACCTGGCAGAACTGGGGTT
GT

>Fertig (GT23G10 micro) TD152-21
GTGTGCTTACAAACATGTACACTACATACCCCATACATTACTTGAGCAATAACAAATCCCCCCCCCCCACACACACACACA
CACACACACACACACACACACACACACACACACACCAGCTCTCCTCTGATATATGAAGCTTTATACTTTGTATAATGGGTAA
CTTTCGCTACCAAAAAAAAATGTTTTATACGTCTTTTTAGAAAACCTTTAGCCTTTTGCATAGAATATTGTTCCTGCTTTTT
TCAATAAGCATGAACATAAATGTTCCTACTGCACTGCCTTGCAGGGAACAATTTGAAACCCCCAGTCAAACACGACTGG
AGAAGTTGGGATTTTCTTCACTCCCACAGCTGGATAACAATTATGTTTGCGGGGTCTTCACTTGCATGTACCCTAGGCA
CCTGGCAGAACTGGGGTTGTC

>TD152-09
GTGTGCTTACAAACATTTACACTACATACCTTTTACATTACTGTAGCAATTGCCCTCCACCCCCCACCCACACACACACCC
ACACACACACACACACACCCACACCA--------
GCTCTCCTCTGATATATGAAGCGTTATACTTTGTATAATGGGTAATGTCCGCTACCAAAAAATAAGTTTTATACGTCTTT
CTCGAAAACCTTTAGCCTTTTGGATAGGATATTTTTCCTGCTTTTTTCAATAAGCATGAACATAAATGTTACTACTGGAC
AGGTCCGCAGGGAACAATCTGAAACCCTCAGTCAAACACGACTGGAGAAGTTGGGATTTTCTCCACCCCCACAGCCGG
ATAACAATTATGTTTGCGGGGTCTACACTTGCATGTACTCTAGGTACCTGGCTGAACTGGGGTTGTT
>

Isolation source, *H. Crispa*
Number of sequences obtained: 1
Length variation: na
Primers
>TD152-88
GGGCAATAAATTCGACGGGAATTATAGATAATAGTTCGTTGTATCTTTTTGTAAATGACTTAGACCATAACCTTAGGAA
GAACTGTATCGACAGCATCGAATCCACCAAATAACAGGAGTCGAGAGATGGTTCGAACGGGCAAAAAATGAGGGTGT
GCATACTTAGGGTACGCGTGCGCACTCACACGCTCATGTACATACACATACACACACACTCACACACACACACACACA
CACACACACCCACACACACACACGCACACACACACACACACACACACACACACACACACACAGCAATGTGTATGGTCGCACGCA
CATGTGTTGAAGGAGTATCATCGAAAATTAGAGTGGTTGTTGATTTTGGGAAGTT
>
Isolation source, *H. Crispa*
Number of sequences obtained: 2
Length variation: yes
Primers
>TD152-51
AGGTCATATACCCGCGCTAGTATATGACTGGCTTTCTACAAAAACAAAAACAACAACAACAAAGACAACAACAACAACA
AAAACAACAACAACAACAACAACAACAACAACAACAAAAACAACAACAACAACAAAAACAACAACAAAAACAACAA
CAACAGCAAAAAAAACAAAACAACAACAACAAATAAAAACAACAACAACAACAGCATACTGAAGTGGTATGCATGAATT
TATAATTCAACCGCAGGTTCCATTCCCCCTAGACACTCGGCTGT

>Fertig (GTT28 micro) TD152-06
GGTCATATACCCGCGCTAGTATATGACTGGCTTCCTACAAAAACAAAAACAACAACAACAACACCAACAACAACAACAA
CAACAAAAACAACAACAACAACAACAACAACAACGACAACAACAACAACAACAACAACAACAACAACAGCTTTGTATGG
ATCCCGAGCCAGATAAGGTTCCACGAAAGCCTCGTGCCGGTGAATCTGTCCAATAAAAACAAAATGGACGACTGGACT
AAACCTAAACCTAACCCTCAACCTTCAACCACGTGTCTGTCTGTGATTGATGCTCCAAAATCCTCCCATCAGGTCTCATG
ATATTCTGAGGATGTTCTCCTCGTGGT

Isolation source, *H. Crispa*
Number of sequences obtained: 1
Length variation: na
Primers

>'HC151_R_micro_schlecht' (1,534)
GGGACTGAATTTGTCTGACTTCAAATCCATCGAGCACAATCAACCAAAATAAACAAAATATCCAATTTCAGAGATAATG
ATTGTGAAATTTAGAACACAAATATATTCCAAGAAATCAACTTACCATATACCAGGTTCTAGTCGTTCACTGACTACATC
GGATTCTCCAAACACGTGACCTGGAAAAAACAACAACAAATTTTTATTTAGAACTGATCGACATACGGGAAGGAGAGA
GAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGGGGAGAGAGA
CACACACAAAAAGAGTGTGTGAGAGAAAGAGAGCGCGCGCGCGAGAGAGAGCGCGCGCTCTCTCTCTCTCTCTCT
CGCGCGCGCGGGCGGGCGCGCGGATATATAGCGAGCGCCCCTATAGAGAGAGAGAGAGAGAGACTTTATATCTCTCTCT
ACATATATATACANA
>

Isolation source: *E. quadricolor*, *H. Crispa*
Number of sequences obtained: 2
Length variation: yes
Primers
>'EQ81_F&R_micro'
GGTTT-
CTTTCACATAAACTGGTACTGTGTATCCAACCTGTAAGTTTGCTTCAACACGTTACATCCAATACACCTTTTACCTTCCCA

ATCAATGGGCTGTGAAATATTCTTATGGATATTGCCTGATAACGACAAGATAACACTGAACTGAACTAGGTATTCTCTC
TCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTTCTCTATCTCTCTTTCTCTCCCTGCCTGACTCTATCTCTCTC
ATGTATAAAGCCACCACTGGATATGCCTAAGAGTTTCCCCAATTATTGCGACAACCTTTATATCTATACTTATCTAAAAC
GAATACATTGATACGATATCAAACATACATGTATTTGAAATACGCTGATGAGGGTGCATCAGGCTAGTAACCGGTGT

>TD152-04
AGGCTTCTTTCACATAAACTGGTACTGTGTATCCAACCTGTAAGTTTGCTTCAACACGTTACATCCAATACACCTTTTAC
CTTCCCAATCAATGGGCTGTGAAATATTCTTATGGATATTGCCTGATAACGACAAGATAACACTGAACTGAACTAGGTA
TTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTC--
TTTCTCTATCTCTCTTTCTCTCCCTGCCTGACTCTATCTCTCTCATGTATAAAGCCACCACTGGATATGCCTAAGAGTTTC
CCCAATTATTGCGACAACCTTTATATCTATACTTATCTAAAACGAATACATTGATACGTTATCAAACATATGCTGTTTTGA
AATACACTGATGAGGGTGCATCAAGCTAGTAACCGGT
>
Isolation source: *H. Crispa*
Number of sequences obtained: 1
Length variation: na
Primers
>TD152-22
ATCACAAACAGAATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTKCTTCTGCTTCTGCTTCTGCTT
CTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCT
TCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTYTTCTTCTTCTTCTTCTTCTTCTTCTTCTT
CTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCCTCCGTCCATGTAATGCTTTAGTAT
AAAGACCAATCACCAGAGGGCGTCCT
>

Isolation source: *E. quadricolor*, *H. Crispa*
Number of sequences obtained: 1
Length variation: na
Primers >

>TD152-31
AGACACGAGGGCAAAAGGCCTAAAGTACGCGTCGCTTGCCATTGTGCCTTAGGCCTCTATGACTGCACTAAAAGCCTC
CTGAGATCGATGTTACTAATGGAAGGGACTCATTCTCGATACTACCAGCTTCCTGGCTCTCTCTCTCTCTCTCTCTCT
CTCTCTCTCTCTCTCTCGCTCTCTCCCTCTCTACCTCTCTTTCTCTTTCTCGTTCTCTCTTTCTCCTTCTTTCTTTCTCTCTCT
ATTTATCTTTCTAAGTGCTGTGTGGGTGTCC
>
Isolation source: *E. quadricolor*
Number of sequences obtained: 2
Length variation: yes
Primers
>'EQ71_F&R_micro'
AACGGTCGTCGTTCTCATCCACTAGTAACGGCCGCCAGTGTGCTGGAATTCGCCCTATAGTGAGTCGTATTACAATTCA
CTGGCCGTCGTTTTACTCCTGTAGCGACATGGTATCCCATGATCGCTACAGCTAAAAAAAAAGAACCAACGGCTCAGTTT
TTCCCTCACCCCCCAACCCCCACCACAAGGTAATAAATAGGATTACCTGACCTGACCTCTTTCTCACTTCTTCTTTTCACT
AAGTCCAGTCAACAGCCTCTCTCTCTCTTTCTCTCTCTCTYTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTC
TCTCTCTCTCTCTCTCTCTCTCTCTCCGTACAGCAGTAACCCCGCCAAACGTCTTATCACCTACAGCTGCAGCACCCACGT
TGTCTAT

>Fertig (GA37 micro) 'TD144-36_F+R_Micro' (1,486)

AACCCCAGGGGAAGGGGGAATCCTGTAGCGACATGGTATCCCATGATCGCTACAGCTAAAAAAAAAGAACCAACGGCT
CAGTTTTTCCCTCACCCCCCAACCCCCACCACAAGGTAATAAATAGGATTACCTGACCTGACCTCTTTCTCACTTCTTCTT
TTCACTAAGTCCAGTCAACAGCCTCTCTCTCTCTTTCTCTCTCTCTTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTC
TCTCTCTCTCTCTCTCT----------
CCGTACAGCAGTAACCCCGCCAAACGTCTTATCACCTACAGCTGCAGCACCCACGTTGTCTAC
>

Isolation source: *E. quadricolor* and ?
Number of sequences obtained: 5
Length variation: yes
Primers

>'EQ67_F&R_micro'
ACCCTAAAGGTGTTTATTTTGATGTCTCTAATTGTCGTCGATGGACGAAGTGCGCACGCAAGATTGCACGACTTCCAAA
AATAATTGTTTTTTCTCAAAATATTATGG-
ATCTTCGAGAACAAGAGGGCTTTGAAAATACACCACGATGTGTTGAGGTGTAGAGATAAGGAAAACATACAGAGAAA
TATAAAAATATGCACACTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCATGGCCCAGGCCTCTCTTTCACAGTCGTAA
TATAAATGTATGTAATAGTTTTCTGCATAATATTATTGTAGATATCTACCAGGTATACTGAATAACCCACGGTTGGTGA
TGAAAGATGCGTTGATATGTTGAAAATAGAACCTCGACTAATCTCGAGTACCCAATATCATCTGATTTTGTCATCAGGG
CGTACGGTGCTACACACTATCGAGTTGCACCAT

>Fertig (GA16 F micro) 'TD149-01+23_(144-07)' (1,672)
~~~CCTAAAAGTGTTTATTTTGATGTCTCTAATTGTCGTCGATGGACGAAGTGTGCACGCAAGATTGCACGACTTCTAA
AAATAATTGTTTTTTCTCAAAATATTATGGGATCTTCGAGAACAAGAGGGCTTTGAAAATACACCACGATGTGTCGAGG
TGTAGAGATAAGGAAAACATACAGAGAAATATAAAAATATGCACTCTCTCTCTCTCTCTCTCTCTCTCTCT~~~~C
CATGGCCCAGGCCTCTCTTTCACAGTCGTAATATAAATGTATGTAATAGTTTTCTGCATAATATTATTGTAGATATCTAC
CAGGTATACTGAATAACCCACGGTTGGTGATGAAAGATGCGTTGATATGTTGAAGATAGAACCTCGACTAATCTCGAG
TACCCAATATCATCTGATTTTGTCATCAGGGCGTACAGTGCTACACGCTATCGAGTTGCACCAC
~~~

>Fertig (GA16 F micro) 'TD144-07_F+R_micro' (1,636)
~~~CCTAAAAGTGTTTATTTTGATGTCTCTAATTGTCGTCGATGGACGAAGTGTGCACGCAAGATTGCACGACTTCTAA
AAATAATTGTTTTTTTCTCAAAATATTATGGGATCTTCGAGAACAAGAGGGCTTTGAAAATACACCACGATGTGTCGAGG
TGTAGAGATAAGGAAAACATACAGAGAAATATAAAAATATGCACTCTCTCTCTCTCTCTCTCTCTCTCTCT~~~~C
CATGGCCCAGGCCTCTCTTTCACAGTCGTAATATAAATGTATGTAATAGTTTTCTGCATAATATTATTGTAGATATCTAC
CAGGTATACTGAATAACCCACGGTTGGTGATGAAAGATGCGTTGATATGTTGAAGATAGAACCTCGACTAATCTCGAG
TACCCAATATCATCTGATTTTGTCATCAGGGCGTACAGTGCTACACGCTATCGAGTTGCACCAC

>Fertig (GA16 F micro) Micro 01b (1,671)
AACCCTAAAAGTGTTTATTTTGATGTCTCTAATTGTCGTCGATGGACGAAGTGTGCACGCAAGATTGCACGACTTCTAA
AAATAATTGTTTTTTTCTCAAAATATTATGGGATCTTCGAGAACAAGAGGGCTTTGAAAATACACCACGATGTGTCGAGG
TGTAGAGATAAGGAAAACATACAGAGAAATATAAAAATATGCACTCTCTCTCTCTCTCTCTCTCTCTCTCT~~~~C
CATGGCCCAGGCCTCTCTTTCACAGTCGTAATATAAATGTATGTAATAGTTTTCTGCATAATATTATTGTAGATATCTAC
CAGGTATACTGAATAACCCACGGTTGGTGATGAAAGATGCGTTGATATGTTGAAGATAGAACCTCGACTAATCTCGAG
TACCCAATATCATCTGATTTTGTCATCAGGGCGTACAGTGCTACACGCTATCGAGTTGCACCAC

>Fertig (GA16 F micro) Contig_1 (1,671)
~~~CCTAAAAGTGTTTATTTTGATGTCTCTAATTGTCGTCGATGGACGAAGTGTGCACGCAAGATTGCACGACTTCTAA
AAATAATTGTTTTTTTCTCAAAATATTATGGGATCTTCGAGAACAAGAGGGCTTTGAAAATACACCACGATGTGTCGAGG
TGTAGAGATAAGGAAAACATACAGAGAAATATAAAAATATGCACTCTCTCTCTCTCTCTCTCTCTCTCTCT~~~~C
CATGGCCCAGGCCTCTCTTTCACAGTCGTAATATAAATGTATGTAATAGTTTTCTGCATAATATTATTGTAGATATCTAC
CAGGTATACTGAATAACCCACGGTTGGTGATGAAAGATGCGTTGATATGTTGAAGATAGAACCTCGACTAATCTCGAG
TACCCAATATCATCTGATTTTGTCATCAGGGCGTACAGTGCTACACGCTATCGAGTTGCACCAC
>
Isolation source: *H. Crispa*
Number of sequences obtained: 1
Length variation: na
Primers

>Fertig (GA26 micro) TD152-69
CAAAGTGGATTCTTCCCTATATCGCAATAAATTATTATCCCCTCTATCTTTCTTCCTCTATATCTGCAGTCTACATGTATC
CCACCTCTCTCTCTCTCTCTCTCTCTCCCTCTTCCCTCTCTCTCTCTCTCTCTCTCTCTTTCGAAAACGGACATAATTATG
CTATAAAAATTATTCTATAGTGTAGGCCTACTTATATATAGACTGACAACACTGACATCAAAATTATAAACACAACCGAG
TAGAACAATTTGTTGCTCCTATATTATACAACTACATGTATAAGCTGTAGATCCAATAACGTTTGAGCCAGAGGCCTTTC
TCGAAGAATTGTCGCCT
>
Isolation source: *E. quadricolor*,
Number of sequences obtained: 2
Length variation: n
Primers

>Fertig (GTAT23 micro) 'TD144-45_F+R_Micro' (1,533)
--
AGCAATAGCGGTCCTACCAGTAAATGATACCTTTATACATACATAAATACATCTACATACATACATACATACATACATAC
ACATACATACATACATACATACATACATACATACATACATACATACATACATACATACATACATAAACATACTTTTTCAGT
GGCAGGATCACCGAAACGAAGAAGGAGCTGTCTCTGGATTGGGATCCACACGACGGCTAGAGTATTTTTATTCCTGG
AAGCAACTATTACAGCTGACTAACACTGGTTCCTAAATTGGCTACTTAGGTACACACGAGCTGTTTTACGTCATAGGTA
CGCTCTCACGAGCGGAGACC

>'EQ503_F&R_micro' (1,513)
ACAGCAATAGCGGTCCTACCAGTAAATGATACCTTTATACATACATAAATACATCTACATACATACATACATACATACAT
ACACATACATACATACATACATACATACATACATACATACATACATACATACATACATACATACATAAACATACTTTTTCA
GTGGCAGGATCACCGAAACGAAGAAGGAGCTGTCTCTGGATTGGGATCCACACGACGGCTAGAGTATTTTTATTCCTG
GAAGCAACTATTACAGCTGACTAACACTGGTTCCTAAATTGGCTACTTAGGTACACACGAGCTGTTTTACGTCATAGGT
ACGCTCTCACGAGCGGAGACC
>

Isolation source: *H. Crispa*
Number of sequences obtained: 1
Length variation: na
Primers

>Fertig (CTT47 micro)'TD152-10
GCAAAGGATTTCCCGATGCAAGGAGCAGATTTGTATTTGTTGAAAGTGTTTTAGAACACAATGCTTAGCTGGAACAAA
AGGATTTTATGGCAAGCATATATATTTGGAAAGGACAAAGAAATGCTACGTGGTTCGTCTTCTTCTTCTTCTTCTTCTTC
TTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCT
TCTTCTTCTTCTCCTTCTTCTTCTTCTTCTCCTCCTTCTTCTCCAACAACATCTTCGTCTTTGAC
>

Isolation source: *H. Crispa*
Number of sequences obtained: 1
Length variation: na
Primers
>Fertig (CA34 micro) TD152-94
ACAGCATGACGTCATGCAATCAGCATCCGGATCCATAATTGTTTTTATTATTTATATTCACACACACACACACACACACA
CACACACACATAGTCACACAACACACACACACACACACACACACACACACACACACACACCCATAGTCACAAAACACACACACA
CCCAGTACTCATTGTGTGAATGGGTCATGTTACCATCGGCATCTGTTTTCTGAGGTCCATTTATGTGC

>
Isolation source: *H. Crispa*
Number of sequences obtained: 1
Length variation: na
Primers
Fertig (AC14 micro) TD152-14
GTGATCTATCTGCCATGTTACCCACGCTGATTTTCCGAGGTGATGCCATCTTGAGAGATCCACTGCGTACACGAGGCAA
GTTGTGGTAAGAATGAAGAAGATGTGAGTGTTTACTCTTAGCCTCCACCTACAACATCCTCTGCTAAACAAGCAGCAAC
AAGAAATCTCCGCCCATACACACACACACACACATACACACTCACACACGAGTGAGCAGGCTCACCTCTCGGTTGGTCG
GTTGGTCGGTTGGCTACATACAGAACATGAATCAACCTGATTACAATACTCAACATGATTTTAGAAAGTTTTACCTCTA
CCTATAAGCTTGCCATTGCTTCTTG

Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
Primers
>Fertig (GT16 micro) 'TD144-29_F+R_micro' (1,555)
AGCAAAGCAAATCGGTATTAGGCCCCAAACCTGTTTATTTTATCACAACAAGTTTTCATGCTTTCGGGAAACATAATTTA
TGTCCTAATTGATCACACACACACACACACACACACACACACATACACTTACTCAGATCTATCTGTCTACCCGCTTCCTTG
TTGGAACATGAATGTCAGTGATTTTGAGTTGCCTACGTTCGGTATTATTAGTTTATGAATAATGCAATGACAAGTAGCC
TACCCTTGCTTGGAGTATCTTGACAGCAGTTTACGAGCCATTTCCACGCGACACCATTCAATATCACCCAGAATACACAC
TCACTGTGTTTTCTGTCTAC

Isolation source: *E. quadricolor* and ?
Number of sequences obtained: 6
Length variation: yes
Primers
>

>Fertig (GAA48GCAGAA11GAA13 micro) 'TD144-14_F+R_micro'(1,541)

GGGAAGGAGTTCCTTAGGGAAGGGAGGATGTCCCCTGAAGGAGAGGGGATGGGTGCCATGACTCCTATCTACCACT
CTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCT
TCTGCTTCTGCTTCTGCTTCTGCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCT
TCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTCCTTCTTCTTCTTCCT
CCGTCCATGTAATGCTTTAGTATAAAGACCAATCACCAGAGGGCGTCC

>Contig_1 (1,583)
~~~~~~
~~--
GGAAGGAGTTCCTTAGGGAAGGGAGGATGTCCCCTGAAGGAGAGGGGATGGGTGCCATGACTCCTATCTACCACTCT
TCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTC
TGCTTCTGCTTCTGCTTCTKCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCttCTTCT
TCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCNTCTTCTTCTTCTCCTTCTTCTTCTTCCTCCGTCCATGTAATGCTT
TAGTATAAAGACCAATCACCAGAGGGCGTCCT

>Micro 02 (1,583)
~~~~~~
~~AGGGAAGGAGTTCCTTAGGGAAGGGAGGATGTCCCCTGAAGGAGAGGGGATGGGTGCCATGACTCCTATCTACC
ACTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCT
GCTTCTGCTTCTGCTTCTGCTTCTKCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCtt
CTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCNTCTTCTTCTTCT

>Contig_1 (1,583)
TACGCCAGCTTGGTACCGGGCTCGGNTCCACTAGTAACGGCCGCCAGTGTGCTGGAATTCGCCCTTGATGAGTCCTGA
GTAAGGGAAGGAGTTCCTTAGGGAAGGGAGGATGTCCCCTGAAGGAGAGGGGATGGGTGCCATGACTCCTATCTAC
CACTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTC
TGCTTCTGCTTCTGCTTCTGCTTCTKCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTC
ttCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCNTCTTCTTCTTCTCCTTCTTCTTCTTCCTCCGTCCATGTA
ATGCTTTAGTATAAAGACCAATCACCAGAGGGCGTCCT

>Fertig (GAA48GCAGAA11GAA13 micro) Micro 02 (1,583)
~~~~~~
~~~GGGAAGGAGTTCCTTAGGGAAGGGAGGATGTCCCCTGAAGGAGAGGGGATGGGTGCCATGACTCCTATCTACC
ACTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTC------
TTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTKCTTCTTCTTCTTCTTCT
TCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCttCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTC
NTCTTCTTCTTCTCCTTCTTCTTCTTCCTCCGTCCATGTAATGCTTTAGTATAAAGACCAATCACCAGAGGGCGTCC
>Fertig (GAA48GCAGAA11GAA13 micro) Contig_1 (1,583)
~~~~~~
~~~GGGAAGGAGTTCCTTAGGGAAGGGAGGATGTCCCCTGAAGGAGAGGGGATGGGTGCCATGACTCCTATCTACC
ACTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTC------
TTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTKCTTCTTCTTCTTCTTCT
TCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCttCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTC
NTCTTCTTCTTCTCCTTCTTCTTCTTCCTCCGTCCATGTAATGCTTTAGTATAAAGACCAATCACCAGAGGGCGTCC
>
Isolation source *H. Crispa*

Number of sequences obtained: 1
Length variation: na
Primers

>Fertig (micro 152-67) TD152-67
GGAATCATTCCCAAGCATATTCTGACACAAAACACACTGACCCTTCACAATACCTTTATCAGAGAGGGAAGTAAAACCA
TATTGTATATAGTCCTCACAGAAGGTTCTTTTAGTCCTACTCGTACCTGCCATCATACTCGTGAATGTGGAAGCCCTGA
AGAGATAGAAGTGAACAATATTCAGCAAACTGTACTGCACACATCTCTCTCTCACACACACACATACACACACACACA
AACACTCACTCCCTCTCACACACATCAAATTTTCTTTAGGTGAGTTCGAGAGCAGAAGCATTGCAGACTACTACCACGG
CAGCTTTAGTCGGTTGATATCTTGGGGATGAGATATCACTGATGGGCCCCACTGCTAAGATAGCTAAATGCGAGTTAG
TTGTTCCAGAGCTCAGTGATGTGG
>
Isolation source: *E. quadricolor*, *H. Crispa*
Number of sequences obtained: 26
Length variation: yes
Primers

>Fertig (GA29 micro) 'TD144-39_F+R_Micro' (1,520)
--
ATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTAGTCTCAACCTCTGATGGGTGTTACAAAGCCATAAC
CACACATGAAGACGTCTAGGGTTTCCTTAGATCCATTTTGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGCGT
GATCTTTCGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCTCTCTCTCTCTCTCTCTCTCTCT
CTCTCCAACCGAGTAGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC
>Fertig (GA29 micro) Micro 05 (1,535)
--
ATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTAGTCTCAACCTCTGATGGGTGTTACAAAGCCATAAC
CACACATGAAGACGTCTAGGGTTTTCTTAGATCCATTTTGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGCGT
GATCTTTTGACGACAGWTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAMTCGCTCTCTCTCTCTCTCTCTCTCTCTC
TCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC
>Fertig (GA29 micro) 'TD144-05_F+R_micro' (1,473)
--
ATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTAGTCTCAACCTCTGATGGGTGTTACAAAGCCATAAC
CACACATGAAGACGTCTAGGGTTTTCTTAGATCCATTTTGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGCGT
GATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCTCTCTCTCTCTCTCTCTCTCTC
CTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>Fertig (CT26b micro) 'TD144-16_F+R_micro' (1,507)
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTAGTCTCAACCTCTGATGGGTGTTACAAAGCCATA
ACCACACATGAAGACGTCTAGGGTTTTCTTAGATCCATTTTGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGCG
TGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCTCTCTCTCTCTCTCTCTCTCTC
TCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>Fertig (CT26b micro) Contig_1 (1,535)
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTAGTCTCAACCTCTGATGGGTGTTACAAAGCCATA
ACCACACATGAAGACGTCTAGGGTTTTCTTAGATCCATTTTGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGCG
TGATCTTTTGACGACAGWTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCTCTCTCTCTCTCTCTCTCTCT
CTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>Fertig (CT26b micro) 'TD144-02_F+R_micro' (1,514)
ACATATCACTGGTAAGGTGGAACGACATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCCATA
ACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGC
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCTCTCTCTCTCCCTCTCTCTCT
CTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGACCACAGCC

>Fertig (GA29 micro) 'TD144-11_F+R_micro' (1,517)
--
ATATCATTGGTAATGTGGAACGATATAGAAAGTCCGAAGGTTCGTCTCGACCTCTGCAGGGTGTTACAGAGCCATAAC
CACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGCGT
GATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCTCTCTCTCTCTCTCTTCTCTCTC
TCTTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC
>Fertig (GA29 micro) 'TD144-06_F+R_micro' (1,479)
--
ATATCATTGGTAATGTGGAACGATATAGAAAGTCCGAAGGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCCATAAC
CACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGCGT
GATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCGCTCTCTCTCTCTCTCTCTCTC
CTWCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>Fertig (CT26b micro) 'TD144-33_F+R_Micro' (1,510)
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCAATA
ACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCGTTACAATACAATAAGGACTTCGC
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCTCTCTCTCTCTCTTCTCTCTC
TCTCTCTCTCTCTCTCTCTTCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>Fertig (CT26b micro) 'TD144-43_F+R_Micro' (1,513)
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCCCTGCAGGGTGTTACAGAGCAATA
ACCACACAYGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCGTTACAATACAATAAGGACTTCGC
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCTCTCTCTCTCTCTTCTCTCTC

TCTCTCTCTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAG
CC

>Fertig (GA29 micro) micro 04 (1,520)—
ATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCAATAAC
CACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCGTTACAATACAATAAGGACTTCGCGT
GATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCTCTCTCTCTCTCTCTTCTCTCTCTC
TCTCTCTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>Fertig (GA29 micro) Contig_1 (1,520)
--
ATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCAATAAC
CACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCGTTACAATACAATAAGGACTTCGCGT
GATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCTCTCTCTCTCTCTCTTCTCTCTCTC
TCTCTCTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>Fertig (GA29 micro) 'TD144-09_F+R_micro' (1,489)
--
ATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCAATAAC
CACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCGTTACAATACAATAAGGACTTCGCGT
GATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCTCTCTCTCTCTCTCTTCWCTCTCT
CTCTCTCTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGC
C

>Fertig (CT26b micro) 'TD144-13_F+R_micro' (1,486)
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTKACAGAGCAATA
ACTACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAGTAACTTCGTTACAATACAATAAGGACTTCGC
GTGATCTTTTGACGACAGTTATTAGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCTCTCTCTCTCTCTCTCTCTCT
CTCTCTCTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACA
GCC
>Fertig (CT26b micro) 'TD144-40_F+R_Micro' (1,507)
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCAATA
ACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCGTTACAATACAATAAGGACTTCGC
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCTCTCTCTCTCTCTCCTCTCTCTC
TCTCTCTCTCTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCAC
AGCC

>Fertig (GA29 micro) 'TD144-17_F+R_micro' (1,489)
--
ATATCACTGGTAAGGTGGAACGATATAGGAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCCATAAC
CACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGCGT
GATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCCCTCTCTCTCTCTCTCTCTCTCTCTCT
CTCTCTCTCTTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCAC
AGCC

>Fertig (GA29 micro) 'TD144-08_F+R_micro' (1,523)
--
ATATCACTGGTAAGGTGGAACGATATAGGAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTKACAGAGCCATAAC
CACACAYGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGCGT
GATCTTTTGACGACAGWTATTGGGTCGGCTCCGGTGTTCGGSGAAGCAATCCCTCTCTCTCTCTCTCTCTCTCTCTCT
CTCTCTCTCTCTCTTCTCTCTCTCTCCAACCGTGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCAC
AGCC

>Fertig (GA29 micro) TD152-07
--
ATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGCGTTACAGAGCAATAAC
CACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCGTTACAATACAATAAGGACTTCGCGT
GATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGGAGCAATCGCTCTCTCTCTCTCTCTCTTCTCTCTCTC
TCTCTCTCTCTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCC
ACAGCC

>Fertig (CT26b micro) 'TD144-31_F+R_micro' (1,521)
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTKACAGAGCCATA
ACCACACAYGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGC
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGATCGGSGAAGCAATCCCTCTCTCTCTCTCTCTCTCTCTCT
CTCTCTCTCTCTCTTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGG
CCACAGCC

>Fertig (GA29 micro) 'TD_144-23_F+R_micro' (1,492)
--
ATATCACTGGTAAGGTGGAACGATATAGGAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCCATAAC
CACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGCGT
GATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGSGAAGCAATCCCTCTCTCTCTCTCTCTCTCTCTCTCT
CTCTCTCTCTCTCTTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGG
CCACAGCC

>Fertig (CT26b micro) 'TD144-38_F+R_Micro' (1,506)

ACATATCACTGGTAAGGTGGAACGATATAGGAAGTCCGAAAGTTCGTCTCAACCTCTGTAGGGTGTKACAGAGCCATA
ACCACACAYGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGC
GTGATCTTTTGACGACAGWTATTGGGTCGGCTCCGGTGTTCGGSGAAGCAATCCCTCTCTCTCTCTCTCTCTCTCTCT
CTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACA
GGCCACAGCC

>Fertig (CT26b micro) 'TD144-44_F+R_Micro' (1,522)
ACATATCACTGGTAAGGTGGAACGATATAGGAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCCATA
ACCACACATGAAGACGTCTAGGGTTTTCATAGACTTATTATGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGC
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCCCTCTCTCTCTCTCCCTCTCTCTCT
CTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCAC
AGGCCACAGCC

>Fertig (CT26 micro) 'TD_144-28_F+R_micro' (1,450)
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTKACAAAGCAATA
ACCACACAYGAAGACGTSTAGGGTTTTCATAGATGTATTATGATCAAATAACTTCTCGTAGTCTTTCTCCCCGAGTTCTT
CTCCTGGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCCAACCGAGTTGTTCACTCGGCTTTC
GCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>Fertig (CT26 micro) Contig_2 (1,362)
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAAAGCAATA
ACCACACATGAAGACGTCTAGGGTTTTCATAGATGTATTATGATCAAATAACTTCTCGTAGTCTTTCTCCCCGAGTTCTT
CTCCTGGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTNNAAC----------
----

>Fertig (CT26 micro) 'TD144-03_F+R_micro' (1,453)
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAAAGCAATA
ACCACACAYGAAGACGTCTAGGGTTTTCATAGATGTATTATGATCAAATAACTTCTCGTAGTCTTTCTCCCCGAGTTCTT
CTCCYGGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCCAACCGAGTTGTTCACTT
GGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>Fertig (CT26 micro) 'TD144-41_F+R_Micro' (1,449)
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTKACAAAGCAATA
ACCACACAYGAAGACGTSTAGGGTKTTCATAGATGTATTATGATCAAATAACTTCTCGTAGTCTTTCTCCCCGAGTTCTT
CTCCYGGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCTCTCTCTCTCTCTCTCCAACCGAGTTGTTC
ACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC
>
Isolation source: *H. Crispa*
Number of sequences obtained: 1
Length variation: na
Primers

>Fertig (TC31 micro) TD152-28
CTAGTAACGGCCGCCAGTGTGCTGGAATTCGCCCTTGATGAGTCCTGAGTAAGCGAAACGAGACCATAAATTTCAACG
TATTTGTAATAATAGAAAGGTTATGTTTGTACTATTGTGTCATGTGTTCGTGTATAATATGCCTCTGAATTTGACTGTG
TCTATGTAGATTGATCTTCCTTGTTTTTCTGTCAGTCGGTAGACCGGTTAGAATTCCTATGCATTCTTTACATCTCCCCTT
TCTCATTATCTATCACACATACTTCAATCTCTCTCTCTCTCTCTCTATCTCTCTCTCTCTCTCTCTCTCTCTCTCT
CTCTCCCTGCCTCCTTCTCTCTAAAGTACGTTCTATCAAAAGTAAATTGTTTTTCCACCCCTTGTCTGGCAGCATGTTACT
CAGGACTCATCAAGGGCGAATTCTGCAGATATCCATCACACTGGCGGCCGCTCGAGCATGCATCTAGAGGGCCCA
>

> Isolation source: *H. Crispa*
Number of sequences obtained: 1
Length variation: na
Primers

>Fertig (AG34 micro) TD152-12
ACACCGGTTACTAGCCTGATGCACCCTCATCAGTGTATTTCAAAACAGCATATATTTGATATCGTATCAATGTTTTCGTT
TTTAGATAAGTATATATTATATAAAGGTTTTCGCAATATTAGATAAACACTTAGGCATATCCAGGGATCACTATAGAGA
GAAGAGAGAGAGAGAGATAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGACCTACTA
TCTTTGCAAGCAGAAAGGTTTTTCGAAATATGCCTCACAGGTAACACTGAAATGAGGTTACTCAAGATCTAGACACGT
GTTCGCGTTAC
>
>
Isolation source: *?*
Number of sequences obtained: 1
Length variation: na
Primers

>Contig_1 (1,646)
GGCCATGTACCCGCGTCAGTATATGGCTGGCTTTCTAATCAAATAAAAAAGACAAATAGAGTAGATAAATACAGCGAA
CACAACTATATATCTCAAGAGACgGGGACGCAAGAGATAGGTAGTCAGTCAGTCAGGCAACCAACAACACCATCCATTA
TAATGAAAATTCCTCTCCACAAAAACAAAACAAAAACACCCGGCAACGTTGGTAACCCTGATCCCTGCTGGCAGGCATG
TGGTCTCASGTGTTTGTGTGTGTGTGTGTGTGTGTGTGCATGTGTGCGTGTAAGTGCGTGCGTGTGTTTGTGTGC
AATTGCCGCTGTTTATTAATGTGTTTGTGTGTGTGTGTGTGCTGTGCGCATGAGTGTGTGCTTGTGTTTGTGTGCAAT
TGTGTCCTGTTTATATATGCGTTTGT----------

Isolation source: *?*

Number of sequences obtained: 1
Length variation: na
Primers

>Micro 06 (1,621)
GCACGGGTGTTTATACATGTGCAGAATTATTCGGTATGCACACTAACGATGACATGATCAATTTGTGAACAATAAAAC
GTTTAGAAACCAGATGACGATGCTGATGTTGAAATAGAGTTTGACGHCTGTTCGGCACAACAGGCATTAGTCCACGTC
TTGTGGTGTGGGTAGTTATTGTATTGGTGTTTATGGCTGTTAGGGGGGGAGCAACGTACTAACTCAGCATCAYTATAA
TAGTCATCAACATCGACAMTATTATAWCTTTATAWMTAGTTCGCAMACCASACAGATaCGATGGATAGACCaSAGAGAG
AGAGAGAGAGAGAGAGAGAGA--
GCGCGCGCGCGCGCGCGTTTCCACTGTAAAGCCAAGCTGGATTTGAAGAGTGAAATGTAG
>

Isolation source: *E. quadricolor*, *H. crispa*
Number of sequences obtained: 17
Length variation: yes
Primers

>'HC144_F+R_micro' (1,504)
~~~~~~ACGCTATTTCATCTTCAATGAATCA
TTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT~~~~~~~~~~~~~~~~~
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CCTTCCCGTATGTCGATCAGTTCTAAATAAAAATTTGTTGTTGTTTTT
TCCAGGTCACGTGTTTGGAGAATCCGATGTAGTCAGTGAACAACTAGAACCTGGTATATGGTAAGTTGATTTCTTGGA
ATATATTTGTGTTCTAAATTTCACAATCATTATCTCTGAAATTGGATATTTTGTTTATTTTGGTTGATTGTGTTCGATGG
ATTTGAAGTCATACAAATTCAGTCCC

>Contig_1 (1,556)
~~~~~~AACGCTATTTCATCTTCAATGAATCA
TTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT~~~~~~
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CCTTCCCGTATGTCGATCAGTTCTAAATAAAAATTTGTTGKTGTTTTT
TCCAGGTCACGTGTTTGGAGAATCCGATGTAGTCAGTGAACGACTAGAACCTGGTATATGGTAAGTTGATTTCTTGGA
ATATATTTGTGTTCTAAATTKCACAATCATTATCTCTGAAATTGGATATTTTGTTTATTTTGGTTGATTGTGTTCGATGG
ATTTGAAGTCATACAAATTCAGTCCCT

>TD152-66
~~~~~~ACGCTATTTCATCTTCAATGAATCA
TTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT~~~~~~~~~~~~~
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CCTTCCCGTATGTCGATCAGTTCTAAATAAAAATTTGTTGTTGTTTTT
TCCAGGTCACGTGTTTGGAGAATCCGATGTAGTCAGTGAACGACTAGAACCTGGTATATGGTAAGTTGATTTCTTGGA
ATATATTTGTGTTCTAAATTTCACAATCATTATCTCTGAAATTGGATATTTTGTTTGTTTTGGTTGATTGTGTTCGATGG
ATTTGAAGTCATACAAATTCAGTCCCT

>'HC153_F+R_micro_' (1,477)
~~~~~~ACGCTATTTCATCTTCAATGAATCA
TTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCC~~~~~~~~~~~~~
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CCTTCCCGTATGTCGATCAGTTCTGAATAAAAATTTGTTGTTGTTTTT
TCCAGGTCACGTGTTTGGAGAATCCGATGTAGTCAGTGAACGACTAGAACCTGGTATATGGTAAGTTGATTTCTTGGA
ATATATTTGTGTTCTAAATTTCACAATCATTATCTCTGAAATTGGATATTTTGTTTATTTTGGTTGATTGTGTTCGATGG
ATTTGAAGTCATACAAATTCAGTCCC

>TD152-85
~~~~~~ACGCTATTTCATCTTCAATGAATCA
TTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT~~~~~~~~~~
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CCTTCCCGTATGTCGATCAGTTCTAAATAAAAATTTGTTGTTGTTTTT
TCCAGGTCACGTGTTTGGAGAATCCGATGTAGTCAGTGAACGACTAGAACCTGGTATATGGTAAGTTGATTTCTTGGA
ATATATTTGTGTTCTAAATTTCACAATCATTATCTCTGAAATTGGATATTTTGTTTATTTTGGTTGATTGTGTTCGATGG
ATTTGAAGTCATACAAATTCAGTCCCT

>TD152-72
~~~~~~ACGCTATTTCATCTTCAATGAATCA
TTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT~~~~~~~~~~
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CCTTCCCGTATGTCGATCAGTTCTAAATAAAAATTTGTTGTTGTTTTT
TCCAGGTCACGTGTTTGGAGAATCCGATGTAGTCAGTGAACGACTAGAACCTGGTATATGGTAAGTTGATTTCTTGGA
ATATATTTGTGTTCTAAATTTCACAATCATTATCTCTGAAATTGGATATTTTGTTTATTTTGGTTGATTGTGTTCGATGG
ATTTGAAGTCATACAAATTCAGTCCCT

>TD152-83
~~~~~~ACGCTATTTCATCTTCAATGAATCA
TTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT~~~~~~~~~~
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CCTTCCCGTATGTCGATCAGCTCTAAATAAAAATTTGTTGTTGTTTTT
TCCAGGTCACGTGTTTGGAGAATCCGATGTAGTCAGTGAACGACTAGAACCTGGTATATGGTAAGTTGATTTCTTGGA
ATATATTTGTGTTCTAAATTTCACAATCATTATCTCTGAAATTGGATATTTTGTTTATTTTGGTTGATTGTGTTCGATGG
ATTTGAAGTCATACAAATTCAGTCCCT

>'HC139_F+R_micro' (1,499)
~~~~~~ACGCTATTTCATCTTCAATGAATCA
TTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT~~~~~~~~~~
~~~~~~

~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CCTTTCCGTATGTCGATCAGTTCTAAATAAAAATTTGTTGTTGTTTTT
TCCAGGTCACGTGTTTGGAGAATCCGATGTAGTCAGTGAACGACTAGAACCTGGTATATGGTAAGTTGATTTCTTGGA
ATATATTTGTGTTCTAAATTTCACAATCATTATCTCTGAAATTGGATATTTTGTTTATTTTGGTTGATTGTGTTCGATGG
ATTTGAAGTCATACAAATTCAGTCCC

>'HC135_F+R_micro' (1,489)
~~~~~~ACGCTATTTCATCTTCAATGAAACA
TTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT~~~~~~~~
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CCTTCCCGTATGTCGATCAGTTCTAAATAAAAATTTGTTGTTGTTTTT
TCCAGGTCACGTGTTTGGAGAATCCGATGTAGCCAGTGAACGACTAGAACCTGGTATATGGTAAGTTGATTTCTTGGA
ATATATTTGTGTTCTAAATTTCACAATCATTATCTCTGAAATTGGATATTTTGTTTATTTTGGTTGATTGTGTTCGATGG
ATTTGAGGTCATACAAATTCAGTCCC

>TD152-70
~~~~~~ACGCTATTTCATCTTCAATGAATCA
TTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTC
TCTCTCTCT~~~~~~~~~~~~~~~~~~~~~~~~CCTTCCCGTATGTCGATCAGTTCTAAATAAAAATTTGTTGTTGTTTTTTC
CAGGTCACGTGTTTGGAGAATCCGATGTAGTCAGTGAACGACTAGAACCTGGTATATGGTAAGTTGATTTCTTGGAAT
ATATTTGTGTTCTAAATTTCACAATCATTATCTCTGAAATTGGATATTTTGTTTATTTTGGTTGATTGTGTTCGATGGAT
TTGAAGTCATACAAATTCAGTCCCT

>'EQ97_F&R_micro'
CCACTAGTAACGGCCGCCAGTGTGCTGGAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAC
ATTTTTTACGTACCTTTCACATCTCCTTCACATTTTGTTTCTCTCTCTCTCCCTCTCTCTCTCTCTCTCTCTCTCTCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCTTCCCGTATGTCGATCAGTTCTAAATAAAAATTTGTTGTTGTTTTTTC
CAGGTCACGTGTTTGGAGAATCCGATGTAGTCAGTGAACGACTAGAACCTGGTATATGGTAAGTTGATTTCTTGGAAT
ATATTTGTGTTCTAAATTTCACAATCATTATCTCTGAAATTGGATATTTTGTTTATTTTGGTTGATTGTGTTCGATGGAT
TTGAAGTCATACAAATTCAGTCCT

>'EQ98_F&R_micro'
~~~~~~AACGCTATTTCATCTTCAATGAAWC
ATTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT~~~~~~~~~
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CCTTCCCGTATGTCGATCAGTTCTAAATAAAAATTTGTTGTTGTTTTT
TTCCAGGTCACGTGTTTGGGGAATCCGATGTAGTCAGTGAACGACTAGAACCTGGTATATGGTAAGTTGATTTCTTGG
AATATATTTGTGTTCTAAATTTCACAATCATTATCTCTGAAATTGGATATTCTGTTTATTTTGGTTGATTGTGTTCGATG
GATTTGAAGTCATACAAATTCAGTCCC

>Fertig (CT34 micro)'TD144-37_F+R_Micro' (1,523)
~~~~~~ACGCTATTTCATCTTCAATGAATCA
TTTTTTGCGTACCTTTCACATCTCCYTCACAYTTTGTTTCTCTYTCTTTCTCTCTCTCTCTCTCTCTCTCTCTCT
CTCTCTCTCTCTCTCTCT~~~~~~~~~~~~~CCTTCCCGTATGTCGATCAGTTCTAAATAAAAATTTGTTGTTGTTTTTTT
CCAGGTCACGTGTTTGGAGAATCCGATGTAGTCAGTGAACGACTAGAACCTGGTATATGGTAAGTTGATTTCTTGGAA
TATATTTGTGTTCTAAATTTCACAATCATTATCTCTGAAATTGGATATTTTGTTTATTTTGGTTGATTGTGTTCGATGGA
TTTGAAGTCATACAAATTCAGTCCC

>Fertig (CT34 micro)'TD144-20_F+R_micro' (1,501)
~~~~~~ACGCTATTTCATCTTCAATGAATCA
TTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----------
----
~~~~~~~~~~~~CCTTCCCGTATGTCGATCAGTTCTAAATAAAAATTTGTTGTTGTTTTTTCCAGGTCACGTGTTTGGAG
AATCCGATGTAGTCAGTGAACGACTAGAACCTGGTATATGGTAAGTTGATTTCTTGGAATATATTTGTGTTCTAAATTT
CACAATCATTATCTCTGAAATTGGATATTTTGTTTATTTTGGTTGATTGTGTTCGATGGATTTGAAGTCATACAAATTCA
GTCCC
>Fertig (CT34 micro)Contig_1 (1,556)
~~~~~~ACGCTATTTCATCTTCAATGAATCA
TTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----------
----
~~~~~~~~~~~~CCTTCCCGTATGTCGATCAGTTCTAAATAAAAATTTGTTGKTGTTTTTTCCAGGTCACGTGTTTGGA
GAATCCGATGTAGTCAGTGAACGACTAGAACCTGGTATATGGTAAGTTGATTTCTTGGAATATATTTGTGTTCTAAATT
KCACAATCATTATCTCTGAAATTGGATATTTTGTTTATTTTGGTTGATTGTGTTCGATGGATTTGAAGTCATACAAATTC
AGTCCC

>Fertig (CT34 micro) Micro 07 (1,556)
~~~~~~ACGCTATTTCATCTTCAATGAATCA
TTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----------
----
~~~~~~~~~~~~CCTTCCCGTATGTCGATCAGTTCTAAATAAAAATTTGTTGKTGTTTTTTCCAGGTCACGTGTTTGGA
GAATCCGATGTAGTCAGTGAACGACTAGAACCTGGTATATGGTAAGTTGATTTCTTGGAATATATTTGTGTTCTAAATT
KCACAATCATTATCTCTGAAATTGGATATTTTGTTTATTTTGGTTGATTGTGTTCGATGGATTTGAAGTCATACAAATTC
AGTCCC

>Fertig (CT34 micro) 'TD144-47_F+R_Micro' (1,523)
~~~~~~ACGCTATTTCATCTTCAATGAATCA
TTTTTTGCGTACCTTTCACATCTCCTTCACATTTTGTTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----------
--
~~~~~~~~~~~~CCTTCCCGTATGTCGATCAGTTCTAAATAAAAATTTGTTGTTGCTTTTTCCAGGTCACGTGTTTGGAG
AATCCGATGTAGTCAGTGAACGACTAGAACCTGGTATATGGTAAGTTGATTTCTTGGAATATATTTGTGTTCTAAATTT
~~~~~~~~~~~~

CACAATCATTATCTCTGAAATTGGATATTTTGTCTATTTTGGTTGATTGTGTTCGATGGATTTGAAGTCATACAAATTCA
GTCCC
>
Isolation source: *H. Crispa*
Number of sequences obtained: 1
Length variation: na
Primers
>New 'HC120_F+R_micro' (1,519)
GGTTACTGCGAGTAAATGATGGTTGATTGCCAAAATGGTAGCCCTCTCTTCACGTCTGGGGTATTGTGTGAAGTACAC
GCGCGAAGTGCCGACTGGACTGACTCACACACACACACACACACACATCACGCACAAAGTAGTTCCTGTTGGAATCAAT
TATATGTTTGCACCTTATAATTATAGGGTTGGGACAATGGGGGTCGAAAATGAATGCATGGTGGATTTAGAGGCCATT
TTTGATGGAAATCATGAAAATGTAGGAATTTTGAGTGAAAAACAATTTTGAGGTCGGTGCGGAGCGATTTTTCAAAAT
GGCCGCCAACATTTGATTTGTTTTTCCCTTGTGCGTTGGAAACAAGTTATACATAGCTTGTAATATGTTCT
>

Isolation source: *E. quadricolor*, *H. Crispa*
Number of sequences obtained: 2
Length variation: yes
Primers

>'HC130_F+R_micro' (1,555)
~~ATGAAGAGATACACTGCAAGGAATTTGCATGTCACCCTACCAAATAAATTAGTTATGACAACCGAATTCTCACACTT
CCGATATCTCTGAAAATGTACTGAGTGCATTGATATGTCCCCCTCACACACACACACACACACACCCCCACACACACACTTT
GTCTCTGTCTCTGTCTCTCTTGTATACATAATGCTCCACTGTTTGGCACTGTAAGACCAATTATCTAATTTAGT~~~CTA
ATACATGTAGGTCAATCCTGAAACATATTGCATAGTATAACATACCTCTCCATGGTACTTGTAGATCAATGTATTCTCCC
TTGACATGCGTAGGAAATCTCAAACAACTGTAGAAAATAAGGCGACGCGAAGGGCGACGTCGGGGTAAAAAATGCGA
CGTCGGGTTAGCTCCCCATACC

>'EQ91_F&R_micro'
AAATTAACAGATACACTGTTTTACTTCTGCATGTCACCCTACCAAATAAATTAGTTATGACAACCGAATTCTCACACTTCC
GATATCTCCGAAAATGTACTGAGTGCATTGATATGTCCCCCTCACACACACACACA~~CCCACACACACACACACTTTGT
CTCTGTCTCTGTCTCTCTTGTATACATAATGCTCCACTGTTTGGCACTGTAAGACCAATTATCTAATTTAGTAGTCTAATA
CATGTAGGTCAATCCTGAAACATATTGCATAGTATAACATACCTCTCCATGGTACTTGTAGATCAATGTATTCTCCCTTG
ACATGCGTAGGAAATCTCAAACAACTGTAGAAAATAAGGCGACGCGAAGGGCGACGTCGGGGTAAAAATGCGACGTC
GGGTTAGCTCCCCATACY

Isolation source: *H. Crispa*
Number of sequences obtained: 1
Length variation: na
Primers
>'HC124_F+R_micro' (1,531)
ACWYMAYTGRSYKYYGTWTYACAAAATAAACACACACACACATACATACATACATACATACATACATACATACATA
CATACATACATACATACATACATACACGCACACACACACACGCACACACACACACACACACATATTTGACACATATGCCA
GGTTTCTACGGCTGGGGAGGACACAACATCTGTACATATGTGCGTGTTTGCAGGACGCACAAACTCAAAACAACAGAT
ACCGCTAACTTTAGATTGTTTGGCAGACTGAAATAGTTTGTCCTACATAGAAAACGGCGCAATCATTGTGAGCCGCTTA
GTCTGATTCAATCAACCTGAGTATTTCCTGCTATGAGTAAGCGTTGAACATCTTCT

Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
Primers
>
>'EQ502_F&R_micro' (1,535)
GTGGAAACACTTACCAACGACAAGAAATTCCTGGGTTCGTGCCGACAAAATGGATCAAAATGCGCTTTTCCACATAGA
AAATTGGGGCGCGGTAATTAGGTCACAGGAAATTTGGTCACCGGAAATTTGGTCACAGTAATTCGGTCACATGGTAAA
TTGGTTAGGGTTATTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTTAGGTTTACGGTTAGGGTTTTGGTTAGGGTTA
GGGTTTAGGTTTACGGTTAGGGTTTTGGTTAGGTTTACGGGACGGGGGCACAAAACTAGGTTGCCGCCCCGAAAGTT
CTGGCGCAGGTTCAGAGATAAGTCTGGTTTCGTT
>
Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
Primers

>'EQ105_F&R_micro' (1,414)
GACATTATCTCAATCCCCTCTCTCTCTCTCTCGCTCTCTCTCTATCTCTCTCTCTCTCTCTCTAATGTAGCTGACAAATGGC
CTTGAGCTACCCGTTCTACATGGGCCCGATGGAAAGTATTTGTTATTATTACGTTTATTGTAGAAATACGTGTAGCACA
GAGAGGACAAAACAACAAATCATGATTCCTCTTGTAATTTTCAACTTGTAGAGACTCCGGTGGGTAAAGTTGGCTGTTT
ATATTTGTGTTATAT

Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
Primers

>'EQ96_F&R_micro'
AAGACTGGGAAATTTTGTCGATAGCCCCGAACATGCAGACTCAAAAACACTGTAAAATCTGAGTTTGTTAGCTAGAGA
AACAACTAGCATAAGTAAATCGGTCAAATGTTCGTGGCACATACATTTCAGTACTAACTCTCGTGAGTAAAGTAATTTA

GTTTGTAGTCTGAGTCTGAGCTTGAGATTATTTGTTTGTTGCTGATCCCCAGGCCAGATCATAGAATTCAAGAGCAAA
GTAAACTTCAATATATAGTACGGTACCATAAGAGACACGCACACACACACCCACACACACAGCCTAAATGTTCGTTTGT
TGAGGATTTGC
>
Isolation source: *H. Crispa*, *E. quadricolor* and ?
Number of sequences obtained: 8
Length variation: yes
Primers
>'HC127_F_micro' (1,435)
~GATCCTGAGTAAAACGAAACCAGACT~ATCNCTGAACCTNCGCCAGAACTTTCGGGGCGGCAACCTAGTTTTGTTGC
CCCCGTCCCGTAAACCT----------
AACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACCCTA
ACCCTAACCCTAATAACCCTAACCAATTTACCATGTGGCCGAATTACTGTGACCAAATTTCCGGTGACCAAATTTCCTGT
GACCTAATTACCGCGCCCCAATTTTCTATGTGGAAAAGCGCATTTTGATCCATTTTGTCGGCACGAACCCAAGAATTTCT
TGTTGTTGGTAAGTGTTTCCAC

>'HC117_F+R_micro' (1,480)

~AACTAAACCAGACTTATCTCTGAACCTGCGCCAGAACTTTCGGGGCGGCAACCTAGTTTTGT~GCCCCCGTCCCGTAA
ACCT----------
AACCAAAACCCTAACCGTAAGCCTAAACCCTAACCCTAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACCCTA
ACCCTAACCCTAATAACCCTAACCAATTTACCATGTGACCGAATTACTGTGACCAAATTTCCGGTGACCAAATTTCCTGT
GACCTAATTACCGCGCCCCAATTTTCTATGTGGAAAAGCGCATTTTGATCCATTTTGTCGGCACGAACCCAAGAATTTCT
TGTTGTTGGTAAGTGTTTCCAC

>'HC137_F+R_micro' (1,494)

~ACTTCACTGGACGTCGTTTTAAACCTGCGCCAGAACTTTCGGGGCGGCAACCTAGTTGTGT~GCCCCCGTCCCGTAAA
CCTAAACCCTAACCCTAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAA----------
CCCTAACCCTAACCCTAATAACCCTAACCAATTTACCATGTGACCGAATTACTGTGACCAAATTTCCGGTGACCAAATTT
CCTGTGACCTAATTACCGCGCCCCAATTTTCTATGTGGAAAAGCGCATTTTGATCCATTTTGTCGGCACGAACCCAAGA
ATTTCTTGTCGTTGGTAAGTGTTTCCAT

>'TD_144-22_F+R_micro' (1,537)
-
~~~~~~~~~~~~AACGAAACCAGACTTATCTCTGAACCTGCGCCAGAACTTTCGGGGCGGCAACCTAGTTTTGT~GCCC
CCGTCCCGTAAACCT~~~~~~~~~~~~~~AACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACCAAAACCCTAAC
CGTAAACCTAAACCCTAACCCTAACCCTAACCCTAACCCTAATAACCCTAACCAATTTACCATGTGACCGAATTACTGTGA
CCAAATTTCCGGTGACCAAATTTCCTGTGACCTAATTACCGCGCCCCAATTTTCTATGTGGAAAAGCGCATTTTGATCCA
TTTTGTCGGCACGAACCCAAGAATTTCTTGTTGTTGGTAAGTGTTTCCAC

>'TD144-04_F+R_micro' (1,509)
-
~~~~~~~~~~~~AACGAAACCAGACTTATCTCTGAACCTGCGCCAGAACTTTCGGGGCGGCAACCTAGTTTTGT~GCCC
CCGTCCCGTAAACCT~~~~~~~~~~~~~~AACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACCAAAACCCTAAC
CGTAAACCTAAACCCTAACCCTAACCCTAACCCTAACCCTAATAACCCTAACCAATTTACCATGTGACCGAATTACTGTGA
CCAAATTTCCGGTGACCAAATTTCCTGTGACCTAATTACCGCGCCCCAATTTTCTATGTGGAAAAGCGCATTTTGATCCA
TTTTGTCGGCACGAACCCAAGAATTTCTTGTTGTTGGTAAGTGTTTCCAC

>'EQ107_F&R_micro' (1,514)

~AACTAAACGGGACTTATCTCTGAACCTGCGCCAGAACTTTCGGGGCGGCAACCTAGTTTTGT~GCCCCCGTCCCGTAA
ACCT----------
AACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACCCTA
ACCCTAACCCTAATAACCCTAACCAATTTACCATGTGACTGAATTACTGTGACCAAATTTCCGGTGACCAAATTTCCTGT
GACCTAATTACCGCGCCCCAATTTTCTATGTGGAAAAGCGCATTTTGATCCATTTTGTCGGCACGAACCCAAAAATTTCT
TGTTGTTGGTAAGTGTTTCCAC

>'EQ95_F&R_micro'
~~~~~~~~~~~~AAACTAAACGGGACTTATCTCTGAACCTGCGCCAGAACTTTCGGGGCGGCAACCTAGTTTTGT~GCC
CCCGTCCCGTAAACCT~~~~~~~~~~~~~~~AACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACCAAAACCCTAA
CCGTAAACCTAAACCCTAACCCTAACCCTAATAACCCTAACCAATTTACCATGTGACCGAATTACTGTG
ACCAAATTTCCGGTGACCAAGTTTCCTGTGACCTAATTACCGCGCCCCAATTTTCTATGTGGAAAAGCGCATTTTGATCC
ATTTTGTCGGCACGAACCCAAGAATTTCTTGTTGTTGGTAAGTGTTTCCAC

>Contig_1 (1,570)
GAGTCCTGAGTAAAACGAAACCAGACTTATCTCTGAACCTGCGCCAGAACTTTCGGGGCGGCAACCTAGTTTTGT~GC
CCCCGTCCCGTAAACCT~~~~~~~~~~~~~~~AACCAAAACCCTAACCGTAAACCTAAACCCTAACCCTAACCAAAACCCTA
ACCGTAAACCTAAACCCTAACCCTAACCCTAATAACCCTAACCAATTCACCATGTGACCGAATTACTGT
GACCAAATTTCCGGTGACCAAATTTCCTGTGACCTAATTACCGCGCCCCAaGTTTCTATGTGGAAAAGCGCATTTTGATC
CATTTTGTCGGCACGAACCCAAGAATTTCTTGTTGTTGGTAAGTGTTTCCACT
>
Isolation source: *H. Crispa*
Number of sequences obtained: 3
Length variation: yes
Primers

Fertig (micro 152-67) TD152-67
~~~~~~~~~~~~

GGAATCATTCCCAAGCATATTCTGACACAAAACACACTGACCCTTCACAATACCTTTATCAGAGAGGGAAGTAAAACCA
TATTGTATATAGTCCTCACAGAAGGTTCTTTTAGTCCTACTCGTACCTGCCATCATACTCGTGAATGTGGAAGCCCTGA
AGAGATAGAAGTGAACAATATTCAGCAAACTGTACTGCACACATCTCTCTCTCACACACACACATACACACACACACA
AACACTCACTCCCTCTCACACACA~~~~TCAAATTTTCTTTAGGTGAGTTCGAGAGCAGAAGCATTGCAGACTACTACCA
CGGCAGCTTTAGTCGGTTGATATCTTGGGGATGAGATATCACTGATGGGCCCCACTGCTAAGATAGCTAAATGCGAGT
TAGTTGTTCCAGAGCTCAGTGATGTGG
>'HC146_F+R_micro' (1,584)
-
GAATCATTCCCAAGCACATTCTGACACAAAATACACTGACCCTTCACAACACATTTATCAGAGAGGGAAGTAAAACCAT
ATTGTATATGGTCCTCACAGAAGGTTCTTTT----------
CTCGTAAATGTGGGAGCCCTGAAGAGATAGAAGTGAAAAATATTCAGC~~ACTGTACTACACACATCACACACACACAC
TAACACACACACACACACACACACACACACTCACTCTCTCACACACACATCAAATTTTCTTTACGTGAGTTCAAGAGC
GGAAGCATTGAAGACTACGACCACGGCAGCTTTAGTCGGTTGATATCTTGGTGATGAGATATCACTGATGGACCCCAC
CGCTAAGAGAGCTAAATTTGAGTTATTTGTAAAAGGACGCCCAGAGAAGGGTAATCCGACTCCATCTAGGGCGAATTC
CAGCACACTGGCGGCCGTTACTAGTGATTCGAGCTACGACCCANGNNGN----------

>'HC136_F+R_micro' (1,524)
GGAATCATTGCCAAGCACATTCTGACACAAAACACACTGACCCTTCACAACACCTTTATCAGAGAGGGAAGTAAAACCA
TATTGTATA--
GTCCTCACAGAAGGTTCTTTTAGTCTTACTCGTATCTGCCATCATACTCGTGAATGTGGGAGCCCTGAAGAGATAGAAG
TGAACAACATTCAGCAAACAATACTGCACACATCACACACACACTCACACACAATCACACACACA~~~~~~~~~~~~~~~
~~~~~~~~~~~~~~~~~TCAAATTTTCTTTAGGTGAGTTCGAGAGCAGAAGCATTGCAGACTACAACCACAGCAGCTTT
AGTCAGTTGATATCTTGGTGATGAGATATCACTGATAGACCCCACCGCTGAGAGAGCTAAATGTGAGTTAGTTGTTCC
AAAGCTCAGTGATGTGG
>
Isolation source: *H. Crispa*
Number of sequences obtained: 2
Length variation: yes
Primers

>'HC133_F+R_micro_' (1,522)
AATTCACTGGCCGTCGATAACCTGAGGGATCATCGGTTCCTGACCTTTTGGAATCCACGACTTCATGAGCCCCTAATTC
AGGTACATACTTTGAACTCTCCGTCGCCGGTTTATTCAGAGCAATATTTACACCACCTAAATATAGACATAAAAGTCCTT
CAATGAGACAAAACCCTTTATAACAAACATAGTATTAGAAAACACACAAATTATTCAACGACTCTCTCTCTCTCTCTCT
CTCTCTCTCTCTCTCTCTCTCTCACTCTGTTTGTCAAACTTTTGAACATACATAAATTATATCAGCAAGTGCTGCCACTTA
CCTGGTAATTTGTAACATTTTGACGGATGACAAAGATTTCCATCAGTGCTAGGTGAATCTACAGCAT

>'HC140_F+R_micro' (1,544)
-
GGTCTAAGCAGTATGATAACCAGGGGGATCATCGGTTCCCGACCTTTTGGAATCCACGACTTCATGAGCCCCTAATTCA
GGTACATACTTTGAACTCTCCGTCGCCGGTTTATTCAGAGCAATATTTACACCACCTAAATATAGACATAAAAGTTCTTC
AATGAGACAAAACCCTTTATAACAAACATAGTATTAGAAAACACACAAATTATTCAACGACTCTCTCTCTCTCTCTCTC
TCTCTCTCTCTCTCTCTCTCTCACTCTGTTTGTCAAACTTTTGAACATACATAAATTATATCAGCAAGTGCTGCCACTTAC
CTGGTAATTTGTAACATTTTGACGGATGACAAAGATTTCCATCAGTGCTAGGTGAAACTACAACAT
>
Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
Primers
>'EQ103_F&R_micro' (1,463)
ACACTCCGGCACTGTTGTACTCTCGCACTCTCGCGCTCACACTCTTGCTCTCTCTATCTCTATGATTCTCTCTCTATATCG
TTCTCACACTCATTTATCGAACTCTCTCCCTCTCTTTCTCATTTCCTTCTCTCTCTCYYYYYYYYYtCTCTCTCTCTCTCTC
TCTCTCTCTCTCTCTCATAAACTTCATACTTTTTACTCTGCATCATCGACTATTCAAGTGGATTGTGTTACTAACAGTACT
TTGGTATCCCATTTATCGTGGCATTGTCGGTTTATGGAAGGTAAAGAGGCGACCAATGAATTGTAATCCGGCCCCCTC
AAGGGCGAATTCCAGCACACTGGCGGCCGTTACTAGTGGAT----------
>
Isolation source: *H. Crispa*
Number of sequences obtained:
Length variation: na
Primers

>TD152-95
ATAGATGAAAATTCTTGAAAAACTAATATAATCGCTCTCTTCCTCTCTCTTTTATTCGCTTTATCCCCTTCTCGTTTAGTC
TCTGACTCTCTTTCTCTCTCTCTCTCTAACTCACTCACTCTCTCTCTTTGTCTCTCTCTCTGTCTCTCTCTCTGTCTCTCTCTCT
ATCGCTCTCTCTCTCGATCCTCCTTCGATCTGGTAGCAATCCTGATCAGGTCATGGATTCTATGACGTCATTCGCCTGCT

Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
Primers
>'EQ72_F&R_micro'
GAACAGACTGGAAGCTGGAAGCAGTTTCAGTCGGTATTTCCACCGGAATTGTTTACATTGTGGTGAGCAGGTGCATTA
TCATATATGAAAACCACACGGGCATTAGGAGCAAGGAACGGGTTTGAAGCAAGAAATCTTAGAATCTCTGGGCAGTCA
TGCTTCCTATTTCTGCAGATTGATGCACAAGGCCAGTCCTAGTTGAGATGGCCATCACAAGGGTCACATTTCTTCCATG
TTGTCCACACACACACACACACACACACACACACACACACACACACCGGTCTGTGCCCCGTTTCTCACTGCTCC
>

Isolation source: *H. Crispa*
~~~~~~~~~~~~~~~~~

Number of sequences obtained: 1
Length variation: na
Primers

>'HC129_F+R_micro' (1,527)
AACTATACCTAATTGGGGGCTTCTGTCGACGCCGCTATAGGTAGTATAGACCGAGGACGTCCTCGGTTGTATCATGGT
CCTCGATTAGAAGGTGTGTATTTACATATGTGTCCTCGATGTACACCTTATACAAACGCAGAACACACCCAGACACACA
CACACACACACACACACACACCCAGTAAACACGTCCCTGGTACGGGGTAAAACAATCAAAGCTGTTATAAAAAATCTTG
TATCATTACCTTTTCGTAACAAACTGTTTTACGGTACGGTAGTTATATTATGTCTGTATCACAGACTTTCACGCAGAATC
ACATTGAAGCTTTCCCACTTTCCACACCATGCTCATTGGTAGAAGTGAAGGCAACC
>
Isolation source: *E. quadricolor* , *H. Crispa,* and *?*
Number of sequences obtained: 21
Length variation: yes
Primers

>'EQ85_F&R_micro'
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAG~TGCACGGTTCAGTACACGATCCAGGCATACTCGGATATC
CAAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATC
AGTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGT

>'EQ108_F&R_micro' (1,526)
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAG~TGCACAGTTCAGTACACGATCCAAGCATACTCGGATATC
CAAGGATAAAGCGCCTGGACAGTGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATC
AGTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGTC

>Fertig (AG18 micro) Contig_1 (1,583)
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAG~TGCACAGTTCAGTACACGATCCAAGCATACTCGGATATC
CAAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATC
AGTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT—
ACAAGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGT

>'EQ101_F&R_micro'
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAACATGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCC
AAGGATAAAGCGCCTGGACGGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATTTAAAAACTATCAAAAATCA
GTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT—
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGT

>'EQ501_F&R_micro' (1,551)
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAG~TGCACAGTTCAGTACACGATCCAAGCATACTCGGATATC
CAAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATC
AGTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT------
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTATGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGT

>Fertig (AG18 micro) 'TD144-35_F+R_Micro' (1,541)
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAG~TGCACAGTTCAGTACACGATCCAAGCATACTCGGATATC
CAAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATC
AGTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTACATGAATGCACACTTACATATATATGTAAGAC
TGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTATCTAACGCTGTGCAGGCTTCAAACTTCAGTCAAT
GTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATGAGCTGAATAATGTATGAATGTTTGAGGAGGTT
GT

>Fertig (AG18 micro) 'TD144-12_F+R_micro' (1,578)
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAG~TGCACAGTTCAGTACACGATCCAAGCATACTCGGATATC
CAAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATC
AGTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTACAAGAATGCACACTTACATATATATGTAAGA
CTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTATCTAACGCTGTGCAGGCTTCAAACTTCAGTCAA
TGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATGAGCTGAATAATGTATGAATGTTTGAGGAGGTT
GT

>Contig_1 (1,583)
~~~~~~~~~~~~~~~~~AGGTACGCTCGGGTGCCTTGAAG~TGCACAGTTCAGTACACGATCCAAGCATACTCGGATATC
CAAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATC
AGTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT-
AACAAGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTA

TCTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACAT
GAGCTGAATAATGTATGAATGTTTGAGGAGGTTGTTTACTCAGGACTCATCAAGGGCGAATTCTGCAGATATCCATCA
CACTGGCGGCCGCTCGAGCATGCAT-
TAGAGGGCCCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGKACTGAAACCG—

>'HC123_F+R_micro' (1,533)
----------GGT-
CGCTGGGGTGCCTTGAACATGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCCAAGGATAAAGCGCCTGGAC
AGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATCAGTCAGTCAGCCTCTCTCTCT
CTCTCTCTCTCTCTCTCTCTCTCT—
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGT

>'HC113_F+R_micro' (1,533)
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAACATGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCC
AAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAA~CTATCAAAAATCA
GTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTACATGAATGCACACTTACATATATATGTAAGACT
GTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTATCTAACGCTGTGCAGGCTTCAAACTTCAGTCAATG
TAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATGAGCTGAATAATGTATGAATGTTTGAGGAGGTTGT

>TD152-24
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAG~TGCACAGTTCAGTACACGATCCAAGCATACTCGGATATC
CAGGGATAAAGCGCCTGGATAGCGCAGTAGGTCTATAATATCTCGTAAGTGAAAATGATCTAAAAACTATCAAAAATC
AGTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT—
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCGAACTTCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGTT

>'HC143_F+R_micro' (1,511)
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAG~TGCACAGTTCAGTACACGATCCAAGCATACTCGGATATC
CAAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGCGATCTAAAAACTATCAAAAATC
AGTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT—
ACATGAATGCACACTTACATATATATGCAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAACGTATGAATGTTTGAGGAGGTTGT

>TD152-60
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAG~TGCACAGTTCAGTACACGATCCAAGCATACTCGGATATC
CAAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATC
AGTCAGTCAGCCTCTCTCTCTCCCTCTCTCTCTCTCTCT—
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGTT

>TD152-76
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAG~TGCACAGTTCAGTACACGATCCAAGCATACTCGGATATC
CAAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATC
AGTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT—
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCCTCAAACTTCCGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGCCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGTT

>TD152-30
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAG~TGCACAGTTCAGTACACGTTCCAAGCATACTCGGATATC
CAAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATC
AGTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCCCTCTCTCTCTCT—
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAATCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGTT

>TD152-37
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAG~TGCACAGTTCAGTACACGATCCAAGCATACTCGGATATC
CAAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACAATCAAAAATC
AGTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----
CCATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTGCGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGTT

>'HC110_F&R_micro' (1,525)
~~~~~~~~~~~~~~~~~GG-
ACGCTCGGGTGCCTTGAAA~TGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCCAAGGATAAAGCGCCTGGA
CAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATCAGTCAGTCAGCCTCTCTCTC
TCTCTCTCTCTCTCTCTCTCTCTCT----
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGT

>TD152-48
~~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAG~TGCACAGTTCAGTACACGATCCAAGCATACTCGGATATT
CAAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAGGTGATCTAAAAACTATCAAAAATC
AGTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCAAACTCCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGTT

>TD152-90
~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAG~TGCACAGTTCAGTACACGATCCAAGCATACTCGGATATC
CAAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATC
AGTCAGTCAGCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT------
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCGGGCTTCAAGCTTCAGTCAATGTAAGTGTCAACCTATAAATTGTCGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGGGGAGGTTGTT

>TD152-02
~~~~~~~~~~~~~~~~GGTACGCTCGGGTGCCTTGAAG~TGCACAGTTCAGTACACGATCCAAGCATATTCGGATATC
CAAGGATAAAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATC
AGTCAGTCAGCCTCTCTCTCTCCCTCTCTCTCTCTCTCTCT------
ACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTACGTGGTATAAGATTGTATAGAACATATTATTTAT
CTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAACCTATAAATTGTTGTATGTCAAAATATGTCACATG
AGCTGAATAATGTATGAATGTTTGAGGAGGTTGTT

>!!!!!!!!!!!!!!!'HC126_F&R_micro' (1,844)
AGGGCTCTGACGGCATAGTACGCTCGGGTGCCTTGAAG~TGCACAGTTCAGTACACGATCCAAGCATACTCGGATATC
CAAGGATAAAGCGCCTGAACAGCGTAGTAGTTCTATGATATCACGCAAGTGAAAGTGGACTAAAAACTCCCAAAAGTC
AGTCAGTCAGTCAGTGTCATTTTGCTCAGCGTTAGCTGGCAAGTTTCAATTGGACATCAACGAAAATTCTAGAAAACTC
ACCGACAGGCGGTAAAACTTGTTGCTGAGCAAAATGACACGAATGACATTTTCCTCAGCATTTTCCTCACTGTGTGCGG
TATGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTCGTTGTCGTTGTCG
TTGTCGTTGTCGTTGTCGTTGTGTYGTYGTYGTTGTYGTYGTTGTCGTTGTCGTTGTCGTTGTCGTTGTCGTTGTCGTTGT
CGTTGTCGTTGTCGTTGTCGTTGTCGTTGTCGTTGTCGTTATTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTTGTT
TTTGTTGTTGTTATGGGTTGTACTGTGACCGATCTTACACCCAATAGTGTAAAACRACGGCCAGTGAATTGTAATMCGA
CTCACTMTAGGGCGAATTCCAGCACACTGGCGGCCGTTACTAGKGATTTAGAACGACGACCAGANCCNNTAATCAT
>
Isolation source: *H. Crispa*
Number of sequences obtained: 1
Length variation: na
Primers
>TD152-96
AGGACATTATCTCAATCCTCTCTCTCTCTCTCGCTCTCTCTCTATCTCTCTCTCTCTCTCTCTCTAATGTAGCTGACAAATG
GCCTTGAGCTACCCGTTCTACATGGGCCCGATGGAAAGTATTTGTTATTATTACGTTTATTGTAGAAATACGTGTAGCA
CAGAGAGGACAAAACAACAAATCATGATTCCTCTTGTAATTTTCAACTTGTAGAGACTCCGGTGGGTAAAGTTGGCTG
TTTATATTTGTGTTATAT
>
Isolation source: *E. quadricolor* , *H. Crispa,* and *?*
Number of sequences obtained: 75
Length variation: yes
Primers
>TD152-32
~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAA~CGATATAGAAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCG
TTACAATACCAAAAGAACTTCTCGTAGTCTTTCCCCCCGAGTTCTTCTCCTGGCTCTCTCTCTCTCTCTCTCTCTCTCTC
TCTCTCTCTCTTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>'HC149_F+R_micro' (1,487)
----------ACTTATCTGTGGGAAGGTGGAA-
CAATATAGGAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGG
GTTTTCATAGATTTATTATGATCAAATAACTTCGTTACGATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATT
GGGTCGGCTCCGGTGTTCGGCGAAGCAATCCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTC
TCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCT

>TD152-45
~~~~~~~~~~----------
~AACATATCACTGGTAAGGTGGAA~CGATATAGGAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCC
ATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGTTACAATACCAAAAGAACTTC
GCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGSGAAGCAATCCCTCTCTCTCTCTCTCTCTCTCT
CTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCT------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>micro 04 (1,520)
GCCCTTGATGAAGTCCTGAGTAAACATATCACTGGTAAGGTGGAAACGATATAGAAAGTCCGAAAGTTCGTCTCAACC
TCTGCAGGGTGTTACAGAGCAATAACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTC
GTTACAATACAATAAGGACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCG
CTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGC
GCTACCTTCGTGCACAGGCCACAGCC
~~~~~~~~~~

>Contig_1 (1,520)
~~~~~~~~~~~~~~~~~~~~~~~~~~ACATATCACTGGTAAGGTGGAAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAGAGCAATAACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCG
TTACAATACAATAAGGACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGC
TCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCCGC
GCTACCTTCGTGCACAGGCCACAGCCT

>Contig_2 (1,362)
~~~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAA~CGATATAGAAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAAAGCAATAACCACACATGAAGACGTCTAGGGTTTTCATAGATGTATTATGATCAAATAACTTCT
CGTAGTCTTTCTCCCCGAGTTCTTCTCCTGGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTNN
AAC

>'EQ100_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~~~~ACATATCACTGGTAAGGTGGAA~CGATATAGAAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAAAGCAATAACCACACATGAAGACGTCTAGGGTTTTCATAGATGTATTATGATCAAATAACTTCT
CGTAGTCTTTCTCCCCGAGTTCTTCTCCTGGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTMTWCTCTCTCT
CTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCCT

>'EQ86_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~~~~ACATATCACTGGTAAGGTGGAA~CGATATAGAAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAAAGCAATAACCACACACGAAGACGTCTAGGGTTTTCATAGATGTATTATGATCAAATAACTTCT
CGTAGTCTTTCTCCCCGAGTTCTTCTCCTSKCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTTCTCTCTCTCTC
TCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCCT

>'EQ87_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~~~~ACATATCACTGGTAAGGTGGAA~CGATATAGGAAGTCCGAAAGTTCGTCTCAACC
TCTGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGACTTATTATGATCAAATAACTTC
GTTACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCC
CTCTCTCTCTCTCCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCCAACCGAGTT
GTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCCT

>'HC147_F+R_micro' (1,489)
~~~~~~~~~~~~~~~~~~~~~~~~~~ACATATCTGTGGTAAGGTGGAA~CGATATAGAAAGTCCGAAAGTTCGTCTCAACC
TCTGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTC
GTTACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCG
CTCGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCC
GCGCTACCTTCGTGCACAGGCCACAGCC

>micro 04 (1,520)
~~~~~~~~~~~~AGTCCTGAGTAAACATATCACTGGTAAGGTGGAAACGATATAGAAAGTCCGAAAGTTCGTCTCAACC
TCTGCAGGGTGTTACAGAGCAATAACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTC
GTTACAATACAATAAGGACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCG
CTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGC
GCTACCTTCGTGCACAGGCCACAGCC

>'EQ83_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~~~~ACATATCACTGGTAAGGTGGAA~CGATATAGAAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAGAGCAATAACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCG
TTACAATACAATAAGGACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGC
TCTCTCTCTCTCTCTTCTCTCTCTCTCCCTCTCTCTCTCTCTCTCTCGCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCC
GCGCTACCTTCGTGCACAGGCCACAGCCT

>'EQ74_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~~~~ACATATCACTGGTAAGGTGGAA~CGATATAGAAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAGAGCAATAACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCG
TTACAATACAATAAGGACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGC
TCTCTCTCTCTCTCWCTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCAACCGAGTTGTCCACTTGGCTTTCGCCC
CCGCGCTACCTTCGTGCACAGGCCACAGCC-

>'EQ76_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~~~~ACATATCACTGGTAAGGTGGAA~CGATATAGAAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGATACAGAGCAATAACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATARCTTCG
TTACAATACAATAAGGACTTCGCGTGGTCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTCCGGCGAAGCAATCKC
TCTCTCTCTCTCTCTCTTYTCTCTCTCTATCTCTCTCTCTCTCTCTCTCTCTCTCCAACCGAGTCGTTCACTTGGCTTTCGCCCC
CGCGCTACCTTCGTGCACAGGCCACAGC—

>'EQ106_F&R_micro' (1,528)
~~~~~~~~~~~~~~~~~~~~~~~~~~ACATATCACTGGTAAGGTGGAA~CGATATAGGAAGTCCGAAAGTTCGTCTCAACC
TCTGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTC
GTTACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCC
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCCCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCT
WYCWTMGTGCACAGGCCACAGCC-

>'HC114_F+R_micro' (1,473)
~~~~~~~~~~~~~~~~~~~~~~~~~~ACATATCACTGGTAAGGTGGAA~CGATATAGAAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCG
TTACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGC

TCGCTCTCTCTCTCTCTCTCTCTCTCTCTTTTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTA
CCTTCGTGCACAGGCCACAGCC-

>'EQ110_F&R_micro' (1,475)
~~~~~~~~~~~~~~~~~~~~~~~ACATATCAGTGGTAAGGTGGAAA~GATATAGAAAGTCCGAAAGTTCGTCTCAACC
TCTGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTC
GTTACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCC
CCGCGCTACCTTCGTGCACAGGCCACAGCC

>'EQ66_F&R'
~~~~~~~~~~~~~~~~~~~~~~~ACATATCACTGGTAAGGTGGAA~CGATATAGAAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAGAGCAATAACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCG
TTACAATACAATAAGGACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGC
TCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCTCTCTCTCCCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCC
CCCGCGCTACCATAGTGCACAGGCAGTGAGCC

>'EQ111_F&R_micro' (1,461)
~~~~~~~~~~~~~~~~~~~~~~~ACATATCACTGGTAAGGTGGAA~CGATATAGAAAGTCCGAAAGTTCGTCTCAACCT
CTGATGGGTGTTACAAAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCG
TTACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTCATTGGGTCGGCTCCGGTGTTCGGCGAAGCGATCGC
TCGCGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCC
GCGCTATCCTTCGTGCACAGGCCACAGCC

>'HC115_F+R_micro' (1,452)
~~~~~~~~~~~~~~~~~~~~~~~ACATATCACTGGTAAGGTGGAA~CGATATAGAAAGTCCGAAAGTTAGTCTCAACCT
CTGATGGGTGTTACAAAGCCATAACCACACATGAAGACGTCTAGGGTTTTCTTAGATCCATTTTGATCAAATAACTTCG
TTACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGC
TCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACA
GGCCACAGCC-

>'EQ93_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~ACATATCACTGGTAAGGTGGAA~CGATATAGAAAGTCCGAAAGTTAGTCTCAACCT
CTGATGGGTGTTACAAAGCCATAACCACACATGAAGACGTCTAGGGTTTTCACAGATTTATTATGATCAAATAACTTCG
TTACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGC
TCTCTCTCTCTCTCTCTTCTCTCTCTCTCTTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCT
ACCTTCGTACACAGGCCACAGCCT

>'EQ88_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGGAAGGTGGAAACGATATAGAAAGTCCGAAAGTTCGTCTCAACC
TCTGCAGGGTGTTACAGAGCAATAACCACACACGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTC
GTTACAATACAATAAGGACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCG
CGCTCTCTCTCTCTCTTTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCC
CCGCGCTACCTTCGTGCACAGGCCACAGCC

>'EQ69_F&R_micro'
~~~~~~TCTCAACCTC
TGCAGGGTGCTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGAATTACTATGATCAAATAACTTCGT
TACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCCCTC
TCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCCTCTCTCTCTTCTCTCTCTCTCCCTCCAACCGAATTGTTCACTTGGCTTTCG
CCCCCGCGCTA---------------------

>TD152-23
~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAAAGCAATAACCACACATGAAGACGTCTAGGGTTTTCATAGATGTATTATGATCAAATAACTT------
----
CTCGTAGTCTTTCTCCCCGAGTTCTTCTCCTGGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTC
TCTCTCT--------------------------------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-38
~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAAAGCAATAACCACACATGAAGACGTCTAGGGTTTTCATAGATGTATTATGATCAAATAACTT------
----
CTCGTAGTCTTTCTCCCCGAGTTCTTCTCCTGGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTC
TCTCTCTCTCT----------------------------
CCAACCGAGTTGTTCACTTGACTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-47
~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAAAGCAATAACCACACATGAAGACGTCTAGGGTTTTCATAGATGTATTATGATCAAATAACTT------
----
CTCGTAGTCTTTCTCCCCGAGTTCTTCTCCTGGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTC
TCTCTCT--------------------------------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-61

~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAAAGCAATAACCACACATGAAGACGTCTAGGGTTTTCATAGATGTATTATGATCAAATAACTT------
----
CTCGTAGTCTTTCTCCCCGAGTTCTTCTCCTGGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTC
TCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCCT

>'EQ86_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAAAGCAATA
ACCACACACGAAGACGTCTAGGGTTTTCATAGATGTATTATGATCAAATAACTT----------
CTCGTAGTCTTTCTCCCCGAGTTCTTCTCCTSKCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTTCTCTCTC
TCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCCT

>'EQ100_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAAAGCAATA
ACCACACATGAAGACGTCTAGGGTTTTCATAGATGTATTATGATCAAATAACTT----------
CTCGTAGTCTTTCTCCCCGAGTTCTTCTCCTGGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTMTWCTCTCT
CTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCCT

>TD152-13
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAAAGCAATAACCACACATGAAGACGTCTAGGGTTTTCATAGATGTATTATGATCAAATAACTT------
----
CTCGTAGTCTTTCTCCCCGAGTTCTTCTCCTGGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTC
TCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCCT

>'EQ504_F&R_micro' (1,444)
~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAAAGCAATA
ACCACACATGAAGACGTCTAGGGTTTTCATAGATGTATTATGATCAAATAACTT----------
CTCGTAGTCTTTCCCCCCGAGTTCTTCTCCTGGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----------
----------CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>'HC122_F+R_micro' (1,455)
~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAAAGCAATA
ACCACACATGAAGACGTCTAGGGTTTTCATAGATGTATTATGATCAAATAACTT----------
CTCGTAGTCTTTCTCCCCGAGTTCTTCTCCTGGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTC
TCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCATAGCGCCGGCCAGTGAGCCTTAATCAGGCTCCATCAAG
GGCGAATTCCAGCACACTGGCGGCCGTTACTAGGGATGCGAGCGACGNCCAGTNCNN----------

>TD152-59
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGGAAGTCCGAAAGTTAGTCTCAACCT
CTGATGGGTGTTACAAAGCCATAACCACACATGAAGACGTCTAGGGTTTTCTTAGATCCATTTTGATCAAATAACTTCG
TTACAATACCAAAGGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGC
TCTCTCTCTCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCCT

>TD152-55
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAATGTGGAACGATATAGAAAGTCCGAAGGTTCGTCTCAACCTC
TGCAGGGTGTTACAGGGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGT
TACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCT
CTCTCTCTCTCTCTCTTCTCTCTCTCTTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCCT

>TD152-16
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTAGTCTCAACCTC
TGATGGGTGTTACAAAGCCATAACCACACATGAAGACGTCTAGGGTTTTCTTAGATCCATTTTGATCAAATAACTTCGT
TACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-33
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAAAGCAATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGT
TACAATACCGAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCCCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTG
GCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-93
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCATTGGTAATGTGGAACGATATAGAAAGTCCGAAGGTTCGTCTCAACCTC
TGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGT
~~~~~~~~~~~~~~~~~~~~~~~~

TACAATACCAAAAGAACTTCGCGTGACCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCT
CTCTCTCTCTCTCTCTTCTCTCTCTCTTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-62
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGGAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCG
TTACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCCCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-74
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAGAGCAATAACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCGT
TACAATACAATAAGGACTTCGCGTTATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCT
CTCTCTCTCGCCTCTCTCTCTCTCTCTCTCTCTCTCTTTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>Contig_1 (1,520)
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAGAGCAATAACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCGT
TACAATACAATAAGGACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCT
CTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-91
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGGAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCG
TTACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCCCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCACTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-80
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGGAAGTCCGAAAGTTCGTCTCAGCCT
CTGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCTAATAACTTCG
TTACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCCCC
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCT----
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-53
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGT
TACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCT
CGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-87
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAGAGCAATAACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTACCAAATAACTTCGT
TACAATACAATAAGGACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCT
CTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGC

>TD152-57
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGT
TACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCT
CGCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-11
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAGAGCAATAACCACACATGAAGACGTCTAGGGATCTCATAGATGTATTATTATCAAATAACTTCGT
TACAATACAATAAGGACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCT
CTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-08
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGGAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCG
TTACAGTACCAAAAGAACTTCGCGTGATCTTCTGACGACAGWTATTGGGTCGGCTCCGGTGTTCGGSGAAGCAATCCC
TCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCC----
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>'EQ65_-F_&_R_micro'
~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTAGTCTCAACCTCTGATGGGTGTTACAAAGCCATA
ACCACACATGAAGACGTCTAGGGTTTTCTTAGATCCATTTTGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGCG
~~~~~~~~~~~~~~~~~~~~~~~~

TGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCTCTCTCTCTCTCTCTCTCTCTC
TCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>Micro 05 (1,535)
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTAGTCTCAACCTC
TGATGGGTGTTACAAAGCCATAACCACACATGAAGACGTCTAGGGTTTTCTTAGATCCATTTTGATCAAATAACTTCGT
TACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGWTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAMTCGCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-07
~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGCGTTACAGAGCAATA
ACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCGTTACAATACAATAAGGACTTCGC
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGGAGCAATCGCTCTCTCTCTCTCTCTTCTCTCTC
TCTCTCTCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCCT

>'HC156_F+R_micro_' (1,476)
~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCCATA
ACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGC
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCGCTCTCTCTCTCTCTCTCTC
TCTCTCTCTCTTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGC

>Micro 05 (1,535)
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTAGTCTCAACCTC
TGATGGGTGTTACAAAGCCATAACCACACATGAAGACGTCTAGGGTTTTCTTAGATCCATTTTGATCAAATAACTTCGT
TACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGWTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAMTCGCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>'HC138_F+R_micro' (1,462)
~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCCATA
ACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGT
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCGCTCTCTCTCTCTCTCTCTC
TCTCTCTCTCTCTTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>'HC107_F&R_micro' (1,463)
~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGCGTTACAGAGCAATA
ACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCGTTACAATACAATAAGGACTTCGC
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCTCTCTCTCTCTCTCCTCTCTC
TCTCTCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>'EQ102_micro' (1,491)
~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGGAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCCATA
ACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGC
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCCCTCTCTCTCTCTCTCTCTCTCTCT
CTCTCCCTCTCTCTCTCTTCTCTCTCTCTCT------
CCAACCGAGTTATTCACTTGGCTTTCGCCCCCGCGCTAGTATAGTGCGCAGGCAGTGAGCCTTAATCAGGCCCCATCAA
GGGCGAATTCCAGCACACTGGCGGCCGTTACTAGGGATGCGAACTACGT----------

>'EQ84_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCAGTGGTAAGGTGGAACGATATAGGAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCG
TTACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCCCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCCTCTTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-42
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTAGTCTCAACCTC
TGATGGGTGTTACAAAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGT
TACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTGTTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCT
CGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC
>TD152-43
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCATTGGTAATGTGGAACGATATAGAAAGTCCGAAGGTTCGTCTCAACCTC
TGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGT
TACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCT
CTCTCTCTCTCTCTTCTCTCTCTCTTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACGGCC

>TD152-50
~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGGAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCG
TTACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCCCC
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-52
~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGCGTTACAAAGCAATAACCACACATGAAGACGTCTAGGGTTTTCATAGATGTATTATGATCAAATAACTTCGT
TACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCT
CGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-19
~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAGAGCAATAACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCGT
TACAATACAATAAGGACTTCGCGTGATCTTTTGACGACAGTTATTGGGTTGGCTCCGGTGTTCGGSGAAGCAATCGCT
CTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCGCAGGCCACAGC

>TD152-78
~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATCTATTATGATCAAGTAACTTCGT
TACAATACCAAATGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCT
CTCTCTCTCCCTCTCTCTCTCTCTCTCTCTCT------------------------------
CCAACCGAGTTGTTCACTCGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGC

>TD152-35
~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAGAGCAATAACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCGT
TACAATACAATAAGGACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGSGAAGCAATCGCT
CTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGC

>TD152-20
~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAGAGCCATAACCACGCATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGT
TACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCT
CGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----------
CCAGCCGAGTTGTTCGCTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>TD152-29
~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTC
TGCAGGGTGTTACAGAGCAATAACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCGT
TACAATACAATAAGGACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGSGAAGCAATCGCT
CTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCCTCGTGCACAGGCCACAGCC

>TD152-44
~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGTAAGGTGGAACGATATAGGAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAGAGCCATAACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCG
TTACAATACCGAAAGAACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGC
TCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>'HC147_F+R_micro' (1,489)
~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCTGTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCCATA
ACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGC
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCGCTCTCTCTCTCTCTCTCTCTC
TCTCTCTCTCTTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

>'EQ87_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGGAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCCATA
ACCACACATGAAGACGTCTAGGGTTTTCATAGACTTATTATGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGC
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCCCTCTCTCTCTCTCCCTCTCTCTCTCT
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCG
CTACCTTCGTGCACAGGCCACAGCCT
>'EQ83_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCAATA
ACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCGTTACAATACAATAAGGACTTCGC
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCTCTCTCTCTCTCTCTTCTCTCTCTC
~~~~~~~~~~~~~~~~~~~~~~~

TCCCTCTCTCTCTCTCTCTCTCGCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCCT

>'EQ74_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCAATA
ACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCGTTACAATACAATAAGGACTTCGC
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCTCTCTCTCTCTCWCTTCTCTCT
CTCTCTCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTCCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC-

>'EQ76_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGATACAGAGCAATA
ACCACACATGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATARCTTCGTTACAATACAATAAGGACTTCGC
GTGGTCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTCCGGCGAAGCAATCKCTCTCTCTCTCTCTCTCTTYTCTCT
CTCTATCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTCGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGC—

>'EQ106_F&R_micro' (1,528)
~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGGAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCCATA
ACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGC
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCCCTCTCTCTCTCTCTCTCTCTCTCT
CTCTCTTCTCTCTCTCCCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTWYCWTMGTGCACAGGCCACAGCC-

>'HC114_F+R_micro' (1,473)
~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCTCTGCAGGGTGTTACAGAGCCATA
ACCACACATGAAGACGTCTAGGGTTTTCATAGATTTATTATGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGC
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCGCTCTCTCTCTCTCTCTCTCTC
TCTCTTTTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC-

>'HC115_F+R_micro' (1,452)
~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTAGTCTCAACCTCTGATGGGTGTTACAAAGCCATA
ACCACACATGAAGACGTCTAGGGTTTTCTTAGATCCATTTTGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGCG
TGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCTCTCTCTCTCTCTCTCTCTC
TCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC-

>'EQ93_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~~-
ACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAAGTTAGTCTCAACCTCTGATGGGTGTTACAAAGCCATA
ACCACACATGAAGACGTCTAGGGTTTTTCACAGATTTATTATGATCAAATAACTTCGTTACAATACCAAAAGAACTTCGC
GTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGCTCTCTCTCTCTCTCTCTTCTCTCTC
TCTCTTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTACACAGGCCACAGCCT

>'EQ88_F&R_micro'
~~~~~~~~~~~~~~~~~~~~~~~~AACATATCACTGGGAAGGTGGAACGATATAGAAAGTCCGAAAGTTCGTCTCAACCT
CTGCAGGGTGTTACAGAGCAATAACCACACACGAAGACGTCTAGGGATTTCATAGATGTATTATTATCAAATAACTTCG
TTACAATACAATAAGGACTTCGCGTGATCTTTTGACGACAGTTATTGGGTCGGCTCCGGTGTTCGGCGAAGCAATCGC
GCTCTCTCTCTCTCTTTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----------
CCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGCGCTACCTTCGTGCACAGGCCACAGCC

Isolation source: *H. Crispa*
Number of sequences obtained: 1
Length variation: na
Primers

>TD152-56
GGTCCCTCAGTTCCTGTCGTACGCCTTCAGTAAGGGCTCTGACGGCATGCTTCGTTGCACAGTAGAAATGGAGACCTG
CACCTCCTGTGACTTGGTGGCCGACAATACTGTAAGAAACAATAAGGTGTTTCTGAGTACACACACACACACGTACACA
CACACGTACATGCATGTGCACACGCACACGCACACGCACACGCACACACACGCACACACACACACACACCTCTGTAAAT
ATATATATACATATATACATATATATATACATACAGTGAAACCTCACTTTTCGTACAGTCCCCTTTTCGTACGCTTCACTT
TTCGTTCACTTTTTTCGTCCTATTTTTGTCTCACTTTTCGTACATTGCCTCATATTTCGTACTTTTTTTGTT
>
Isolation source: *E. quadricolor* and *?*
Number of sequences obtained: 5
Length variation: no
Primers

>Contig_1 (1,646)
ACACAAACGCATATATAAACAGGACACAATTGCACACAAACACAAGCACACACTCATGCGCACAGCACACACACACACA
CAAACACATTAATAAACAGCGGCAATTGCACACAAACACACGCACGCACTTACACGCACACATGCACACACACACACAC
~~~~~~~~~~~~~~~~~~~~~~~~

ACACACACACAAACACSTGAGACCACATGCCTGCCAGCAGGGATCAGGGTTACCAACGTTGCCGGGTGTTTTTGTTTTG
TTTTTGTGGAGAGGAATTTTCATTATAATGGATGGTGTTGTTGGTTGCCTGACTGACTGACTACCTATCTCTTGCGTCC
CcGTCTCTTGAGATATATAGTTGTGTTCGCTGTATTTATCTACTCTATTTGTCTTTTTTATTTGATTAGAAAGCCAGCCAT
ATACTGACGCGGGTACATGGCCT
>Contig_1 (1,646)
--
ACAAACGCATATATAAACAGGACACAATTGCACACAAACACAAGCACACACTCATGCGCACAGCACACACACACACACA
AACACATTAATAAACAGCGGCAATTGCACACAAACACACGCACGCACTTACACGCACACATGCACACACACACACACAC
ACACACACAAACACSTGAGACCACATGCCTGCCAGCAGGGATCAGGGTTACCAACGTTGCCGGGTGTTTTTGTTTTGTT
TTTGTGGAGAGGAATTTTCATTATAATGGATGGTGTTGTTGGTTGCCTGACTGACTGACTACCTATCTCTTGCGTCCCc
GTCTCTTGAGATATATAGTTGTGTTCGCTGTATTTATCTACTCTATTTGTCTTTTTTATTTGATTAGAAAGCCAGCCATA
TACTGACGCGGGTACATGGCC

>'TD144-21_F+R_micro' (1,621)
ACACAAACGCATATATAAACAgGACACAATTGCACACAAACACAAGCACACACTCATGCGCACAGCACACACACACACAC
AAACACATTAATAAACAGCGGCAATTGCACACAAACACACGCACGCACTTACACGCACACATGCACACACACACACACA
CACACACACAAACACCTGAGACCACATGCCTGCCAGCAGGGATCAGGGTTACCAACGTTGCCGGGTGTTTTTGTTTTG
TTTTTGTGGAGAGGAATTTTCATTATAATGGATGGTGTTGTTGGTTGCCTGACTGACTGACTACCTATCTCTTGCGTCC
CCGTCTCTTGAGATATATAGTTGTGTTCGCTGTATTTATCTACTCTATTTGTCTTTTTTATTTGATTAGAAAGCCAGCCA
TATACTGACGCGGGTACATGGCCT

>Contig_1 (1,646)
--
ACAAACGCATATATAAACAGGACACAATTGCACACAAACACAAGCACACACTCATGCGCACAGCACACACACACACACA
AACACATTAATAAACAGCGGCAATTGCACACAAACACACGCACGCACTTACACGCACACATGCACACACACACACACAC
ACACACACAAACACSTGAGACCACATGCCTGCCAGCAGGGATCAGGGTTACCAACGTTGCCGGGTGTTTTTGTTTTGTT
TTTGTGGAGAGGAATTTTCATTATAATGGATGGTGTTGTTGGTTGCCTGACTGACTGACTACCTATCTCTTGCGTCCCc
GTCTCTTGAGATATATAGTTGTGTTCGCTGTATTTATCTACTCTATTTGTCTTTTTTATTTGATTAGAAAGCCAGCCATA
TACTGACGCGGGTACATGGCC

>Contig_1 (1,646)
ACACAAACGCATATATAAACAGGACACAATTGCACACAAACACAAGCACACACTCATGCGCACAGCACACACACACACACA
CAAACACATTAATAAACAGCGGCAATTGCACACAAACACACGCACGCACTTACACGCACACATGCACACACACACACACAC
ACACACACACAAACACSTGAGACCACATGCCTGCCAGCAGGGATCAGGGTTACCAACGTTGCCGGGTGTTTTTGTTTTG
TTTTTGTGGAGAGGAATTTTCATTATAATGGATGGTGTTGTTGGTTGCCTGACTGACTGACTACCTATCTCTTGCGTCC
CcGTCTCTTGAGATATATAGTTGTGTTCGCTGTATTTATCTACTCTATTTGTCTTTTTTATTTGATTAGAAAGCCAGCCAT
ATACTGACGCGGGTACATGGCCT
>
Isolation source: *H. Crispa*
Number of sequences obtained: 1
Length variation: na
Primers
>TD152-34
ACACAAAATACAAAACTAGATTAGATTTGTTCCGATCCTGGAACTCATGAATAAATTTGTACTATTGGTAGGGGGAGG
CTGGATGGCCTATGCTGTTTCTTGCAGTCAGAGCTCATCCATAGACAACATGCACACCCGAATGTACATGCACACACAA
ACACACACACACACACACACAAACACACGAACACACACACACACAATTGCGGACATACACACAAAACAGTACACACAAAT
ACGTATAAAGACATGTGTACACACATATGCAATCAAACACTAACAAACAAACACATACAAACACAAACATGTAAACACA
CATACACACGTTTTTCTTTCCT
>
Isolation source: *H. Crispa*
Number of sequences obtained: 1
Length variation: na
Primers
>TD152-03
ACTACTTCTTCTCAAAAACATCTTCTCAAAAACACACACACACACACACACACACACACACATATATAGGTTCTTCATATA
TTATCCAGCAATTCCTTCTAATACCAAAAACCACTAGGCCTATCTCAAATGACAAGCTAATAAATGAAAACAGTGTTTAC
TGTATCACAAAATGGTCCAAATTGCATAAGGCATTGGTTTATCAAGCAATAAATAGAATCAAATAACAATTCATAAGAT
TATTTTTTGAAATTCCAAAATATCATTTTTAGTGCCACATGGTACTTTCTGGAGAAATGTGTGGTCCT
>
Isolation source: *H. Crispa*
Number of sequences obtained: 2
Length variation: yes
Primers
>'HC109_F&R_micro' (1,430)
GAATTCGCCCTTGATGAGTCCTGAGTAAACACACCCCTTTAGTCCTCCCCCACCTCCACACACACACACACGCACACACA
CACACACACATCTGGCCACACCCACACATGTACATACATACCCCTACACACCCGGCCACAATAGAGGGAGGTTGAGGGT
TAGCTTTAGGATTTTTCTGGTCTTCCATTTCTACTCTACATTTCATTTTCATTTACTGTGGTGATATTCTCACGATTCCTT
TCTCTTCCTTTTCTTTTCTTATTACCGATGCATTGAATGTGATAGGTAACATATAGGTCGGTACTATTTCATAAACAAAC
AAACAAACACACACACACATTCATCTGTCC

>TD152-77
----------ACACACCCCTTTAGTCCTCCCCCACCTCCACACACACACACACACGCACACACACACACA—
TCTGGCCACACCCACACATGTACATACATACCCCTACACACCCAGCCACAATAGAAGGAGGTTGAGGGTTAGCTTTAGG
ATTTTTCTGGTCTTCCATTTCTACCCTACATTTCATTTTCATTTACTGTGGTGATATTCTCACGATTCCTTTCTCTTCCTTT
TCTTTTCTTATTACCGATGCATTGAATGTGATAGATGACATATAGGTCGGTACTATTTCATAAACAAACAAACAAACACA
CACACACATTCATCTGTCCT

>

Isolation source: *H. Crispa*
Number of sequences obtained: 1
Length variation: na
Primers
>TD152-79
GGCAGATTACTCATGCTCGTAAGGATAAGCGTTTTATTCCATTGTTCTCGTTGGTGCCTAACAAGACTCTCCAATATAC
AGTAATCACACAAACACGTGCAGGCATAGACTCACACACACACACACACACACACACACACACATATGTACACTCTCTCGCT
CGCCCGCTTTCTAGCTCTCTGTATTTTACTCGCTCTCTTTTGCTCTCTCCCTCTCTCTCTTGCTCACTCTCTCATTTGTTGT
CTTT
>
Isolation source: *H. Crispa*
Number of sequences obtained: 1
Length variation: na
Primers
>TD152-84
AGGCTAGGATACAATTGCTGCTGGGAAATTATAAACCCTGCAAACAAAAACTGTAATCACTGTCAAACAAACACAGAG
CAGCCTCTCTTACACTATTTACTGGAATGCCACATAACAGAGCAACTCAGAGAAAACTTCAATGAAAGCTGCAATATAA
CTAGTCTCTATGCAAGAAAAACAGCTTCTCTCTTGGTTAGACACATTTTCTTCCAAATGGACAGACTCCAAAATATCCTC
CAAACCTTCCTGCCTCCAAGATAACTATAAAAAGAACCATCCTTACCCTTACTCTTATCCTAACCCAACTCCTTATCTTCTC
CTATCAACACACATACACACTTATGTACATGCGTAACACACACACACACACACACACACACACACACACACACACACA
CCACATGCACATAAGTGCACCCACACCTAAATCATATTTCACACAATTCATTCTATCCTGATGCCCTAGGGCCTAGGGGC
>
Isolation source: *H. Crispa*
Number of sequences obtained: 1
Length variation: na
Primers
>TD152-86
AGGTACTCAAACTATTCTGTTTTCAGATTTATTTTATTTCTGTCCGGATCGTCCAGACAATTATTTTTGACAGTAGGTAC
CTGGGTCACCAATACACACGCTCACCCACACACACACACACACACACACACACACACACAACTCAAAAACACACACAACTAAAACA
CACACACACAACTCAAACACACACACACGCACACAACTAAAACACACACACACACAACTCAAACACACACACACACACAAATCACA
TACACACACAACTCAAAACTGGAGATCCAGATCATCCCGAGCAGATAGAAGTTTTGTATAACCGTCAACAACTACGATT
TATTGTATTCTAACGATCCATACATGCGGGTCGTCTAAGGCCATGTACCGGCGCCAGTATATGACTGGC
>
Isolation source: *H. Crispa*
Number of sequences obtained: 1
Length variation: na
Primers
>TD152-25
ACTTACAATACCCACAAACACACCACACACACACATACACACACACACACACACACACACACACACACACACACACACACACA
CACACACACACACACACACACACACACACACACTCAATGTGTTACAGTGGGAGAGGGCTCTCCTGAAGTAGGCGCCGATGGTGG
TATCTAGGTATTCTCTGGGGTCACTCACTTTGTCCGTTCAGGCAGATCCACATATTTGATGGTTTCACATACATCCTGAT
GTTGATGCTGTCGGTATCTTTCTTGAGTAGGCCGTTCTCCT
>
Isolation source: *H. Crispa*
Number of sequences obtained: 3
Length variation: na
Primers
>'HC104_F&R_micro' (1,563)
GTGTGCTTACAAACATGTACACTACATACCCCATACATTACTTGAGCAATAACAAATCCCCCCCCCCCCACACACACACACAC
ACACACACACACACACCA----------
GCTCTCCTCTGATATATGAAGCTTTATACTTTGTATAATGGGTAACTTTCGCTACCAAAAAAA-
TGTTTTATACGTCTTTTTAGAAAACCTTTAGCCTTTTGCATAGAATATTGTTCCTGCTTTTTTCAATAAGCATGAACATAA
ATGTTCCTACTGCACTGCCTTGCAGGGAACAATTTGAAACCCCCAGTCAAACACGACTGGAGAAGTTGGGATTTTCTTC
ACTCCCACAGCTGGATAACAATTATGTTTGCGGGGTCTTCACTTGCATGTACCCTAGGCACCTGGCAGAACTGGGGTT
GT

>Fertig (GT23G10 micro) TD152-21
GTGTGCTTACAAACATGTACACTACATACCCCATACATTACTTGAGCAATAACAAATCCCCCCCCCCCACACACACACACA
CACACACACACACACACACACACACACACACACACCAGCTCTCCTCTGATATATGAAGCTTTATACTTTGTATAATGGGTAA
CTTTCGCTACCAAAAAAAATGTTTTATACGTCTTTTTAGAAAACCTTTAGCCTTTTGCATAGAATATTGTTCCTGCTTTTTT
TCAATAAGCATGAACATAAATGTTCCTACTGCACTGCCTTGCAGGGAACAATTTGAAACCCCCAGTCAAACACGACTGG
AGAAGTTGGGATTTTCTTCACTCCCACAGCTGGATAACAATTATGTTTGCGGGGTCTTCACTTGCATGTACCCTAGGCA
CCTGGCAGAACTGGGGTTGTC

>TD152-09
GTGTGCTTACAAACATTTACACTACATACCTTTTACATTACTGTAGCAATTGCCCTCCACCCCCCACCCACACACACACCC
ACACACACACACACACACCCACACCA--------
GCTCTCCTCTGATATATGAAGCGTTATACTTTGTATAATGGGTAATGTCCGCTACCAAAAAATAAGTTTTATACGTCTTT
CTCGAAAACCTTTAGCCTTTTGGATAGGATATTTTTCCTGCTTTTTTCAATAAGCATGAACATAAATGTTACTACTGGAC
AGGTCCGCAGGGAACAATCTGAAACCCTCAGTCAAACACGACTGGAGAAGTTGGGATTTTCTCCACCCCCACAGCCGG
ATAACAATTATGTTTGCGGGGTCTACACTTGCATGTACTCTAGGTACCTGGCTGAACTGGGGTTGTT
>
Isolation source: *H. Crispa*
Number of sequences obtained: 1
Length variation: na
Primers
>TD152-88

GGGCAATAAATTCGACGGGAATTATAGATAATAGTTCGTTGTATCTTTTTGTAAATGACTTAGACCATAACCTTAGGAA
GAACTGTATCGACAGCATCGAATCCACCAAATAACAGGAGTCGAGAGATGGTTCGAACGGGCAAAAAATGAGGGTGT
GCATACTTAGGGTACGCGTGCGCACTCACACGCTCATGTACATACACATACACACACACACTCACACACACACACACACA
CACACACACCCACACACACACACGCACACACACACACACACACACACACACACACACACACACAGCAATGTGTATGGTCGCACGCA
CATGTGTTGAAGGAGTATCATCGAAAATTAGAGTGGTTGTTGATTTTGGGAAGTT
>
Isolation source: *H. Crispa*
Number of sequences obtained: 2
Length variation: na
Primers
>TD152-51
AGGTCATATACCCGCGCTAGTATATGACTGGCTTTCTACAAAAACAAAAACAACAACAACAAAGACAACAACAACAACA
AAAACAACAACAACAACAACAACAACAACAACAACAAAAACAACAACAACAACAACAACAAAAACAACAACAAAAACAACAA
CAACAGCAAAAAAAACAAAACAACAACAACAAATAAAAACAACAACAACAACAGCATACTGAAGTGGTATGCATGAATT
TATAATTCAACCGCAGGTTCCATTCCCCCTAGACACTCGGCTGT

>Fertig (GTT28 micro) TD152-06
-
GGTCATATACCCGCGCTAGTATATGACTGGCTTCCTACAAAAACAAAAACAACAACAACAACACCAACAACAACAACAA
CAACAAAAACAACAACAACAACAACAACAACGACAACAACAACAACAACAACAACAACAACAACAGCTTTGTATGG
ATCCCGAGCCAGATAAGGTTCCACGAAAGCCTCGTGCCGGTGAATCTGTCCAATAAAAACAAAATGGACGACTGGACT
AAACCTAAACCTAACCCTCAACCTTCAACCACGTGTCTGTCTGTGATTGATGCTCCAAAATCCTCCCATCAGGTCTCATG
ATATTCTGAGGATGTTCTCCTCGTGGT
>
Isolation source: *H. Crispa*
Number of sequences obtained: 1
Length variation: na
Primers
>'HC151_R_micro_schlecht' (1,534)
GGGACTGAATTTGTCTGACTTCAAATCCATCGAGCACAATCAACCAAAATAAACAAAATATCCAATTTCAGAGATAATG
ATTGTGAAATTTAGAACACAAATATATTCCAAGAAATCAACTTACCATATACCAGGTTCTAGTCGTTCACTGACTACATC
GGATTCTCCAAACACGTGACCTGGAAAAAACAACAACAAATTTTTATTTAGAACTGATCGACATACGGGAAGGAGAGA
GAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGGGGAGAGAGA
CACACACAAAAAGAGTGTGTGAGAGAAAGAGAGCGCGCGCGCGAGAGAGAGAGCGCGCGCTCTCTCTCTCTCTCT
CGCGCGCGCGGGCGGGCGCGCGGATATATAGCGAGCGCCCCTATAGAGAGAGAGAGAGAGACTTTATATCTCTCTCT
ACATATATATACANA
>
Isolation source: *H. Crispa* and *E. quadricolor*
Number of sequences obtained: 2
Length variation: yes
Primers
>'EQ81_F&R_micro'
GGTTT-
CTTTCACATAAACTGGTACTGTGTATCCAACCTGTAAGTTTGCTTCAACACGTTACATCCAATACACCTTTTACCTTCCCA
ATCAATGGGCTGTGAAATATTCTTATGGATATTGCCTGATAACGACAAGATAACACTGAACTGAACTAGGTATTCTCTC
TCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTTCTCTATCTCTCTTTCTCTCCCTGCCTGACTCTATCTCTCTC
ATGTATAAAGCCACCACTGGATATGCCTAAGAGTTTCCCCAATTATTGCGACAACCTTTATATCTATACTTATCTAAAAC
GAATACATTGATACGATATCAAACATACATGTATTTGAAATACGCTGATGAGGGTGCATCAGGCTAGTAACCGGTGT

>TD152-04
AGGCTTCTTTCACATAAACTGGTACTGTGTATCCAACCTGTAAGTTTGCTTCAACACGTTACATCCAATACACCTTTTAC
CTTCCCAATCAATGGGCTGTGAAATATTCTTATGGATATTGCCTGATAACGACAAGATAACACTGAACTGAACTAGGTA
TTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTC--
TTTCTCTATCTCTCTTTCTCTCCCTGCCTGACTCTATCTCTCTCATGTATAAAGCCACCACTGGATATGCCTAAGAGTTTC
CCCAATTATTGCGACAACCTTTATATCTATACTTATCTAAAACGAATACATTGATACGTTATCAAACATATGCTGTTTTGA
AATACACTGATGAGGGTGCATCAAGCTAGTAACCGGT
>
Isolation source: *H. Crispa,*
Number of sequences obtained: 1
Length variation: na
Primers

>TD152-22
ATCACAAACAGAATCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTKCTTCTGCTTCTGCTTCTGCTT
CTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTT
TCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTYTTCTTCTTCTTCTTCTTCTTCTTCTTCTT
CTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCCTCCGTCCATGTAATGCTTTAGTAT
AAAGACCAATCACCAGAGGGCGTCCT
>
Isolation source: *H. Crispa, E. quadricolor*
Number of sequences obtained: 2
Length variation: yes
Primers

>'EQ73_F&R_micro'

AGCTCAACGGCCGCACCCCTCCCTTGCATGCCACATATTCACGCCTATCAAGACACAAGACCCAAAAGCAAGAAGGCTC
TCTCACTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTTCT----

CAATAATTCCATGTGGCAATAATTCTATGTCGCAATAATTTCATGTCGCAATAATTATATGTTTTACAAACATGCACAAC
GCACAGAACATAATGTCATAGAAGAAATGTTTTCATATGGGCTCAGCATGCAATACGTGTGCATCTATATGTCAGTATG
CCGCCTGAATCGATGAAATATAAGACTAGGAAAATTCCTTGAAC

>TD152-49
TTGTTTTACCCCGTACCAGGGACGTGTTTACTGGGTGTGTGTGTGTGTGTGTGTGTGTGTCTGGGTGTGTTCTGC
GTTTGTATAAGGTGTACATCGAGGACACAAGCGAAACCAAAGCACCCCTCTCTTGCATGCCACATATTCACGCCTATCA
AGACACAAGACCCAAAAGCAAGAAGGCTCTCTCTCTCCCTCTTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTTCT
CTCTCTCTCAATAATTCCATGTGGCAATAATTCCATGTCGCAATAATTTCATGTCGCAATAATTATATGTTTTACAAACAT
GCACAACGCACAGAACATAATGTCATAGAAGAAATGTTTTCATATGGGCTCAGCATGCAATACGTATGCATCTATATGT
CAGTATGCCGCCTGAATCGATGAAATATAAGACTAGGAAAAGTCCTTGAACT
>
Isolation source: *H. Crispa,*
Number of sequences obtained: 1
Length variation: na
Primers

>TD152-31
AGACACGAGGGCAAAAGGCCTAAAGTACGCGTCGCTTGCCATTGTGCCTTAGGCCTCTATGACTGCACTAAAAGCCTC
CTGAGATCGATGTTACTAATGGAAGGGACTCATTCTCGATACTACCAGCTTCCTGGCTCTCTCTCTCTCTCTCTCTCT
CTCTCTCTCTCTCTCTCGCTCTCTCCCTCTCTACCTCTCTTTCTCTTTCTCGTTCTCTCTTTCTCCTTCTTTCTTTCTCTCTCT
ATTTATCTTTCTAAGTGCTGTGTGGGTGTCC

Isolation source: *H. Crispa,*
Number of sequences obtained: 1
Length variation: na
Primers
>Fertig (TC31 micro) TD152-28
GCGAAACGAGACCATAAATTTCAACGTATTTGTAATAATAGAAAGGTTATGTTTGTACTATTGTGTCATGTGTTCGTGT
ATAATATGCCTCTGAATTTGACTGTGTCTATGTAGATTGATCTTCCTTGTTTTTCTGTCAGTCGGTAGACCGGTTAGAA
TTCCTATGCATTCTTTACATCTCCCCTTTCTCATTATCTATCACACATACTTCAATCTCTCTCTCTCTCTCTCTATCTCTC
TCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCCTGCCTCCTTCTCTCTAAAGTACGTTCTATCAAAAGTAAATTGTTTTT
CCACCCCTTGTCTGGCAGCATGT
>
Isolation source: *E. quadricolor*
Number of sequences obtained: 2
Length variation: yes
Primers
>'EQ71_F&R_micro'
AACGGTCGTCGTTCTCATCCACTAGTAACGGCCGCCAGTGTGCTGGAATTCGCCCTATAGTGAGTCGTATTACAATTCA
CTGGCCGTCGTTTTACTCCTGTAGCGACATGGTATCCCATGATCGCTACAGCTAAAAAAAAAGAACCAACGGCTCAGTTT
TTCCCTCACCCCCCAACCCCCACCACAAGGTAATAAATAGGATTACCTGACCTGACCTCTTTCTCACTTCTTCTTTTCACT
AAGTCCAGTCAACAGCCTCTCTCTCTCTTTCTCTCTCTCTYTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTC
TCTCTCTCTCTCTCTCTCTCTCTCCGTACAGCAGTAACCCCGCCAAACGTCTTATCACCTACAGCTGCAGCACCCACGT
TGTCTAT

>Fertig (GA37 micro) 'TD144-36_F+R_Micro' (1,486)

AACCCCAGGGGAAGGGGGAATCCTGTAGCGACATGGTATCCCATGATCGCTACAGCTAAAAAAAAAGAACCAACGGCT
CAGTTTTTCCCTCACCCCCCAACCCCCACCACAAGGTAATAAATAGGATTACCTGACCTGACCTCTTTCTCACTTCTTCTT
TTCACTAAGTCCAGTCAACAGCCTCTCTCTCTCTTTCTCTCTCTCTTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTC
TCTCTCTCTCTCTCTCT----------
CCGTACAGCAGTAACCCCGCCAAACGTCTTATCACCTACAGCTGCAGCACCCACGTTGTCTAC
>

Isolation source: *E. quadricolor* and ?
Number of sequences obtained: 5
Length variation: yes
Primers

>Micro 01b (1,671)
AACCCTAAAAGTGTTTATTTTGATGTCTCTAATTGTCGTCGATGGACGAAGTGTGCACGCAAGATTGCACGACTTCTAA
AAATAATTGTTTTTTCTCAAAATATTATGGGATCTTCGAGAACAAGAGGGCTTTGAAAATACACCACGATGTGTCGAGG
TGTAGAGATAAGGAAAACATACAGAGAAATATAAAAATATGCACTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----
CCATGGCCCAGGCCTCTCTTTCACAGTCGTAATATAAATGTATGTAATAGTTTTCTGCATAATATTATTGTAGATATCTA
CCAGGTATACTGAATAACCCACGGTTGGTGATGAAAGATGCGTTGATATGTTGAAGATAGAACCTCGACTAATCTCGA
GTACCCAATATCATCTGATTTTGTCATCAGGGCGTACAGTGCTACACGCTATCGAGTTGCACCACT

>Micro 01 (1,671)
AACCCTAAAAGTGTTTATTTTGATGTCTCTAATTGTCGTCGATGGACGAAGTGTGCACGCAAGATTGCACGACTTCTAA
AAATAATTGTTTTTTCTCAAAATATTATGGGATCTTCGAGAACAAGAGGGCTTTGAAAATACACCACGATGTGTCGAGG
TGTAGAGATAAGGAAAACATACAGAGAAATATAAAAATATGCACTCTCTCTCTCTCTCTCTCTCTCTCTCTCT----
CCATGGCCCAGGCCTCTCTTTCACAGTCGTAATATAAATGTATGTAATAGTTTTCTGCATAATATTATTGTAGATATCTA
CCAGGTATACTGAATAACCCACGGTTGGTGATGAAAGATGCGTTGATATGTTGAAGATAGAACCTCGACTAATCTCGA
GTACCCAATATCATCTGATTTTGTCATCAGGGCGTACAGTGCTACACGCTATCGAGTTGCACCACT

>'EQ67_F&R_micro'

-
ACCCTAAAGGTGTTTATTTTGATGTCTCTAATTGTCGTCGATGGACGAAGTGCGCACGCAAGATTGCACGACTTCCAAA
AATAATTGTTTTTTCTCAAAATATTATGG-
ATCTTCGAGAACAAGAGGGCTTTGAAAATACACCACGATGTGTTGAGGTGTAGAGATAAGGAAAACATACAGAGAAA
TATAAAAATATGCACACTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCATGGCCCAGGCCTCTCTTTCACAGTCGTAA
TATAAATGTATGTAATAGTTTTCTGCATAATATTATTGTAGATATCTACCAGGTATACTGAATAACCCACGGTTGGTGA
TGAAAGATGCGTTGATATGTTGAAAATAGAACCTCGACTAATCTCGAGTACCCAATATCATCTGATTTTGTCATCAGGG
CGTACGGTGCTACACACTATCGAGTTGCACCAT

>Contig_1 (1,671)
AACCCTAAAAGTGTTTATTTTGATGTCTCTAATTGTCGTCGATGGACGAAGTGTGCACGCAAGATTGCACGACTTCTAA
AAATAATTGTTTTTTCTCAAAATATTATGGGATCTTCGAGAACAAGAGGGCTTTGAAAATACACCACGATGTGTCGAGG
TGTAGAGATAAGGAAAACATACAGAGAAATATAAAAATATGCACTCTCTCTCTCTCTCTCTCTCTCTCTCT----
CCATGGCCCAGGCCTCTCTTTCACAGTCGTAATATAAATGTATGTAATAGTTTTCTGCATAATATTATTGTAGATATCTA
CCAGGTATACTGAATAACCCACGGTTGGTGATGAAAGATGCGTTGATATGTTGAAGATAGAACCTCGACTAATCTCGA
GTACCCAATATCATCTGATTTTGTCATCAGGGCGTACAGTGCTACACGCTATCGAGTTGCACCAC

>Fertig (GA16 F micro) 'TD144-07_F+R_micro' (1,636)
--- CCTAAAAGTGTTTATTTTGATGTCTCTAATTGTCGTCGATGGACGAAGTGTGCACGCAAGATTGCACGACTTCTAAAAA
TAATTGTTTTTTCTCAAAATATTATGGGATCTTCGAGAACAAGAGGGCTTTGAAAATACACCACGATGTGTCGAGGTGT
AGAGATAAGGAAAACATACAGAGAAATATAAAAATATGCACTCTCTCTCTCTCTCTCTCTCTCTCTCT----
CCATGGCCCAGGCCTCTCTTTCACAGTCGTAATATAAATGTATGTAATAGTTTTCTGCATAATATTATTGTAGATATCTA
CCAGGTATACTGAATAACCCACGGTTGGTGATGAAAGATGCGTTGATATGTTGAAGATAGAACCTCGACTAATCTCGA
GTACCCAATATCATCTGATTTTGTCATCAGGGCGTACAGTGCTACACGCTATCGAGTTGCACCAC
>
Isolation source: *H. Crispa,*
Number of sequences obtained: 1
Length variation: na
Primers
>Fertig (GA26 micro) TD152-69
CAAAGTGGATTCTTCCCTATATCGCAATAAATTATTATCCCCTCTATCTTTCTTCCTCTATATCTGCAGTCTACATGTATC
CCACCTCTCTCTCTCTCTCTCTCTCTCCCTCTTCCCTCTCTCTCTCTCTCTCTCTCTCTCTTTCGAAAACGGACATAATTATG
CTATAAAAATTATTCTATAGTGTAGGCCTACTTATATATAGACTGACAACACTGACATCAAAATTATAAACACAACCGAG
TAGAACAATTTGTTGCTCCTATATTATACAACTACATGTATAAGCTGTAGATCCAATAACGTTTGAGCCAGAGGCCTTTC
TCGAAGAATTGTCGCCT

Isolation source: *E. quadricolor*
Number of sequences obtained: 2
Length variation: no
Primers
>Fertig (GTAT23 micro) 'TD144-45_F+R_Micro' (1,533)
--
AGCAATAGCGGTCCTACCAGTAAATGATACCTTTATACATACATAAATACATCTACATACATACATACATACATACATAC
ACATACATACATACATACATACATACATACATACATACATACATACATACATACATACATACATACATAAACATACTTTTTCAGT
GGCAGGATCACCGAAACGAAGAAGGAGCTGTCTCTGGATTGGGATCCACACGACGGCTAGAGTATTTTTATTCCTGG
AAGCAACTATTACAGCTGACTAACACTGGTTCCTAAATTGGCTACTTAGGTACACACGAGCTGTTTTACGTCATAGGTA
CGCTCTCACGAGCGGAGACC

>'EQ503_F&R_micro' (1,513)
ACAGCAATAGCGGTCCTACCAGTAAATGATACCTTTATACATACATAAATACATCTACATACATACATACATACATACAT
ACACATACATACATACATACATACATACATACATACATACATACATACATACATACATACATACATACATAAACATACTTTTTCA
GTGGCAGGATCACCGAAACGAAGAAGGAGCTGTCTCTGGATTGGGATCCACACGACGGCTAGAGTATTTTTATTCCTG
GAAGCAACTATTACAGCTGACTAACACTGGTTCCTAAATTGGCTACTTAGGTACACACGAGCTGTTTTACGTCATAGGT
ACGCTCTCACGAGCGGAGACC
>
Isolation source: *H. Crispa,*
Number of sequences obtained: 1
Length variation: na
Primers
>Fertig (CTT47 micro)'TD152-10
GCAAAGGATTTCCCGATGCAAGGAGCAGATTTGTATTTGTTGAAAGTGTTTTAGAACACAATGCTTAGCTGGAACAAA
AGGATTTTATGGCAAGCATATATATTTGGAAAGGACAAAGAAATGCTACGTGGTTCGTCTTCTTCTTCTTCTTCTTC
TTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCT
TCTTCTTCTTCTCCTTCTTCTTCTTCTTCTCCTCCTTCTTCTCCAACAACATCTTCGTCTTTGAC
>
Isolation source: *E. quadricolor*
Number of sequences obtained: 4
Length variation: yes
Primers

>Fertig (AG16CG8 micro) 'TD144-18_F+R_micro' (1,564)
---TACATTTCA-
TCTTCAAATCCAGCTTGGCTTTACAGTGGAAACGCGCGCGCGCGCGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT
—
GGTCTATCCATCGTATCTGTCTGGTTTGCGAACTAGTTATAAAGTTATAATATTGTCGATGTTGATGACTATTATAATG
ATGCTGAGTTAGTACGTTGCTCCCCCCCTAACAGCCATAAACACCAATACAATAACTACCCACACCACAAGACGTGGACT

AATGCCTGTTGTGCCGAACAGACGTCAAACTCTATTTCAACATCAGCATCGTCATCTGGTTTCTAAACGTTTTATTGTTC
ACAAATTGATCATGTCATCGTTAGTGTGCATACCGAATAATTCTGCACATGTATAAACACCCGTGC

>Fertig (AG16CG8 micro) Micro 06 (1,621)
--
CTACATTTCACTCTTCAAATCCAGCTTGGCTTTACAGTGGAAACGCGCGCGCGCGCGCTCTCTCTCTCTCTCTCTCTC
TCTCTCTCTSt—
GGTCTATCCATCGtATCTGTSTGGTKTGCGAACTAKWTATAAAGWTATAATAKTGTCGATGTTGATGACTATTATARTG
ATGCTGAGTTAGTACGTTGCTCCCCCCCTAACAGCCATAAACACCAATACAATAACTACCCACACCACAAGACGTGGACT
AATGCCTGTTGTGCCGAACAGDCGTCAAACTCTATTTCAACATCAGCATCGTCATCTGGTTTCTAAACGTTTTATTGTTC
ACAAATTGATCATGTCATCGTTAGTGTGCATACCGAATAATTCTGCACATGTATAAACACCCGTGC

>Fertig (AG16CG8 micro) 'TD_144-27_F+R_micro' (1,563)
---TACATTTCA-
TTTTCAAATCCAGCTTGGCTTTACAGTGGAAACGCGCGCGCGCGCGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT
CTGGTCTATCCATCGTATCTGTCTGGTTTGCGAACTAGTTATAAAGTTATAATATTGTCGATGTTGATGACTATTATAAT
GATGCTGAGTTAGTACGTTGCTCCCCCCCTAACAGCCATAAACACCAATACAATAACTACCCACACCACAAGACGTGGA
CTAGTGCCTGTTGTGCCGAACAGACGTCAAACTCTATTTCAACATCAGCATCGTCATCTGGTTTCTAAACGTTTTATTGT
TCACAAATTGATCATGTCATCGTTAGTGTGCATACCGAATAATTCTGCACATGTATAAACACCCGTGC

>'HC108_F&R_micro' (1,552)
-ACTACATTTCA-
TTTTCAAATCCAGCTTGGCTTTACAGTGGAAACGCGCGTGCGCGCGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTC
TGGTCTATCCATCGTATCTGTCTGGTTTGCGAACTAGTTATAAAGTTATAATATTGTCGATGTTGATGACTATTATAAT
GATGCTGAGTTAGTACGTTGCTCCCCCCCTAACAGCCATAAACACCAATACAATAACTACCCACACCACAAGACGTGGA
CTAATGCCTGTTGTGCCGAACAGACGTCAAACTCTATTTCGACATCAGCATCGTCATCTGGTTTCTAAACGTTTTATTGT
TCACAAATTGATCATGTCATCGTTAGTGTGCATACCGAATAATTCTGCACATGTATAAACACCCGTGC

Isolation source: *H. Crispa,*
Number of sequences obtained: 1
Length variation: na
Primers
>Fertig (CA34 micro) TD152-94
ACAGCATGACGTCATGCAATCAGCATCCGGATCCATAATTGTTTTATTATTTATATTCACACACACACACACACACA
CACACACACATAGTCACACAACACACACACACACACACACACACACACACACACACCCATAGTCACAAAACACACACACA
CCCAGTACTCATTGTGTGAATGGGTCATGTTACCATCGGCATCTGTTTTCTGAGGTCCATTTATGTGC

Isolation source: *H. Crispa,*
Number of sequences obtained: 1
Length variation: na
Primers
>Fertig (AC14 micro) TD152-14
GTGATCTATCTGCCATGTTACCCACGCTGATTTTCCGAGGTGATGCCATCTTGAGAGATCCACTGCGTACACGAGGCAA
GTTGTGGTAAGAATGAAGAAGATGTGAGTGTTTACTCTTAGCCTCCACCTACAACATCCTCTGCTAAACAAGCAGCAAC
AAGAAATCTCCGCCCATACACACACACACACACATACACACTCACACACGAGTGAGCAGGCTCACCTCTCGGTTGGTCG
GTTGGTCGGTTGGCTACATACAGAACATGAATCAACCTGATTACAATACTCAACATGATTTTAGAAAGTTTTACCTCTA
CCTATAAGCTTGCCATTGCTTCTTG
>
Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
Primers
>Fertig (GT16 micro) 'TD144-29_F+R_micro' (1,555)
AGCAAAGCAAATCGGTATTAGGCCCCAAACCTGTTTATTTTATCACAACAAGTTTTCATGCTTTCGGGAAACATAATTTA
TGTCCTAATTGATCACACACACACACACACACACACACACACATACACTTACTCAGATCTATCTGTCTACCCGCTTCCTTG
TTGGAACATGAATGTCAGTGATTTTGAGTTGCCTACGTTCGGTATTATTAGTTTATGAATAATGCAATGACAAGTAGCC
TACCCTTGCTTGGAGTATCTTGACAGCAGTTTACGAGCCATTTCCACGCGACACCATTCAATATCACCCAGAATACACAC
TCACTGTGTTTTCTGTCTAC

Isolation source: *E. quadricolor* and ?
Number of sequences obtained: 6
Length variation: yes
Primers >

>Fertig (GAA48GCAGAA11GAA13 micro) 'TD144-14_F+R_micro' (1,541)

GGGAAGGAGTTCCTTAGGGAAGGGAGGATGTCCCCTGAAGGAGAGGGGATGGGTGCCATGACTCCTATCTACCACT
CTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCT
TCTGCTTCTGCTTCTGCTTCTGCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCT
TCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTCCTTCTTCTTCTTCCT
CCGTCCATGTAATGCTTTAGTATAAAGACCAATCACCAGAGGGCGTCC

>Contig_1 (1,583)
~~~~~~
~~--
GGAAGGAGTTCCTTAGGGAAGGGAGGATGTCCCCTGAAGGAGAGGGGATGGGTGCCATGACTCCTATCTACCACTCT
TCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTC
TGCTTCTGCTTCTGCTTCTKCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCttCTTCT
~~~~~~

TCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCNTCTTCTTCTTCTCCTTCTTCTTCTTCCTCCGTCCATGTAATGCTT
TAGTATAAAGACCAATCACCAGAGGGCGTCCT

>Micro 02 (1,583)
~~~~~~
~~AGGGAAGGAGTTCCTTAGGGAAGGGAGGATGTCCCCTGAAGGAGAGGGGATGGGTGCCATGACTCCTATCTACC
ACTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCT
GCTTCTGCTTCTGCTTCTGCTTCTKCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCtt
CTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCNTCTTCTTCTTCT

>Contig_1 (1,583)
TACGCCAGCTTGGTACCGGGCTCGGNTCCACTAGTAACGGCCGCCAGTGTGCTGGAATTCGCCCTTGATGAGTCCTGA
GTAAGGGAAGGAGTTCCTTAGGGAAGGGAGGATGTCCCCTGAAGGAGAGGGGATGGGTGCCATGACTCCTATCTAC
CACTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTC
TGCTTCTGCTTCTGCTTCTGCTTCTKCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTC
ttCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCNTCTTCTTCTTCTCCTTCTTCTTCTTCCTCCGTCCATGTA
ATGCTTTAGTATAAAGACCAATCACCAGAGGGCGTCCT

>Fertig (GAA48GCAGAA11GAA13 micro) Micro 02 (1,583)
~~~~~~
~~~GGGAAGGAGTTCCTTAGGGAAGGGAGGATGTCCCCTGAAGGAGAGGGGATGGGTGCCATGACTCCTATCTACC
ACTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTC------
TTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTKCTTCTTCTTCTTCTTCT
TCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCttCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTC
NTCTTCTTCTTCTCCTTCTTCTTCTTCCTCCGTCCATGTAATGCTTTAGTATAAAGACCAATCACCAGAGGGCGTCC

>Fertig (GAA48GCAGAA11GAA13 micro ) Contig_1 (1,583)
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
~~~GGGAAGGAGTTCCTTAGGGAAGGGAGGATGTCCCCTGAAGGAGAGGGGATGGGTGCCATGACTCCTATCTACC
ACTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTC------
TTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTGCTTCTKCTTCTTCTTCTTCTTCT
TCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCttCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTC
NTCTTCTTCTTCTCCTTCTTCTTCTTCCTCCGTCCATGTAATGCTTTAGTATAAAGACCAATCACCAGAGGGCGTCC
>

Isolation source: *H. Crispa,*
Number of sequences obtained: 1
Length variation: na
Primers
>Fertig TD152-15 (micro 152-15)
CATAAATCGATTCATTTCAATATCATCAGAACAGGAAGAAAGTCTACGTAAATGCGATCTGATGACGAAAAACAACAAC
AAAAAGACACAGACAACAACAACAACAACAAAAACAACAACAACAACAACAACAACAACAACAACAACAACGACAACAA
CAACAACAACATCAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACA
ACAACAACAACAGCGACAACAACAACAGCAACAACAACAATAATAATAATTAGAAACCCAATGGCGAGTTTTATTGGC
TCTCCCGTGTACTGACCTCACCCCCC

Isolation source: *E. quadricolor,* : *H. Crispa,* and ?
Number of sequences obtained: 7
Length variation: no
Primers >

>Fertig (micro neu 1+2) 'TD144-30_F+R_micro' (1,550)
GTGGAAACACTTACCAACAACAAGAAATTCTTGGGTTCGTGCCGACAAAATGGATCAAAATGCGCTTTTCCACATAGAA
AATTGGGGCGCGGTAATTAGGTCACAGGAAATTTGGTCACCGGAAATTTGGTCACAGTAACTCGGTCACATGGTAAAT
TGGTTAGGGTTATTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTTAGGTTTACGGTTAGGGTTTTGGTTAGGGTTA
GGGTTTAGGTTTACGGTTAGGGTTTTGGTTAGGTTTACGGAACGGGGGCACAAAACTAGGTTGCCGCCCCGAAAGTT
CTGGCGCAGGTCCAGAGATAAGTCTGGTTTCGTT

>Contig_1 (1,570)
GTGGAAACACTTACCAACAACAAGAAATTCTTGGGTTCGTGCCGACAAAATGGATCAAAATGCGCTTTTCCACATAGAA
ACtTGGGGCGCGGTAATTAGGTCACAGGAAATTTGGTCACCGGAAATTTGGTCACAGTAATTCGGTCACATGGTGAAT
TGGTTAGGGTTATTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTTAGGTTTACGGTTAGGGTTTTGGTTAGGGTTA
GGGTTTAGGTTTACGGTTAGGGTTTTGGTTAGGTTTACGGGACGGGGGCACAAAACTAGGTTGCCGCCCCGAAAGTT
CTGGCGCAGGTTCAGAGATAAGTCTGGTTTCGTT

>TD152-27
GTGGAAACACTTACCAGCAACAAGAAATTCTTGGGTTCGTGCCGACAAAATGGATCAAAATGCGCTTTTCCACATAGA
AAATTGGGGCGCGGTAATTAGGTCACAGGAAATTTGGTCACCGGAAATTTGGTCACAGTAACTCGGTCACATGGTAA
ATTGGTTAGGGTTATTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTTAGGTTTACGGTTAGGGTTTTGGTTAGGGTT
AGGGTTTAGGTTTACGGTTAGGGTTTTGGTTAGGTTTACGGGACGGGGGCACAAAACTAGGTTGCCGCCCCGAAAGT
TTTGGCGCAGGTTCAGAGATAAGTCTGGTTTCGTTT

>TD152-39
GTGGAAACACTTACCAACAACAAGAAATTCTTGGGTTCGTGCCGACAAAATGGATCAAAATGCGCTTTTCCACATAGAA
AATTGGGGCGCGGTAATTAGGTCACAGGAAATTTGGTCACCGGAAATTTGGTCACAGTAATTCGGTCACATGGTAAAT
TGGTTAGGGTTATTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTTAGGTTTACGGTTAGGGTTTTGGTTAGGGTTA
GGGTTTAGGTTTACGGTTAGGGTTTTGGTTAGGTTTACGGGACGGGGGCACAAAACTAGGTTGCCGCCCCGAAAGTT
CTGGCGCAGGTTCAGAGATAAGTCTGGTTTCGTTT
~~~

>TD152-26
GTGGAAACACTTACCAGCAACAAGAAATTCTTGGGTTCGTGCCGACAAAATGGATCAAAATGCGCTTTTCCACATAGA
AAATTGGGGCGCGGTAATTAGGTCACAGGAAATTTGGTCACCGGAAATTTGGTCACAGTAACTCGGTCACATGGTAA
ATTGGTTAGGGTTATTAGGGTTAGGGTTAGGGTTAGGGTTTAGGTTTACGGTTAGGGTTTTGGTTAGGGTT
AGGGTTTAGGTTTACGGTTAGGGTTTTGGTTAGGTTTACGGGACGGGGGCACAAAACTAGGTTGCCGCCCCGAAAGT
TTTGGCGCAGGTTCAGAGATAAGTCTGGTTTCGTTT

>TD152-58
GTGGAAACACTTACCAACAACAAGAAATTCTTGGGTTCGTGCCGACAAAATGGATCAAAATGCGCTTTTCCACATAGAA
AATTGGGGCGCGGTAATTAGGTCACAGGAAATTTGGTCACCGGAAATTTGGTCACAGTAATTCGGTCACATGGTAAAT
TGGTTAGGGTTATTAGGGTTAGGGTTAGGGTTAGGGTTTAGGTTTACGGTTAGGGTTTTGGTTAGGGTTA
GGGTTTAGGTTTACGGTTAGGGTTTTGGTTAGGTTTACGGGACGGGGGCACAAAACTAGGTTACCGCCCCGAAAGTT
CTGGCGCAGGTTCAGAGATAAGTCTGGTTTCGTTT

>TD152-05
GTGGAAACACTTACTAACAACAAGAAATTCTTGGGTTCGTGCCGACAAAATGGATCAAAATGCGCTTTTCCACATAGAA
AATTGGGGCGCGGTAATTAGGTCACAGGAAATTTGGTCACCGGAAATTTGGTCACAGTAATTCGGTCACATGGTAAAT
TGGTTAGGGTTATTAGGGTTAGGGTTAGGGTTAGGGTTTAGGTTTACGGTTAGGGTTTTGGTTAGGGTTA
GGGTTTAGGTTTACGGTTAGGGTTTTGGTTAGGTTTACGGGACGGGGGCACAAAACTAGGTTGCCGCCCCGAAAGTT
CTGGCGCAGGTTCAGAGATAAGTCTGGTTTCGTT
>
Isolation source:?
Number of sequences obtained: 2
Length variation: yes
Primers
:
>Contig_1 (1,646)
----GTTTNAGTACGACGTTGTAAA-
CGACGGCCAGTGAATTGTAATACGACTCACTATAGGGCGAATTGGGCCCTCTAGATGCATGCTCGAGCGGCCGCCAGT
GTGATGGATATCTGCAGAATTCGCCCTTGATGAGTCCTGAGTAAGGCCATGTACCCGCGTCAGTATATGGCTGGCTTT
CTAATCAAATAAAAAAGACAAATAGAGTAGATAAATACAGCGAACACAACTATATATCTCAAGAGACgGGGACGCAAG
AGATAGGTAGTCAGTCAGTCAGGCAACCAACAACACCATCCATTATAATGAAAATTCCTCTCCACAAAAACAAAACAAA
AACACCCGGCAACGTTGGTAACCCTGATCCCTGCTGGCAGGCATGTGGTCTCASGTGTTTGTGTGTGTGTGTGTGTGT
GTGTGTGCATGTGTGCGTGTAAGTGCGTGCGTGTGTTTGTGTGCAATTGCCGCTGTTTATTAATGTGTTTGTGTGTGT
GTGTGTGCTGTGCGCATGAGTGTGTGCTTGTGTTTGTGTGCAATTGTGTCCTGTTTATATATGCGTTTGTGTT

>Contig_1 (1,535)
--CGGTTTCMGTMCGACGTTGTAAAACGACGGC-
MGTGAATTGTAATACGACTCACTATAGGGCGAATTGGGCCCTCTAGATGCATGCTCGAGCGGCCGCCAGTGTGATGG
ATATCTGCAGAATTCGCCCTTGATGAGTCCTGAGTAAACATATCACTGGTAAGGTGGAACGATATAGAAAGTCCGAAA
GTTAGTCTCAACCTCTGATGGGTGTTACAAAGCCATAACCACACATGAAGACGTCTAGGGTTTTCTTAGATCCATTTTG
ATCAAATAACTTCGTTACAATACCAAAAGAACTTCGCGTGATCTTTTGACGACAGWTATTGGGTCGGCTCCGGTGTTC
GGCGAAGCAMTCGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCAACCGAGTTGTTCACTTGGCTTTCGCCCCCGC
GCTACCTTCGTGCACAGGCCACAGCCT

Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
Primers
>'EQ109_F&R_micro' (1,538)

ATGATGATGCNNNNCTGGTACGTMGNTCGTGATCCACTAGTAACGGCCGCCAGTGTGCTGG----------
AATTCGCCCTAKAGKGAGTCGGATTACAATTCACTGGCCGTCGTTTTACATCACAACCATAACCCTAACTACAACCACAA
CCACAGTCACAAGCACAACCATAACCACAACCACAACTACAACAACAACAACAATAACAACAGCAACAACAACAACAACA
ACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAACAAC
CACAATTACAACCACAACCACAACCAAAACCATAACTACAACCACCACAACCACAACCACCACAACCATAACCACAACCAC
AACCACAACCATAACCATAACT

Isolation source: *?*
Number of sequences obtained: 1
Length variation: na
Primers

>Contig_1 (1,621)
-CCGGTTTCMGTMCGACGTTGTAAAACGACGGC-
MGTGAATTGTAATACGACTCACTATAGGGCGAATTGGGCCCTCTAGATGCATGCTCGAGCGGCCGCCAGTGTGATGG
ATATCTGCAGAATTCGCCCTTGATGAGTCCTGAGTAAGCACGGGTGTTTATACATGTGCAGAATTATTCGGTATGCAC
ACTAACGATGACATGATCAATTTGTGAACAATAAAACGTTTAGAAACCAGATGACGATGCTGATGTTGAAATAGAGTT
TGACGHCTGTTCGGCACAACAGGCATTAGTCCACGTCTTGTGGTGTGGGTAGTTATTGTATTGGTGTTTATGGCTGTT
AGGGGGGGAGCAACGTACTAACTCAGCATCAYTATAATAGTCATCAACATCGACAMTATTATAWCTTTATAWMTAGTT
CGCAMACCASACAGATaCGATGGATAGACCaSAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGCGCGCGCGCGCGC
GTTTCCACTGTAAAGCCAAGCTGGATTTGAAGAGTGAAATGTAGTT

Isolation source: *H. Crispa,*
Number of sequences obtained: 4
Length variation: yes
Primers

>TD152-41
ACAAAAAAGCCCATAAAAATAGTAAATGTTCGTCTCGGAGTAATACAATTTGATGCACATACATCAGAAAGACTATCTA
GAGCACCATTTGAGTATAAGAAGTGAAAACAGCCGGGAGGTAGGAAAGTGTAATATGGAGTCTCAGCGCGTTCGATT
ACTTACTCGAGCCTAAAGCGTTCGTTTACTTGATAAGGTCGCAAGTCGCGCTTACTCGAGACTAACATATGATAGTGAA
TACATAATAATGCAAATATGGATGAAGGCCCGCTCTGTGCTATTTTTATTTCTTGTTTATTGATGTTTTATTCCATGAAA
AGCGTGATGTCCTTGCCCCCCCCCCACACACACACACA~~~~~~~CTTTTAGCAGTGGTACGTATCCTACGCGCCT

>'HC131_R_micro' (1,599)
ACAAAAAAGCCCATAAAAATAATAAATGTTCGTCTCGGAGTAATACAATTTGATGCACATACATCAGAAAGACTATCTA
GAGCACCATTTGAGTATAAGAAGTGAAAACAACCGGGAGGTAGGAAAGTGTAATATGGGGTCTCAGCGCGTTCGATT
ACTTACTCGAGCCTAAAGCGTTCGTTTACTTGATAAGGTCGCAAGTCGCGCTTACTCGAGACTAACATATGATAGTGAA
TACATAATAATGCAACTATGGATGAAGGCCCGCTCTGTGCTATTTTTATTTCTTGTTTATTGATGTTTTATTCCATGAAA
AGCGTGATGTCCTTGCCCCCCCCCCCCCCCACACACACACACACACATTTTTAGCAGGGGTACTTATCCAACCCCC

>'HC142_F+R_micro' (1,541)
ACAAAAAAGCCCATAAAAATAATAAATGTTCGTCTCGGAGTAATACGATTTGATGCACATACACCAGAAAGACTATCTA
GAGCACCATTTGAGTATAAGAAGTGAAAACAGCCGGGAGGTAGGAAAGTGTAATATGGAGTCTCAGCGCGTTCGATT
ACTTACTCGAGCCTAAAGCGTTCGTTTACTTGATAAGGTCGCAAGTCGCGCTTACTCGAGACTAACATATGATAGTGAA
TACGTAATAATGCAAATATGGATGAAGGCCCGCTCTGTGCTATTTTTATTTCTTGTTTATTGATGTTTTATTCCATGAAA
AGCGTGATGTCCTTGCCCCCCCCCCCCACACACACACACA~~~~~CTTTTAGCAGTGGTACGTATCCTACGCGCC

>'HC112_F+R_micro' (1,567)
ACATAAAAGCCCATAAAAATAACAAATGTTCGTCTCGGAGTAATACAATTTGATGCACATACATCAGAAAGACTATCTA
GAGCACCATTTGAGTATAAGAAGTGAAAACAGCCGGGAGGTAGGAAAGTGTAATATGGAGTCTCAGCGCGTTCGATT
ACTTACTCGAGCCTAAAGCGTTCGTTTACTTGATAAGGTCGCAAGTCGCGCTTACTCGAGACTAACATATGATAGTGAA
TACATAATAATGCAACTATGGATGAAGGCCCGCTCTGTGCTATTTTTATTTCTTGTTTATTGATGTTTTATTCCATGAAA
AGCGTGATGTCCTTGCCCCCCCCCCCCCCACACACACACACACA~CTTTTAGCCGTGGTACGTATCCTACGCGCT

Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
Primers

>'TD144-34_F+R_Micro' (1,540)
AACAAAGGTCCCTCGGTTTTAGCCACACTCAACTTCCCCTCCTCCCTCTCATCATTGTTTTCCCTTTCACCATCCATTGAA
TTCCCCCTCACCACTTCCAACTTCCGACCACACCTCTTAGATCATCTACAGTATATTCCATTCTCTTCCCCTCACACTYTCA
CCCCAYWCATTTCCCTTTACCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTATTTATCTA
TCTATCTATCTATCTATCTATCCTTCATATGTCATTCAGCGTTCTTTATTGTTTGCTTTGTTTGTTTGTGATTTCCTGTTAC
AGCTGGTTGATGGCTAAGAAGTTTTGTAATGCATGTTAGGGTCC

Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
Primers

>'TD144-15_F+R_micro' (1,523)
ACTAAAACTGACTTGTAAGGTAAAAATAGTGGACAAGATTACTGTAAGTATTAGGCCTTACATATGTGTTTTTTTTTATT
CATGCTTATTTTACTATTATACTTATTATTATTCAGTATTTATACCGTATGTTTTTTTATGTAAATTTTTATATTTATCTAC
ATTATTTATAAGCTAATATTTATCATTTATACAACCACCCACTTCTCCCCAAACACCCACTAAACAACCAGACACCAACAC
ACGTGCGCGCTCACACACGCACACACACGCACACACACGCGCGCACGCTATACATGTATTTTTTTATATCTAATATAAAA
ACTATAAATGCC

Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
Primers

>'TD144-46_F+R_Micro' (1,432)

ACAGTAGGTACATCCAGGCGCTAAATAATAACTCTTCCCAACTCCCACACACACACACACACACACACACACACACACAC
ACACACACACACACACACACACACACACACAYATACACACACACACACACACACACACACACACACACACACCCACACA
CATACACACACACACACGCCCATACACACACACACTCACACACGCGCGCGCGCAGAATTGTAAATGTGGATATCGGTCG
TGATTCGACGCTGGTCC

Isolation source: *H. Crispa,*
Number of sequences obtained: 1
Length variation: na
Primers
>TD152-71
ACACCTAAACGAAAAGGTCAAACTAGACGCACACGCACACACACACACACACACACACATACATACATACATACATACATAC
ACAGTATACACACAGCACACGTATACACGCAGGCGAATCACTATCACCAAGGATTCATTCATAGCTAGATAATGACTTC
AACTGATTACTGCAAATAGAAACAAAATGAATTACTGGATCGTGCGTTCGAATCCTGGTGACTTTGCAACGAATTTTTT
ATGCCTGAAATCAATACTCAAGCATCCGCCCACCTGTCAGTTTGACCTGGTATCCGTTGTTAGTCGAATGTAATGGTCC
CACCTAGCAGAAGTTCTTGTCTTTGTATTGATCT

Isolation source: *E. quadricolor*
Number of sequences obtained: 1

Length variation: na
Primers

>'EQ68_F&R_micro'
AGAGTTACAGCTGCGGCGTGCCTTGTAACGATGCACAGTTCAGTACACGATCCAAGCATACTCGGATATCCAAGGATA
AAGCGCCTGGACAGCGCAGTAGGTCTATGATATCTCGTAAGTGAAAGTGATCTAAAAACTATCAAAAATCAGTCAGTC
AGCCCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTACATGAATGCACACTTACATATATATGTAAGACTGTGCAGGCCTAC
GTGGTATAAGATTGTATAGAACATATTATTTATCTAACGCTGTGCAGGCTTCAAACTTCAGTCAATGTAAGTGTCAACC
TATAAATTGTTGTATGTCAAAATATGTCACATGAGCTGAATAATGTATGAATGTTTGAGGAGGTTGT

Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
Primers
>'EQ67_F&R_micro'
ACCCTAAAGGTGTTTATTTTGATGTCTCTAATTGTCGTCGATGGACGAAGTGCGCACGCAAGATTGCACGACTTCCAAA
AATAATTGTTTTTTCTCAAAATATTATGGATCTTCGAGAACAAGAGGGCTTTGAAAATACACCACGATGTGTTGAGGTG
TAGAGATAAGGAAAACATACAGAGAAATATAAAAATATGCACACTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCCAT
GGCCCAGGCCTCTCTTTCACAGTCGTAATATAAATGTATGTAATAGTTTTCTGCATAATATTATTGTAGATATCTACCAG
GTATACTGAATAACCCACGGTTGGTGATGAAAGATGCGTTGATATGTTGAAAATAGAACCTCGACTAATCTCGAGTAC
CCAATATCATCTGATTTTGTCATCAGGGCGTACGGTGCTACACACTATCGAGTTGCACCAT
>
Isolation source: *H. Crispa,*
Number of sequences obtained: 1
Length variation: na
Primers

>TD152-46
AACCTACGAATGTGTTCTAATGTATTACATTGAATTTGCCTCTCACTCACCCACTCACTCACTCACTCACTCACTCACTCA
CTCACTCACTCACTCACTCAATCACTCACTCACTCACTCACTCACTCACTCTCTATCTCTTGTTCTCCTCTCTCTATGTCTCT
CTCTTTCCTCTCCCTCTGTTTCTCTCTCTCTCTTTCTCTCACTCTCTATCTGTCTGTCTGTCTGTCTGTCTGTCGGTCTATC
TCCCTCTCTCTCTCTCTCTCTCCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTC

Isolation source: *H. Crispa,*
Number of sequences obtained: 1
Length variation: na
Primers

>'HC106_F&R_micro' (1,558)
AGAAAAGAGAAGCAAACCTCTCTAGACAGGTTTCTCATGAGACAGCAAGCACAGTCTGTATGAGAGGAAGCAGCACCT
GCAGCAGGGTCTAGTGGGTAGCCTACATAAGGCAGAGGATGGAATCAACACCTGAAATGAATATCGTGCCTGAAGTC
TTTATGGAGGGGGCTCCCCTTCCCAACAATAACTTCTCTTCTTCTCCTCCTCCTTTTCCACCTTCTCCACCTTCTCCTCCTC
GTCTTCCTCCTCCTCCTCCTCCTTCTCCTCCTCCTTATCCTTCTCCTCCTCTTCATTTCCTACTCCTCCTTTTCCTCCTTC
TCCTCATCCTCCTTATACTCCTCGTAGTTGTCATCGTCGTCGTCATCGTCGTCGTCGTACTCCTCCTCCTCCTCCTCCT
CCTCCTCC

Isolation source: *E. quadricolor*
Number of sequences obtained: 1
Length variation: na
Primers

>'EQ112_F&R_micro' (1,704)
ACACACACAAGCAGACGTACACACAGAGGTACACGCACAAACACGCACACACACTCACACACACACACACACACGCACA
CGCACTCTTTCACACACAACTCACACCACACACTTGCTCTCTCGATCCCTCACTCAACCCAATCCGGTCAAAAATTTACCG
TATAGAAGGCACGCTTCGCAGCTTCTCAAAACCGGTTGATGGGCCAGAACTGCTTTGCTGTAAAGATAGGCAAAAAAA
TTTCAATCGCCAGAGTATCCAGCGGAAGCACTAACGGTACAAAATTATCCATAATTATGAAATAGAAATAGGAACTGCT
CCTCCATTCTCCCTAATCCTGTTCATCCCGAGACTTTACCCTCAGCTCCCGGAATGGATGTAGGTGCTCCCTCCCTAACC
AAGTCCTTCACCACAGTCCTCCCCTAATCCCTCGAAACTCCTCCTTCGCCCTCAGAGCCATTACC