# MOLECULAR INVESTIGATION OF THE DISTRIBUTION OF TYPHLATYA SPP. IN CAVE SYSTEMS WITHIN THE YUCATÁN PENINSULA, MEXICO

An Undergraduate Research Scholars Thesis

by

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#### **ABSTRACT**

Molecular Investigation of the Distribution of *Typhlatya* spp. in Cave Systems within the Yucatán Peninsula, Mexico

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Stygobitic species such as the anchialine cave shrimp *Typhlatya* have adapted to life in freshwater and marine cave systems around the world. Limited research has been conducted on the ecological, morphological and genetic diversity of the four known species of the Yucatán Peninsula, Mexico: T. dzilamensis, T. pearsei, T. mitchelli and T. campecheae. A total of eighteen specimens were obtained from Dr. Thomas Iliffe's collection housed in the Marine Biospeleology Lab at Texas A&M University at Galveston, representing seven cave systems (Temple of Doom, Systema Paamul, Carwash, Crustacea, Nayah, Sabak Ha, and Kankirixche). Specimens were photo-documented and identified to species level following diagnostic characters published in the taxonomic literature. Both the 16S rRNA and the nuclear internal transcribed spacer (ITS) gene were sequenced. After analyzing the sequences, phylogenetic trees and genetic distance tables were generated to discern the phylogenetic relationship among the specimens and compared to our initial morphological diagnosis. The phylogenetic analyses of 16S showed five distinct clades: T. dzilamensis, T. mitchelli A and B, T. pearsei, and Typhlatya sp. Data acquired from this study will be part of a larger study that will provide more insight into the biogeography and connectivity of cave systems within the Yucatán Peninsula.

### **DEDICATION**

This thesis is dedicated to our families, especially our parents, David, Delise, Alexandra, and Guadalupe, and sisters, Sasha and Jazmin. We are extremely grateful for their continual support of our dreams of becoming scientists and for nurturing us into the women we are today. It is also dedicated to the Terry Foundation who believed in us and financially invested in our undergraduate degrees and this thesis throughout the past four years. We will always be thankful. Lastly, we dedicate this thesis to our niece and nephew, Jade and Jace. It is our hope that when they grow up, they follow their dreams like we followed ours.

#### **ACKNOWLEDGEMENTS**

We would like to begin by thanking God, who led us to Texas A&M and to this project.

He has given us the means to complete this thesis and we hope to bring Him glory in our work.

We would like to thank our advisor, Dr. Thomas Iliffe, for seeing potential in us and taking us on as Undergraduate Research Scholars. Dr. I taught us the wonders of cave biology. We thank him for always pushing us forward, providing assistance whenever needed, and showing us that there will always be more discoveries to be made.

We would also like to thank our graduate student advisor, Lauren Ballou, for going above and beyond for us during this project. She sacrificed her time and energy to assist in this project and the completion of this thesis would not have been possible without her. A special thanks goes to Dr. Elizabeth Borda for guiding us and helping us throughout this research and Dr. Pia Miglietta for allowing access to her laboratory and resources.

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#### **NOMENCLATURE**

BLAST Basic Local Alignment Search Tool

COI Cytochrome Oxidase I

dNTP Deoxyribonucleotide triphosphate

EDTA Ethylenediaminetetraacetic acid

ESU Evolutionary Significant Unit

EtOH Ethanol

GenBank NIH genetic sequence database

ITS Internal Transcribed Spacer

LAS Leica Application Suite

MEGA7 Molecular Evolutionary Genetics Analysis version 7.0

MUSCLE Multiple Sequence Comparison by Log-Expectation

NaCl Sodium chloride

NCBI National Center for Biotechnology Information

PCR Polymerase Chain Reaction

PhyML Phylogenetic inferences using Maximum Likelihood

TAE Tris/Acetic acid/Ethylenediaminietetraacetic acid

TBE Tris/Borate/Ethylenediaminietetraacetic acid

Tris-HCl Tris(hydroxymethyl)aminomethane hydrochloride

#### **CHAPTER I**

#### INTRODUCTION

#### Geography of the Yucatán

Anchialine caves are diverse inland systems formed beneath karst landscapes or volcanic terrain (Holthius, 1973). One of their defining characteristics is that they have a subsurface connection to the sea. The majority of these cave systems are formed due to the mixing of freshwater masses and saltwater input, which together dissolves limestone (Back et al., 1986). Others emerge from volcanic lava pouring from volcanoes that carves out underground caves commonly known as lava tubes (Iliffe, 1987). Anchialine caves have disjunct distribution around the world, are typically are located within a few kilometers from coastlines, and include biodiversity hotspots in Yucatán, the Bahamas, Canary Islands and Australia (Iliffe, 1992).

Among the Yucatán Peninsula's numerous cave systems, Sistema Ox Bel Ha was previously recognized as the longest underwater cave in the world and is a relatively well-studied area for anchialine systems (Iliffe, 2009). At present, another one of the Yucatán's cave systems, Sistema Sac Actun, has recently been documented as the longest in the world (Leadbeater, 2018). A highly significant geological event occurred in the region during the Cretaceous period, 65 million years ago. An asteroid struck the Yucatán Peninsula causing a large explosion and forming the Chicxulub impact crater, spanning a diameter of approximately 200 kilometers (Morgan et al., 2000). As sedimentary rocks were deposited over the crater, a semi-circle of fault lines in limestone rock above the crater edge caused their rapid dissolution (Collins et al., 2002). This dissolution led to collapse of karstic bedrock and the formation of surface pools called cenotes, or sinkholes, along its perimeter now known as the "Ring of Cenotes". Cenotes are coupled to the Yucatán 's

anchialine caves, providing a connection between subterranean groundwater and the surface (Bauer-Gottwein et al., 2011). Anchialine caves are the main reason that surface rivers are absent, making the aquifer the primary source of water in the region (Pohlman et al., 1997).

With over 2,000 known sinkholes in the Yucatán Peninsula, anchialine caves have numerous entry points permitting research on hydrology and biology to be conducted (Calderon-Gutierrez et al., 2016). These caves are home to some of the many unique and little-studied organisms in the Yucatán, providing opportunities for study of ecology of cave-limited anchialine species known as stygobionts.

#### **Ecology of cave animals**

Cave organism ecology has been a topic of interest since cave exploration began in Yucatán during the late 19th century (Barr Jr, 1968). In the 1930s, the Carnegie Institution of Washington pioneered investigation of stygobitic fauna inhabiting caves in the Yucatán Peninsula (Pearse & Banks, 1938). Since then, there have been discoveries of 41 cave species found in the Yucatán with 39 arthropods belonging to the Class Crustacea and two fish belonging to the Class Actinopterygii (Iliffe, 2002; Suarez-Morales & Iliffe, 2005).

Of all aquatic organisms dwelling in caves, crustaceans are the most abundant taxon with over 2,900 cave species currently identified worldwide (Protas, 2011). They have adapted to thrive in aphotic environments with restricted food sources through specialized feeding structures and sensory organs (Howarth, 1993). In addition, these fauna thrive in total darkness and over the course of evolutionary history, some have lost their eyes and pigmentation (Iliffe & Kornicker, 2009). Apart from their unique adaptations, many stygobionts are also known for their global distribution. One such genus is the shrimp, *Typhlatya*, a taxon which includes 17 described species found throughout the world (Alvarez, Iliffe &Villalobos, 2005).

#### Typhlatya in the Yucatán

This study focused on the four atyid shrimps of the genus *Typhlatya* endemic to the Yucatán Peninsula: *T. mitchelli, T. pearsei, T. dzilamensis,* and *T. campecheae. Typhlatya* are especially fascinating because they are found not only in Mexico, but in regions extending from the Mediterranean, through the Caribbean, to the Galapagos Islands (Alvarez et al., 2005). They live in a range of salinities from fresh to saltwater, as well as in caves located in both inland and coastal regions (Alvarez et al., 2005). Due to their global distribution in aquatic caves, it is likely that the ancestors of *Typhlatya* originated in the ocean (Creaser, 1936). Due to their narrow range, seven *Typhlatya* species are Red Listed by the International Union for Conservation of Nature (IUCN) as vulnerable or critically endangered. Although multiple ecological and taxonomic analyses have been performed on *Typhlatya* (Jaume & Bréhier, 2005; Richard, De Grave & Clark, 2012), there is a deficit in molecular investigations.

The general distribution of *Typhlatya* in the Yucatán can begin to be examined by examining the type localities of the four species. The first Mexican species to be described was *T. pearsei* in 1932 from Balam Canche Cave and Santa Elena Cave, Yucatán, Mexico (Creaser, 1932). This species is the most widely dispersed atyid shrimp in the Yucatán recorded from at least 21 localities (Hobbs, 1979). In 1973, 25 female *T. mitchelli* were initially obtained from the Cenote Kabahchen in Mani, Yucatán (Hobbs & Hobbs, 1976). Since then, this species has been found in multiple sinkholes in the Mexican state of Quintana Roo as well (Hunter et al., 2008). Hobbs & Hobbs (1976) also discovered large populations of *T. campecheae* in Grutas de Xtacumbilxunam, Bolonchenticul, Campeche. Unlike the other two species, which are considered to be widespread in the Yucatán, *T. campecheae* has only been found in its type locality and Cenote de Cantemo, Campeche (Hobbs & Hobbs, 1976; Mercado-Salas et al., 2013). Lastly, the most recent of the four

species described is *T. dzilamensis*, collected from three cenotes near Dzilam de Bravo, Yucatán: Cenote Cervera, Cenote Dzilamway, and Cenote Buya Uno (Alvarez et al., 2005).

Studies of specific mitochondrial and nuclear genes have the potential of providing information on the biogeographic distributions of cave fauna within the Yucatán Peninsula. Caves within the Yucatán appear to be disconnected and are in many cases remote and inaccessible. Little is known about the ecology of *Typhlatya* and other anchialine cave species due to the complex nature of these habitats. In the absence of ecological data of *Typhlatya*, knowledge of their distribution and dispersal mechanisms must be determined in alternate ways. In our study, we carried out genetic analysis of mitochondrial 16S rRNA and nuclear ITS genes from 18 specimens of *Typhlatya* collected from several Yucatán anchialine caves to aid in identifying species and determining geographical connections between populations. This genetic data may also provide insight into the current distribution of *Typhlatya* in the Yucatán, critical to responsible conservation of these species.

The Yucatán Peninsula has a thriving ecotourism industry due in part to the abundance of cenotes in the region. Adverse anthropogenic impacts on cave systems, including water pollution, garbage dumps, and resort and residential developments, could have on the diverse range of species within it is poorly understood. Molecular sequencing of *Typhlatya* genes has been limited and even conflicting, leading to apparent misidentification of pseudocryptic species such as in studies by Hunter et al. (2008) and Botello et al. (2013). Thus, we will attempt to conclusively identify species of *Typhlatya* by molecular means. Another objective is to determine if current morphological descriptions allow for accurate identification and differentiation. The results of our study will provide the foundation for a more extensive investigation, analyzing DNA sequences of a larger sample size.

#### **CHAPTER II**

#### **METHODS**

#### **Specimen inventory**

Our study began with an inventory of preserved specimens in the personal collection of Dr. Tom Iliffe, Marine Biospeleology Lab, Texas A&M University at Galveston. These specimens varied in species, preservative, storage location, and date and locality of collection. All information gathered from the original vial, dive logs and expedition journals were recorded in an Excel document. The original specimen ID numbers were recorded, and alternate IDs assigned to reflect the new inventory system. The new ID contained the initials of the collector, Thomas Iliffe (i.e. TI), as well as a number, starting with 001. The first vial inventoried was identified as TI001, followed by TI002, TI003, etc. Each vial was labeled twice, once on the outside with a strip of painter's tape, and on the inside with a labeled strip of Rite in the Rain paper. For this study, 18 specimens purported to be from the genus *Typhlatya* were chosen from collections in the Yucatán Peninsula.

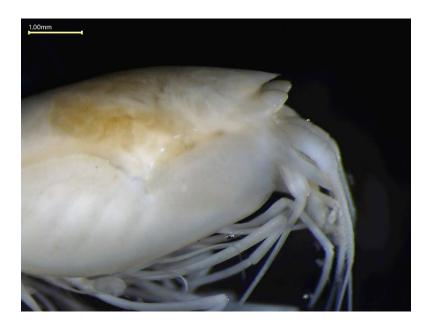
#### **Photo-documentation of specimens**

All specimens of *Typhlatya* were preserved in 85% ethanol and photographed under a dissecting microscope. Pictures were taken with a Leica M80 microscope using a Leica MC170 HD camera and the LAS core program. The 85% Ethanol (EtOH) solution was made by adding 7.5 mL of water to 42.5 mL of EtOH in a 50 mL bottle, then mixed by shaking. For vials containing only one specimen, the entire contents (specimen and ethanol) were poured into a plastic petri dish. Fresh 85% EtOH solution was poured into the vial until the specimen was completely covered. This prevented drying of tissue as well as the glare of light that occurred on

uncovered portions of the shrimp. In vials with more than one specimen, individuals were placed in separate vials and labeled accordingly to eliminate confusion. For example, if the original vial was TI008, the vials would be renamed TI008A, TI008B, TI008C, etc. depending on the number of specimens originally in the vial. Full-body photographs were taken as well as close-up photos that focused on certain distinguishable features of species within the *Typhlatya* genus (Figure 1; Hobbs & Hobbs Jr., 1976; Alvarez et al., 2005). Such features included the rostrum and carapace lengths, general size of specimens, and coloration of the body and eyes. Specimens chosen for this study ranged in size from 12-20 mm. Cave shrimp differ morphologically from their marine counterparts mainly in their general lack of pigment in their bodies and eyes. Morphological differences between species of cave shrimp can be difficult to discern, which is why close-up photos were necessary for identification purposes (Figure 2). Readily observable differences that can be used to distinguish Yucatán *Typhlatya* species include length of rostrum, body coloration, and carapace length and size.



**Figure 1.** Full body photograph of a *T. dzilamensis* specimen. Specimen TI034 from Cenote Crustacea, Quintana Roo.



**Figure 2.** Close-up picture of a *T. dzilamensis* specimen. Specimen TI034, showing the rostrum length and eye color in more detail.

#### **DNA** extraction

DNA from each sample was isolated by removing pleopods under the dissecting microscope. Forceps were sterilized with Ethanol (EtOH) and a flame, and pleopods were removed from only one side of the specimen when possible, depending on its size. Specimens under three millimeters in size were not selected for this study because full bodies would be required for successful DNA extraction. Using the Qiagen DNeasy kits according to the manufacturer's protocol (QIAGEN Inc., Middiddauga, ON), pleopods were placed in a chilled 1.5 mL Eppendorf tube containing 600 µL of Solution 2 (2% SDS, 50mM Tris-HCl, 20mM EDTA), a lysate. After adding the limbs, three microliters of proteinase K were added to the tube containing the lysate and limbs. The tube was placed in an incubator at 55°C and left overnight.

After at least eight hours of incubation, 3  $\mu$ L of RNAse A (20 mg/mL) were added to the Eppendorf tube. The solution was mixed by inverting gently twenty-five times. Once mixed, the tube was incubated at 37°C for one hour. Following incubation, the sample was placed on ice for five minutes. To precipitate the proteins 200  $\mu$ L of saturated salt (5M NaCl) were added to the

tube and mixed well. Tubes were placed in a centrifuge for six minutes. If particles were seen suspended in the supernatant, the solution was centrifuged for several more minutes to ensure complete separation. Once completely separated, the tube contained a protein pellet at the bottom and a liquid supernatant directly above, containing the DNA. The supernatant was decanted by pouring the contents of the tube into a clean 1.5 mL Eppendorf tube, leaving the protein pellet in the original tube. After discarding the original tube, 600 µL 100% isopropanol were added to the supernatant. The solution was inverted gently fifty times and centrifuged for ten minutes at high speed. After decanting the 100% isopropanol, 600 µL of 70% EtOH were added to the tube. Again, the tube was mixed gently by inverting and was then centrifuged at high speed for five minutes. Once centrifuged, the ethanol was decanted from the tube, and the tube was left under a fume hood until completely dried. After all of the ethanol evaporated from the tube, its contents were rehydrated with 30 μL of water. A Thermo Scientific NanoDrop 2000 spectrophotometer was used to quantify the amount of DNA as well as protein contamination. Once the DNA had been isolated and quantified, Polymerase Chain Reactions (PCR) were completed to amplify the DNA. PCR reactions were used to amplify fragments of the 16S gene and the nuclear ITS gene (Table 1) using the primers displayed in Table 2.

**Table 1.** Successful amplification sample size of the two genes by specimen ID.

Cenote Name/Specimen ID	16S	ITS			
Temple of Doom					
TI005	1	1			
Systema Paamul					
TI008A	1	1			
TI008B	1	1			
TI008C	1	1			
TI008D	1	1			
TI008E	1	1			
Carwash (FW)					
TI012	1	1			
Carwash (SW)					
TI013A	1	1			
TI013B	1	1			
TI015	1	1			
Cenote Crustacea					
TI034	1	1			
Cenote Nayah					
TI157A	1	0			
TI157B	1	1			
TI161	1	1			
Cenote Sabak Ha					
TI166A	1	1			
TI166B	1	1			
Cenote Kankirixche					
TI169	1	0			
TI172	1	1			
Total	18	16			

#### **PCR** reactions and purification

Polymerase chain reactions

Prior to beginning the Polymerase Chain Reaction (PCR) protocol, dilutions of the extracted DNA were created so that both undiluted and diluted samples could be run to evaluate which dilution, if any, yielded the best results. The four dilutions created were 1:1, 1:10, 1:50, and 1:100. All materials required for PCR were placed in a CL-1000 Ultraviolet Crosslinker for

four minutes to ensure sterility of equipment. To create the master mix for the PCR reactions, GoTaq® Green (composed of GoTaq® DNA Polymerase, dNTPs, MgCl<sub>2</sub> and reaction buffer), nuclease free water, and the forward and reverse primers were combined in a 1.5 mL Eppendorf tube. Each primer selects for a different region of genetic code, making it gene and/or species-specific (Table 2). Master mix was aliquoted (24  $\mu$ L) into labeled 0.2 mL polypropylene PCR tube strips and 1  $\mu$ L of DNA template (diluted and/or undiluted) was added to each tube with the exception of negative controls. Depending on the gene, various PCR cycles were used to amplify the DNA (Appendix A).

**Table 2.** Name, source, and nucleotide sequences of all primers used.

Amplified fragment	Primer	Reference	Primer sequence (5'-3')
Mitochondrial			
16S rRNA	16SarL	Simon et al., 1991	CGCCTGTTTATCAAAAACAT
	16SbrH	Simon et al., 1991	CCGGTCTGAACTCAGATCACGT
Nuclear			
ITS	ITSF	Chu et al., 2001	CACACCGCCCGTCGCTACTA
	ITSR	Chu et al., 2001	ATTTAGCTGCGGTCTTCATC

#### Gel electrophoresis

PCR product was then tested for successful amplification using gel electrophoresis. To create the gel, 0.7 grams of agarose and 70 mL of 10% 1X TBE and TAE solution were combined in an Erlenmeyer flask. The flask was microwaved in two thirty-second intervals, or until the solute was no longer visible. While gently stirring the flask, 2  $\mu$ L SYBR Safe Gel Stain were added, and the contents of the flask were poured into the center of the electrophoresis chamber. After waiting approximately twenty minutes for the gel to set, 3  $\mu$ L of Lucigen Gel-Ready 1kb DNA ladder were added to the first well, followed by 3  $\mu$ L of each sample run through the PCR in the additional wells. The electrophoresis machine was preset to 110 V and

each gel ran for twenty minutes. Once completed, the gel was analyzed for successful amplification using the Benchtop Single UV Transilluminator.

#### Purification of PCR products

Following PCR, the samples were purified for sequencing. Using the Exo-SAP-IT protocol (Affymetrix USB), Exo-SAP-IT reagent (2 µL) was mixed with 10 µL of post-PCR reaction product in 0.2 mL polypropylene PCR tubes for each sample. The tubes were incubated at 37°C for 15 minutes to degrade the remaining primers and nucleotides. This was followed by incubation at 80°C for 15 minutes to inactivate the ExoSAP-IT reagent. Following incubation, all samples were packaged and sent to the Texas A&M Corpus Christi Genomics Core Lab for sequencing.

#### Sequence analysis

Once sequences were received from the Corpus Christi lab, raw sequences (Appendix B and Appendix C) were imported into the Geneious 11.1.2 program as DNA Electropherogram (.ab1) files (http://www.geneious.com, Kearse et al., 2012). Once imported, National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) was used to ensure the sequences generated belonged to the genus of interest. Both forward and backward reads were run for each specimen, and a De Novo Assembly was performed with medium sensitivity to make a consensus for each sequence. Once the sequences were assembled, text files of each consensus sequence were generated and were aligned using MUSCLE. A Maximum Likelihood phylogenetic tree was created with bootstrap values for the mitochondrial 16S rRNA gene using the General Time Reversible (GTR + G + I) Model (Nei and Kumar, 2000) in PhyML with Smart Model Selection (LeFort, Longueville & Gascuel, 2017). In order to identify each specimen at the species level, the 16S gene was used because of the quality of the sequences

present. Sequences obtained from specimens in this study were concatenated with sequences obtained from GenBank to confirm species identifications. Preliminary identifications were based on the morphological features of each specimen as reported in the original species descriptions.

#### **CHAPTER III**

#### **RESULTS**

#### Morphological identification

Three of the specimens (TI013A, TI013B, TI015) were initially identified as *T. campecheae* based only on morphological observations (Table 3). Characteristics of these specimens included the possession of long rostrums extending past the eyes but ending before they reached the second podomere of the antennular peduncle. The carapace lengths ranged from 3.0 to 4.0 mm and were similar to the original *T. campecheae* male and female holotypes, which were approximately 3.9 mm long (Hobbs & Hobbs, 1976). The specimens' general body colors were white with prominent hepatic grooves running along the majority of their entire carapace lengths. The primary characteristic considered in specimen identification of all specimens in this study was rostrum length.

**Table 3.** Specimen identification based on morphology.

Specimen ID	Cave Locality	Morphological ID	Genetic ID
TI005	Temple of Doom	T. dzilamensis	T. dzilamensis
TI008A	Systema Paamul	T. mitchelli*	N/A
TI008B	Systema Paamul	T. pearsei	T. pearsei
TI008C	Systema Paamul	T. mitchelli	T. mitchelli
TI008D	Systema Paamul	T. mitchelli	T. mitchelli
TI008E	Systema Paamul	T. mitchelli	T. mitchelli
TI012	Car Wash (FW)	T. mitchelli	T. mitchelli
TI013A	Car Wash (SW)	T. campecheae	T. dzilamensis
TI013B	Car Wash (SW)	T. campecheae	T. dzilamensis
TI015	Car Wash (SW)	T. campecheae	T. dzilamensis
TI034	Cenote Crustacea	T. dzilamensis	T. dzilamensis
TI157A	Cenote Nayah	T. mitchelli*	N/A
TI157B	Cenote Nayah	T. mitchelli	Typhlatya sp.
TI161	Cenote Nayah	T. mitchelli*	N/A

TI166A	Cenote Sabak Ha	T. pearsei	T. pearsei
TI166B	Cenote Sabak Ha	T. pearsei	T. pearsei
TI169	Cenote Kankirixche	T. mitchelli*	N/A
TI172	Cenote Kankirixche	T. mitchelli	Typhlatya sp.

<sup>\*</sup>Specimens for which there were no 16S rRNA sequences were identified based solely on their morphology.

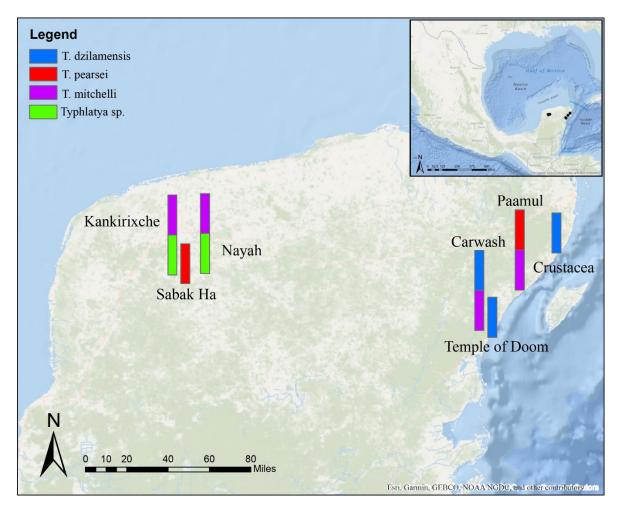
Two specimens (TI005, TI034) were identified as *T. dzilamensis*. They had short rostrums that did not extend anteriorly past their eyes, along with carapace lengths between 6.0 and 7.0 mm. The carapace and telson lengths were similar to those of the original *T. dzilamensis* holotype, allotype, and paratypes (Alvarez et al., 2005). Three specimens (TI008B, TI166A, TI166B) were identified as *T. pearsei* because they displayed certain unique characteristics observed in the species. These specimens were the easiest to identify because *T. pearsei* has a long rostrum extending past the eyes and the second podomere attached to the end of the antennular peduncle, as did all specimens studied in the original species description (Hobbs, 1979). Carapace lengths of specimens also were 3.0 to 4.0 mm. In conjunction with the above characteristics, the general yellow body coloration of persevered specimens was also a factor in the identification of *T. pearsei*.

Ten specimens (TI008A, TI008C, TI008D, TI008E, TI0012, TI157A, TI161, TI169, TI157B, TI172) were identified as *T. mitchelli*. These specimens possessed short rostrums that did not extend anteriorly past their eyes, but curved up at the ends. They had thick carapaces that were approximately 3.0 to 5.0 mm in length, which compared to that of the female holotype (4.8 mm) collected from the type locality (Hobbs & Hobbs, 1979). The presence of visible hepatic grooves and large first maxillipeds in the test samples also aided in identification, as these traits are common in *T. mitchelli*.

#### **Distribution**

Specimens were collected from seven cave systems located on either the Caribbean coast of the Yucatán in Quintana Roo or within the inland portion of the state of Yucatán (Figure 3). Out of the four described species endemic to the Yucatán Pensinsula, three were identified in this study: *T. mitchelli*, *T. dzilamensis*, and *T. pearsei*. The fourth species, *T. campecheae*, was not found. Two specimens of a possible undescribed species, *Typhlatya* sp., were present. The distribution of these species is displayed in Figure 3, with each bar representing a cave locality. *T. mitchelli* was found in two coastal caves, Systema Paamul and Cenote Carwash as well as two inland caves, Cenote Nayah and Cenote Kankirixche. *T. pearsei* was present in the coastal cave Systema Paamul and the inland cave Cenote Sabak Ha.

The distribution of *T. dzilamensis* was limited to three coastal caves, Cenote Crustacea, Cenote Carwash, and Temple of Doom. In this study, the range in distribution of *T. dzilamensis* was greatly expanded, with five specimens found in the Eastern coast within the state of Quintana Roo. These specimens were also found within the saltwater layer of these cenotes. Similarly, the distribution of *Typhlatya* sp. was limited to two inland caves, Cenote Kankirixche and Cenote Nayah. The distance between the coastal and inland caves ranges from approximately 250-350 kilometers.

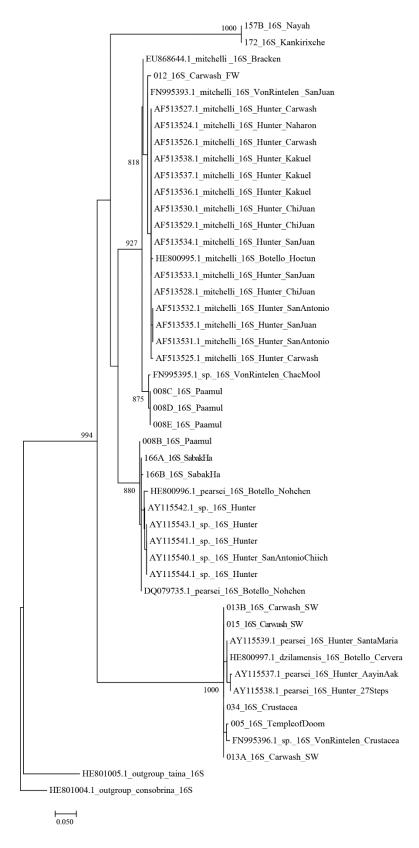


**Figure 3.** Distribution of *Typhlatya* within the Yucatán Peninsula. Map includes the distribution of the four *Typhlatya* species throughout seven caves.

#### Variation in the 16S rRNA gene

Five main clades were identified as *T. mitchelli* A and B, *T. dzilamensis*, *T. pearsei*, and *Typhlatya* sp. (Figure 4). The first clear group of specimens on the tree was TI157B from Cenote Nayah and TI172 from Cenote Kankirixche, which grouped together in 1000 of the trees generated. Specimens TI012 (Carwash FW) and TI008E, TI008C, and TI008D (Systema Paamul) clustered together, grouped with the known *T. mitchelli* GenBank sequences. Two different clades appeared within the *T. mitchelli* species. One clade contained TI008C, TI008D, TI008E, and a closely related GenBank sequence (*T. mitchelli* A). The second clade contained

TI012 and several GenBank species (*T. mitchelli* B). Based on the tree, TI166A and TI166B (Cenote Sabak Ha), and TI008B (Systema Paamul) grouped together in 880 of the trees generated. These three specimens also grouped together with the known *T. pearsei* GenBank sequences. The last cluster on the tree contained 005 (Temple of Doom), TI013A, TI013B, and TI015 (Carwash SW), and TI034 (Cenote Crustacea). The bootstrap value for these specimens was 1000, and when compared to the GenBank sequences were identified as *T. dzilamensis*. The only other described species currently known to inhabit the Yucatán peninsula is *T. campecheae*. No specimens were identified as this species. The two outgroups present in the tree are *T. taina* and *T. consobrina*, two species native to Cuba that are both sister groups to the Yucatán Species.



**Figure 4.** Maximum Likelihood Tree of successful 16S sequences. Tree includes fourteen of the eighteen specimens as well as GenBank sequences.

A pairwise distance table was generated after grouping specimens together by species (Table 4). The generated table was based on nucleotide substitutions (transitions and transversions) between group averages. The highest distance values were seen between *T*. *dzilamensis* and all other groups. Average pairwise distance among the five major clades ranged between 5.85% - 23%. *Typhlatya dzilamensis* and *Typhlatya* sp. was the highest distance computed at 23.6%. Between *T. dzilamensis* and the other four groups, values ranged from 15.5% to 23.6%. High distance values were also seen between the *Typhlatya* sp. and all other species, ranging from 13.5% to 23.6%. The lowest pairwise distance was 1.8%, between the *T. mitchelli* A and B subclades.

**Table 4.** 16S rRNA pairwise distance among the *Typhlatya* clades.

	1	2	3	4	5
1. T. dzilamensis					
2. T. pearsei	0.155				
3. T. mitchelli A	0.182	0.057			
4. T. mitchelli B	0.172	0.060	0.018		
5. Typhlatya sp.	0.236	0.139	0.135	0.141	

#### Variation in the ITS gene

The nuclear ITS gene showed considerably more variation than the 16S rRNA gene. Sequences aligned well when grouped within species, but between species there were many differences. The sequences identified as *T. dzilamensis* showed multiple and sizable insertions and deletions (indels, Appendix C). These indels were seen throughout the ITS sequences and resulted in large gaps where the *T. dzilamensis* sequences differed from the other species. Mean genetic distances for the ITS gene were also generated (Table 5).

When comparing *T. mitchelli*, *T. pearsei*, and the unidentified *Typhlatya* sp., the highest genetic distance was 11.7% between the *Typhlatya* sp. and *T. pearsei*. The genetic distance between *T. mitchelli* and *T. pearsei* was 7.6%, while the genetic distance between *T. mitchelli* and the *Typhlatya* sp. was 7.5%. Significantly higher distance values were seen between *T. dzilamensis* and the other three species. Values ranged from 22.6% between *T. dzilamensis* and the *Typhlatya* sp. to 27.5% between *T. dzilamensis* and *T. pearsei*.

**Table 5.** ITS pairwise genetic distance among *Typhlatya* clades.

	1	2	3	4
1. T. pearsei				
2. T. mitchelli	0.076			
3. <i>Typhlatya</i> sp.	0.117	0.075		
4. T. dzilamensis	0.275	0.231	0.226	

#### **CHAPTER IV**

#### **DISCUSSION**

#### Morphological and genetic identifications

Each specimen was initially identified based on its morphological features. These identifications were later compared to the phylogenetic identifications (Table 3). The purpose of this dual-identification system was to test the utility of identification based on morphology alone within the *Typhlatya* genus. Two specimens (TI005, TI034) initially identified as *T. dzilamensis* and three specimens (TI008B, TI166A, TI166B) thought to be *T. pearsei* were correct according to their genetic identifications. Of the ten specimens morphologically identified as *T. mitchelli*, eight (TI008A, TI008C, TI008D, TI008E, TI0012, TI157A, TI161, TI169) were accurate. The other two specimens (TI157B, TI172) were phylogenetically identified as *Typhlatya* sp., an undescribed species. Without knowledge of the cave locality, three specimens (TI013A, TI013B, TI015) were misidentified morphologically as *T. campecheae* that were identified genetically as *T. dzilamensis*.

Identification of species based solely on morphological features can lead to misidentification and conflicting genetic results. The presence of pseudocryptic species is evident in the GenBank sequences within Figure 4. Based on morphological misidentification during this study, *T. dzilamensis* and *T. campecheae* were difficult to distinguish due to their similar rostrum lengths and body colorations. There were no specimens characterized as *T. campecheae* from these 18 samples. This species has only been found in Cenote de Cantemo in Campeche, Mexico, which is its type locality (Hobbs & Hobbs, 1976; Mercado-Salas et al., 2013). None of the 18 samples were collected from this cave. A pseudocryptic species observed

here is *Typhlatya* sp. because it has often been misidentified as *T. mitchelli*. The undescribed species, even though they were collected from two different cenotes, Kankirixche and Nayah, looked very similar to each other and were initially misidentified due to their short, curved rostrums and thick carapaces as similar to *T. mitchelli*. Further identification and description of the *Typhlatya* sp. is ongoing.

In the bottom-most clade (Figure 4), Hunter et al. (2008) misidentified several specimens as *T. pearsei* that were later identified by Botello et al. (2013) as *T. dzilamensis*. The specimen collected by Botello et al. (2013) was collected from Cenote Cervera, one of the caves in which *T. dzilamensis* was originally collected and described, confirming the identity of all specimens within this clade as *T. dzilamensis*. In a separate clade, Hunter et al. (2008) misidentified several specimens as *Typhlatya* sp. that were later identified as *T. pearsei* by Botello et al. (2013). These conflicting identifications are a result of entering preliminary morphology-based species identifications without first comparing the sequences to other known GenBank sequences. Lajus et al. (2015) analyzed the potential inadequacy of traditional morphological techniques in copepod taxonomy and the dilemma of "cryptic or pseudocryptic" species. In their study, detailed morphological analysis and multivariate statistical approaches proved to be efficient techniques for morphological discrimination of cryptic copepod species. Traditional morphological descriptions of *Typhlatya* may require a similar approach.

Currently, there are no ITS sequences present for *Typhlatya* in any sequencing databases. Since sequencing of the ITS gene has not been done before, GenBank comparisons as were completed with the 16S rRNA gene (Figure 4) were not possible. The variation found in the ITS gene between species in the Yucatán warrants further exploration through sequencing of additional specimens.

#### **Distribution**

Previously, *T. dzilamensis* specimens have been found only in the central northernmost coast of the state of Yucatán, in three caves within Dzilam de Bravo (Alvarez et al., 2005). In this investigation, the range of *T. dzilamensis* was doubled to include three caves along the Caribbean coast of Quintana Roo. *T. dzilamensis* specimens were collected in both the saltwater and freshwater layers, meaning this species can inhabit both brackish and saltwater. All other species found in the Yucatán are limited to the freshwater layer (Alvarez et al. 2005). The presence of genetically similar specimens found in cave systems separated geographically by considerable distance begs the question of the dispersal potential of *Typhlatya*. Similar results were found in research conducted by Webb (2003), where *T. mitchelli* samples collected from vast geographical areas throughout the Yucatán had very low amounts of variation.

In all trees generated from the mtDNA sequences, specimens 157B and 172 emerged as a separate clade. A high amount of genetic distance was seen between this *Typhlatya* sp. and the other three species in this study (Tables 4 and 5). Hebert et al. (2004) proposed that the average interspecific divergence in the COI gene should be 10 times the average intraspecies divergence in order to designate the species as an Evolutionary Significant Unit (ESU). In this investigation, the specimens identified as *Typhlatya* sp. had identical sequences. The lowest interspecific divergence was between *Typhlatya* sp. and *T. mitchelli* B at 13.5%, well over 10 times the average intraspecific divergence. Based on the Maximum Likelihood tree as well as the pairwise distance tables, *T. mitchelli* is the known species most closely related to *Typhlatya* sp. However, when considering the nearest relative to *T. mitchelli*, *T. pearsei* has the shortest genetic distance.

#### **Future directions**

This will be part of a larger study that will delve more into the biogeography of Typhlatya in the Yucatán utilizing an extensive sample size. It will include additional GenBank and past experimental sequence data from other Texas A&M-affiliated research. Future studies that could provide more robust experiments and results on species biogeography and anchialine cave system geologic history include: 1) Expanding regional molecular analyses of Typhlatya to marine species inhabiting other parts of the Caribbean and Mediterranean. There may be differences in the biogeography depending on whether the species has colonized in fresh, brackish, or saltwater. 2) Performing similar analyses on other stygobiont genus', such as Remipedia, in order to gain stronger insight on the subsurface connectivity of cave systems in the Yucatán and other regions 3) Supplementary study on the reasoning behind the molecular distinction between T. mitchelli A and T. mitchelli B produced in the Maximum Likelihood tree (Figure 3). 4) Execute a more intensive look at the variations in the ITS gene between Typhlatya species in the Yucatán and other regions. 5) Apart from biogeographical analyses, expand on other Typhlatya-specific data sets suggesting dispersal, evolutionary, colonization theories in fixed regions. 5) Further description of unidentified *Typhlatya* sp. collected from Cenotes Nayah and Kankirixche, comparison to Typhlatya sp. from other studies, and search for additional specimens from other cenotes located in the Yucatán Peninsula.

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#### **APPENDIX A**

#### **PCR** protocols

## ITS Protocol

Lid: 105°C

Volume: 25 µL

- 1. 94°C, 1:30
- 2. 94°C, 0:30
- 3. 56.8°C, 0:30
- 4. 72°C, 0:30
- 5. GOTO step 2, 30X
- 6. 72°C, 5:00
- 7. 12°C, ∞

#### **ITS-GRAD Protocol**

Lid: 105°C

Volume: 25 µL

- 8. 94°C, 1:30
- 9. 94°C, 0:30
- 10. 52°C 62°C, 0:30
- 11. 72°C, 0:30
- 12. GOTO step 2, 30X
- 13. 72°C, 5:00
- 14. 12°C, ∞

#### 16S Protocol

Lid: 105°C

Volume: 25 µL

- 1. 94°C, 3:00
- 2. 94°C, 0:45
- 3. 47°C, 0:30
- 4. 72°C, 1:00
- 5. GOTO step 2, 34X
- 6. 72°C, 5:00
- 7. 12°C, ∞

## APPENDIX B

Legend	
Number Sites	549
Identical	
Missing	?
Indel	-

## 16S sequences

005_16S	T	C	Α	Α	Α	A	A	C	A	T	G	T	C	T	A	T	T	C	G	Α	A
008B16S	-	-	-	-														T			
008C_16S	-	-	-	-														T		T	
008D_16S	-	-	-	-	-													T		T	
008E_16S	-	-	-	-														T		T	
012_16S	-	-	_	-	_													T		T	
013A_16S	-	-	_	_																	
013B_16S	-	-	_	_	_	_															
015_16S	-	-	_	-	_	_	-	-	-	-	-										
034_16S	-	-	-	-	-	-	-	-	-	-	-										
157B_16S	-	-	_	_												Α		T		T	
166A_16S	_	-	_															T			
166B_16S	_	_	_	_	_	_												T			
172_16S	_	_	_	_												A		T		T	
_																					
005_16S	T	A	A	A	T	T	T	T	T	T	A	G	T	C	T	G	G	C	C	T	G
008B16S		T	C																		
008C_16S		T	T																		
008D_16S		T	T																		
008E_16S		T	T																		
012_16S		C	T																		
013A_16S																					
013B_16S																					
015_16S																					
034_16S																					
157B_16S		T	T		Α																
166A_16S		T	C																		
166B_16S		T	C																		
172_16S		T	T		A																
005.450	~	~	~		~	_				_	_		_	_				~	~	~	~
005_16S	C	C	C	A	C	T	G	A	A	T	T	A	T	T	A	A	A	G	G	G	C
008B16S	•	•	•	•	٠	•	•	•	T	•	•	•	•	•	•	•	•	٠	•	•	•
008C_16S	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	•	•	•	•
008D_16S	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	•	•	•	•
008E_16S	•	•	•	•	٠	•	•	•	G	٠	•	•	•	•	•	•	•	٠	•	•	•
012_16S	•	•	•	•	٠	•	•	•	G	٠	•	•	•	•	•	•	•	٠	•	•	•
013A_16S	•	٠	•	•	٠	•	•	•	•	٠	•	•	•	•	•	•	٠	٠	٠	٠	•
013B_16S	•	٠	•	•	٠	•	•	•	•	٠	•	•	•	•	•	•		٠	٠	٠	•
015_16S	•	•	•	•	•	•	•	•	•	•	•	•		•	•	•	R	•	•	•	•
034_16S	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
157B_16S	•	•	•	•	•	•		G	G	•	•	G	•	•		•	•	•	•	•	•
166A_16S	•	•		•	•	•	•		T			•	•		•	•	•	•	•	•	•
166B_16S	•		•	•	•				T			•				•	•	•	•	•	
172_16S			•		•			G	G			G	•				•	•		•	

005_16S	C	G	C	G	G	T	A	T	T	T	G	G	A	C	C	G	T	G	C	T	A
008B16S																					
008C_16S																					
008D_16S																					
008E_16S																					
012_16S																					
013A_16S																					
013B_16S																					
015_16S																					
034_16S																					
157B_16S									C												
166A_16S																					
166B_16S																					
172_16S	·	·	·	į.	Ō	·	Ō	Ī	C	Ō		·	Ī		Ō		·	·	Ō	Ō	·
172_105	•	•	•	•	•	•	•	•	Ü	•	•	•	•	•	•	•	•	٠	•	•	•
005_16S	A	G	G	T	A	G	C	A	T	A	A	T	C	A	A	T	A	G	T	A	T
008B16S		•	•								•	•		•		•	•	•		C	•
008C_16S		•	•		•		•	•		•		•	•		•		•	•		C	•
008D_16S																				C	
008E_16S																				C	
012_16S																				C	
013A_16S		•										•					•			C	
013B_16S																				C	
015_16S																				C	
034_16S																				C	
157B_16S																				C	
166A_16S																				C	
166B_16S																				C	
172_16S																				C	
005_16S	T	T	T	A	A	T	T	G	G	A	G	T	C	T	A	G	T	A	T	G	A
008B16S	C														G		A				
008C_16S	C											A			G		A				
008D_16S	C	•										A			G		A				•
008E_16S	C	•										A			G		A				
012_16S	C														G		Α				
013A_16S																					
013B_16S																					
015_16S																					
034_16S																					
157B_16S	C											A			G		A				
166A_16S	C														G		A				
166B_16S	C														G		A				
172_16S	C		•									A			G		A	•			•
005_16S	A	C	G	G	T	С	G	G	A	T	G	G	A	A	T	A	T	G	A	Т	T
008B16S		T	J	J	•		J	J	. 1	•		A		4.1	•	4.1		A	. 1	•	•
008B10S 008C_16S		T	•	•	•	T	•	•	•	•		A		•	•	•	•	A	•	•	•
008C_10S 008D_16S	•		•	•	•		•	•	•	•	•	1	•	•	•	•	•		•	•	•
		Т				т						Δ						Δ			
008E_16S		T T	•		•	T T	٠	•				A A						A A		٠	٠

012_16S		T				T						Α						A			
013A_16S																					
013B_16S																					
	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
015_16S	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
034_16S	•	٠	•	٠	٠	•	٠	٠	•	•	•	٠	٠	٠	٠	٠	٠	•	٠	•	٠
157B_16S		T										A	G					A			
166A_16S		T										A						A			
166B_16S		T										A						A			
172_16S		T										A	G					A			
172_100	•	-	•	•	•	•	•	•	•	•	•		•	•	•	•	•	••	•	•	•
007 169	<b></b>	~	<b></b>	~	<b></b>	<b></b>	~	~		~	-	~	-				<b></b>		-		~
005_16S	T	G	T	C	T	T	C	G	A	G	T	G	T	A	A	A	T	A	T	T	G
008B16S						G		Α		Α		A			G	T	-				
008C_16S						G	T	Α		Α		Α			G		-				
008D_16S						G	T	Α		Α		A			G		_				
008E_16S						G	T	Α		Α		A			G		_				
012_16S	•	•	•	•	•	A	•	A		A		A	•	•	T	•		•	•	•	•
	•	•	•	•	•	A	•	А	•	A	•	Α	•	•	1	•	-	•	•	•	•
013A_16S	•		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
013B_16S	•						•		•		•	•									
015_16S																					
034_16S																					
157B_16S						G		A	G	A		A				T	_	T			
166A_16S	•	•	•	•	•	G				A	•	A	•	•	G	T	_	•	•	•	•
	•	•	•	•	•		•		•		•		•	•	G			•	•	•	•
166B_16S	•	•	•	•	•	G	•	٠.		A	•	A	•	•	G	T	-		•	•	•
172_16S	•	•	•	•	٠	G	•	A	G	A	•	A	•	•	•	T	-	T	•	•	٠
005 160																					
005_16S	A	Α	T	T	T	T	Α	C	T	T	T	T	G	A	G	T	G	A	Α	Α	Α
	A	A	T	T	T	T	A	C	T .	T	T	T .		A	G	T	G	A	A	A	A
008B16S	A .		T .	T .	T .	T .	A .	C .		T .	T .		A	A .	G	T .	G	A .	A .	A .	A .
008B16S 008C_16S	A	A	T	T	T	T	A	C		T	T		A A	A	G	T	G	A	A	A	A
008B16S 008C_16S 008D_16S	A	A	T	T	T	T	A	C	T	T	T		A A A	A	G	T	G	A	A	A	A
008B16S 008C_16S 008D_16S 008E_16S	A	A	T	T	T	T	A	C	T	T	T		A A A	A	G	T	G	A	A	A	A
008B16S 008C_16S 008D_16S 008E_16S 012_16S	A	A	T	T	T	T	A	C	T	T	T		A A A	A	G	T	G	A	A	A	A
008B16S 008C_16S 008D_16S 008E_16S	A	A	T	T	T	T	A	C	T	T	T		A A A	A	G	T	G	A	A	A	A
008B16S 008C_16S 008D_16S 008E_16S 012_16S	A	A	T	T	T	T	A	C	T	T	T		A A A	A	G	T	G	A	A	A	A
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S	A	A	T	T	T	T	A	C	T	T	T		A A A	A	G	T	G	A	A	A	A
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S	A	A	T	T	T	T	A	C	T	T	T		A A A	A	G	T	G	A	A	A	A
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S 034_16S	A	A	T	T	T	T	A	C	T	T	T		A A A	A	G	T	G	A	A	A	A
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S 034_16S 157B_16S	A	A	T	T	T	T	A	C	T	T	T		A A A A	A	G	T	G	A	A	A	A
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S 034_16S 157B_16S 166A_16S	A	A	T	T	T	T	A	C	T	T	T		A A A A	A	G	T	G	A	A	A	A
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S 034_16S 157B_16S 166A_16S 166B_16S	A	A	T	T	T	T	A	C	T	T	T		A A A A	A	G	T	G	A	A	A	A
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S 034_16S 157B_16S 166A_16S	A	A	T	T	T	T	A	C	T	T	T		A A A A	A	G	T	G	A	A	A	A
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S 034_16S 157B_16S 166A_16S 166B_16S 172_16S	A	A	T	T	T	T	A	C		T	T		A A A A	A	G	T	G	A	A	A	A
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S 034_16S 157B_16S 166A_16S 166B_16S	A	A	T	T	T	T	A	C	T	T	T		A A A A	A	G	T	G	A	A	A	A
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S 034_16S 157B_16S 166A_16S 166B_16S 172_16S 005_16S												T	A A A A A A A								
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S 034_16S 157B_16S 166A_16S 166B_16S 172_16S 005_16S 008B16S											G A	T A	A A A A A A A			G A					
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S 034_16S 157B_16S 166A_16S 166B_16S 172_16S 005_16S 008B16S 008C_16S										T A A	G A A	T A A	A A A A A A A T T			G A A					
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S 034_16S 157B_16S 166A_16S 166B_16S 172_16S 008B16S 008C_16S 008D_16S										T A A A A	G A A A A	T A A A A	A A A A A								
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S 034_16S 157B_16S 166A_16S 166B_16S 172_16S 008B16S 008B16S 008C_16S 008D_16S 008E_16S													A A A A A A A A A A T T T T T								
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S 034_16S 157B_16S 166A_16S 166B_16S 172_16S 008B16S 008C_16S 008C_16S 008E_16S 012_16S										T A A A A	G A A A A	T A A A A	A A A A A								
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S 034_16S 157B_16S 166A_16S 166B_16S 172_16S 008B16S 008C_16S 008C_16S 008E_16S 012_16S 012_16S													A A A A A A A A A A T T T T T								
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S 034_16S 157B_16S 166A_16S 166B_16S 172_16S 008B16S 008C_16S 008C_16S 008E_16S 012_16S													A A A A A A A A A A T T T T T								
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S 034_16S 157B_16S 166A_16S 166B_16S 172_16S 008B16S 008C_16S 008C_16S 008E_16S 012_16S 013A_16S 013A_16S 013B_16S													A A A A A A A A A A T T T T T								
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S 034_16S 157B_16S 166A_16S 166B_16S 172_16S 008B16S 008C_16S 008C_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S													A A A A A A A A A A T T T T T								
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S 034_16S 157B_16S 166A_16S 166B_16S 172_16S 008B16S 008C_16S 008C_16S 008E_16S 012_16S 013A_16S 013A_16S 013B_16S													A A A A A A A A A A T T T T T								

166A_16S 166B_16S										A A	A A	A A	T T			A A	A A				
172_16S	•									G		A	•			A	A				
005_16S	C	G	A	T	A	A	G	A	C	C	C	T	G	T	G	A	A	G	C	T	T
008B16S																					
008C_16S																					
008D_16S																					
008E_16S																					
012_16S																					
013A_16S																					
013B_16S																					
015_16S																					
034_16S																					
157B_16S																					
166A_16S																					
166B_16S																					
172_16S										•			•								
005_16S	Т	A	Т	Т	Т	Т	Т	G	C	T	Т	Т	G	A	Т	Т	Т	Т	A	G	G
008B16S	1		1	1	1	1		A	T		A			-	1	1	1	1		A	T
008C_16S	•	•	•	•	•	•	•	A	T		G		•	_		•	•	•	•	A	A
008D_16S	•	•	•	•	•	•	•	A	T		G		•	_	_	•	•	•	•	A	A
008E_16S	•	•	•	•	•	•	•	A	T	•	G	•	•	_	_	•	•	•	•	A	A
012_16S	•	•	•	•	•	•	•	A	T	•	G	•	•	_	_	•	•	•	•	A	A
012_10S 013A_16S	•	•	•	•	•	•	•	$\Lambda$	1	•	G	•	•			•	•	•	•	А	А
013A_103 013B_16S	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
015 <u>1</u> 16S	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
034_16S	•	•	•	•	•	•	•		•	•	•	•	•	•	•	•	•	•	•	•	•
157B_16S	•	•	•	•	•	•	•	А	T		А		•	-	G	G	C	•	•	А	T
166A_16S	•	•	•	•	•	•	•	A	T		A		•	_	G	J		•	•	A	T
166B_16S	•	•	•	•	•	•	•	A	T	•	A	•	•		•	•	•	•	•	A	T
172_16S								A	T		A			_	G	G	C			A	T
	_	_												_					_	_	
005_16S	G	C	T			A	T	A	A	T	T	G	T	G	T		G	T	T	G	G
008B16S	Α	T	•	T	G	•		•	G	•	•	•	•	•		G	A	•	•	A	Α
008C_16S	A	T	•	T	G	•	G		G	•		A	G			G	A	•	•	A	A
008D_16S	A	T	•	T	G	•	G	•	G	•	•	A	G	•		G	A	•	•	A	A
008E_16S	A	T	•	T	G	•	G	•	G	•	•	A	G	•		G	A	•	•	A	A
012_16S	A	T	•	T	G	•	G	•	G	•	•	A	G	•		G		•	•	A	A
013A_16S	•	•	•	•	•	•		•	G	•	•	٠	•	•		•	٠	•	•	•	•
013B_16S	•	•	•	•	•	•		•	G	•	•	•	•	•		•	•	•	•	•	•
015_16S	•	•	•	•	•	•		•	G	•	•	٠	•	•		•	٠	•	•	•	•
034_16S	•	•	•	•	•	•			G	•		•	•			•	•	•	•	•	•
157B_16S	A	T	•	T		•		•		•	•		•	•		G	T	•	•	A	A
166A_16S	A	T		T	G	•	•	•	G	•	•		•	•	•	G	A	•	•	A	A
166B_16S	A	T		T	G				G							G	A			A	A
172_16S	A	T	•	T	•		•	٠	٠	•	٠	•	•	٠	•	G	T	•	•	A	A
005_16S	G	T	T	T	A	A	G	T	T	T	A	G	C	A	G	A	T	A	T	T	T
008B16S	A				G								T		A		A				A

008C_16S	Α				G							A	T		A	G	A				A
008D_16S	A				G							A	T		A	G	Α				A
		•	•	•		•	•	•	•	•	•			•				•	•	•	
008E_16S	A			•	G		•		•	•	•	A	T	•	A	G	A	•	•		A
012_16S	A				G							Α	T		A		A				A
013A_16S															Α						
	•	•	•	•	•	•	•	•	•	•	•	•	•	•		•	•	•	•	•	•
013B_16S	•	•	•	•	•	•	•	•	•	•	•	•	•	•	A	•	•	•	•	•	•
015_16S															Α						
034_16S															A						
 157B_16S	A				G				G			A	T		A	G	A				A
		•	•	•		•	•	•	U	•	•	Л						•	•	•	
166A_16S	A			•	G		•			•	•		T	•	A		A				A
166B_16S	Α				G								T		Α		Α				G
172_16S	A				G				G			A	T		A	G	A				A
172_100		•	-	•		•	•	•	Ū	•	•		-	•				·	•	•	
005 160	~	~	-	-	~	~	~	~	~	~		~	-	~	~			<b></b>		-	
005_16S	G	G	T	T	G	G	G	G	C	G	A	C	T	G	G	A	A	T	A	T	A
008B16S	Α	A										T									
008C_16S												T			A						
008D_16S	•	•	•	•	•	•	•	•	•	•		T			A	•	•	·	•	•	•
	•	•	•	•	•	•	•	•	•	•	٠		٠	•		•	•	•	•	•	•
008E_16S												T		•	A					•	
012_16S												T		Α	A						
013A_16S																					
	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
013B_16S	•	•	•	٠	•	•	•	•	•	•	٠	•	•	•	•	•	•	•	•	•	•
015_16S																					
034_16S																					
157B_16S														A	A	G					T
	•	٠.	•	•	•	•	•	•	•	•	•		•	A	A	U	•	•	•	•	1
166A_16S	A	A		•	•		•			•	•	T		•	•	•					•
166B_16S	Α	Α										T									
172_16S														Α	Α	G					T
172_100	•	•	•	•	•	•	•	•	•	•	•	•	•			_	•	•	•	•	-
005 160	-		<b></b>	~	<b></b>			<u> </u>	-	~	<b></b>	<b></b>	<b></b>	æ	<b></b>			<b></b>		_	<b></b>
005_16S	T	T	T	G	T	A	A	C	T	G	T	T	T	T	Т	G	T	T	A	G	T
005_16S 008B16S	T .	T	T A	G T	T	A .	A .	C	T	G	T	T	T	T	T .	G A	T A	T	A	G	T
008B16S	T .	T .	A	T		A .	A .	C .	T	G	T .	T	T .	T	T	A	A	T	A	G	T .
008B16S 008C_16S	T	T	A A	T T	T	A	A	C .	T	G	T	T	T	T	T	A A	A A	T	A	G	T
008B16S 008C_16S 008D_16S	T	T	A A A	T T T		A	A	C	T	G	T	T	T	T	T	A A A	A A A	T	A	G	T
008B16S 008C_16S 008D_16S 008E_16S	T	T	A A A	T T T		A	A	C	T	G	T	T	T	T	T	A A A	A A A	T	A	G	T
008B16S 008C_16S 008D_16S	T	T	A A A	T T T		A	A	C	T	G	T	T	T	T	T	A A A	A A A	T	A	G	T
008B16S 008C_16S 008D_16S 008E_16S 012_16S	T	T	A A A	T T T		A	A	C	T	G	T	T	T	T	T	A A A	A A A	T	A	G	T
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S	T	T	A A A	T T T		A	A	C	T	G	T	T	T	T	T	A A A	A A A	T	A	G	T
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S	T	T	A A A	T T T		A	A	C	T	G	T	T	T	T	T	A A A	A A A	T	A	G	T
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S	T	T	A A A	T T T		A	A	C	T	G	T	T	T	T	T	A A A	A A A	T	A	G	T
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S	T	T	A A A	T T T		A	A	C	T	G	T	T	T	T	T	A A A	A A A	T	A	G	T
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S 034_16S	T	T	A A A A	T T T T		A	A	C	T	G	T	T	T	T	T	A A A A	A A A A		A		T
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S 034_16S 157B_16S	T	T	A A A A	T T T T		A	A	C	T	G	T	T	T	T	T	A A A A	A A A A	G	A	G	T
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S 034_16S 157B_16S 166A_16S	T	T	A A A A A	T T T T T		A	A	C	T	G	T	T	T	T	T	A A A A A A	A A A A		A		T
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S 034_16S 157B_16S	T	T	A A A A	T T T T		A	A	C	T	G	T	T	T	T	T	A A A A A A A	A A A A	G	A		T
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S 034_16S 157B_16S 166A_16S			A A A A A	T T T T T		A	A	C	T	G	T	T		T		A A A A A A	A A A A	G			T
008B_16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S 034_16S 157B_16S 166A_16S 166B_16S			A A A A A A A	T T T T T T		A	A	C	T	G	T	T		T		A A A A A A A	A A A A A			T	T
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S 034_16S 157B_16S 166A_16S 166B_16S 172_16S			A A A A A A A A A A A A A A A A A	T T T T T T T T T T												A A A A A A A A A A A A A A A A	A A A A A A	G		T	
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S 034_16S 157B_16S 166A_16S 166B_16S 172_16S 005_16S			A A A A A A A	T T T T T T		A		G			T	T T		T T		A A A A A A A	A A A A A			T	T
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S 034_16S 157B_16S 166A_16S 166B_16S 172_16S 005_16S 008B16S			A A A A A A A A A A A A A A A A A	T T T T T T T T T T				G A		G A						A A A A A A A A A A A A A A A A	A A A A A A	G		T	
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S 034_16S 157B_16S 166A_16S 166B_16S 172_16S 005_16S			A A A A A A A A A A A A A A A A A	T T T T T T T T T T				G								A A A A A A A A A A A A A A A A	A A A A A A	G		T	
008B16S 008C_16S 008D_16S 008E_16S 012_16S 013A_16S 013B_16S 015_16S 034_16S 157B_16S 166A_16S 166B_16S 172_16S 005_16S 008B16S 008C_16S			A A A A A A A A A A A A A A A A A	T T T T T T T T T T				G A A								A A A A A A A A A A A A A A A A	A A A A A A	G		T	
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015_16S																					
034_16S																					
157B_16S			G						G		G	G	C			A	G	-		T	A
166A_16S								A		Α								-			
166B_16S								A		A								_			
172_16S			G						G		G	G	C			A	G	_		T	A
005_16S	A	T	T	G	A	T	C	C	T	G	T	G	T	T	G	T	G	G	A	T	T
008B16S	G							T				A			A	C					
008C_16S	G							T				A			A		A				
008D_16S	G							T				A			A		A				
008E_16S	G							T				A			A		Α				
012_16S	G							T				A			A		A				
013A_16S																					
013B_16S																					
015_16S																					
034_16S																					
157B_16S	G		•	•		•			•	•	-	T			A	C	A	-	•	-	C
166A_16S	G		•	•	•	•	•	T	•	•	•	A	•		A	C		•	•	•	
166B_16S	G	•	•	•	•	•	•	T	•	•	•	A	•		A	C		•	•	•	•
172_16S	G	•	•	•	•	•	•	•	•	•	•	T	•	•	A	C	A	•	•		C
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008B16S																					
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005_16S	G	G	A	T	A	A	C	A	G	C	G	T	A	A	T	T	T	T	T	C	T
008B16S																	C			T	
008C_16S																	C			T	
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005_16S	T	G	A	G	A	G	T	T	C	T	T	A	T	C	G	A	C	A	G	A	A
008B16S																					
008C_16S																			A		
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008E_16S																			A		
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166B_16S	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
172_16S	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	A	•	•
172_102	·	•	•	·		•	•	•	•	·	•	·		•	•		·	•		·	•
005_16S	A	T	A	T	T	T	G	C	G	A	C	C	T	C	G	A	T	G	T	T	G
008B16S	G	•												•	•		•	•	•	•	•
008C_16S	G																				
008D_16S	G																				•
008E_16S	G																				•
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166A_16S	G																				
166B_16S	G																				
172_16S	G															٠					•
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005_16S	A	A	T	T	A	A	G	G	T	T	T	C	A	T	C	A	A	G	A	T	G
008B16S	•	•	•	•	•	•	٠	A	•	٠	•	٠	•	•	T	•	•	٠	G	•	٠
008C_16S	•	•	•	•	•	•	٠	A	•	•	•	٠	•	•	•	•	•	•	G	•	٠
008D_16S	•	٠	•	•	•	•	٠	A	•	٠	•	٠	•	•	٠	٠	٠	•	G	•	٠
008E_16S	•	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	٠	•	G	•	•
012_16S	•	•	•	•	•	•	•	A	•	•	•	•	•	•	•	•	•	•	G	•	•
013A_16S	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	٠	•	•	•	•
013B_16S	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	٠	•	•	•	•
015_16S	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
034_16S	•	•	•	•	•	•	٠	•	•	•	•			•	•	•	•	٠	•	•	٠
157B_16S	•	•	•	•	•	•	•	A	•	•	•	•	G	C	T	•	G	•	G	•	•
166A_16S		•	•	•				A						•	T	•	٠	•	G	•	•
166B_16S		•						A						•	T		•	•	G		•
172_16S				•	•	•	٠	A	٠	•	•	•	G	C	T	•	G	•	G	•	٠
005_16S	C	A	G	A	A	G	T	C	T	T	G	C	T	T	G	T	A	G	G	T	C
005_16S 008B16S	C T	A	G	A T	A	G	T	C	T	T	G	C A	T	T	G	T .	A .	G	G	T	C
		A					T	C	T	T		A		T	G	T	A	G	G	T	
008B16S	T	A		T			T	C	T	T				T	G	T	A	G	G	T	
008B16S 008C_16S	T T	A		T G	G		T	C	T	T		A A		T	G	T	A	G	G	T	

012_16S	T			G	G							A									
013A_16S	_																				
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034_16S	•	•	•	•	•	•	•	•	•	•	•		•	•	•	•	•	•	•	•	•
157B_16S	T	•			•	•	C	•				А			•		G			•	•
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172_16S	T	•	•	•	•	•	C	•	Ү	•		A	•	•	•	•	G	•	•	•	•
172_100	-	•	•	•	·	•	•	•	-	•	•		-	-	•	•		•	-	•	•
005_16S	T	G	T	T	C	G	A	C	C	T	T	T	Α	A	A	A	T	C	T	T	Α
008B16S																		T			
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008C_16S							-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
008D_16S							-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
008E_16S							-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
012_16S							-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
013A_16S								T	G	A	-	-	-	-	-	-	-	-	-	-	-
013B_16S						-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
015_16S						-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
034_16S							-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
157B_16S								T	G	A	G	T	N	N	C	C	A	N	Α	C	C
166A_16S								T	G	A	-	-	-	-	-	-	-	-	-	-	-
166B_16S						-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
172_16S								T	G	A	-	-	-	-	-	-	-	-	-	-	-
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166A\_16S - - - - 166B\_16S - - - - 172\_16S - - - -

Legend							A	ΡF	E	ND]	IX	C										
	134	4																				
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166B_ITS		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	G	T	C	T	T
166A_ITS		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
008B_Fonly_ITS		N	N	N	N	N	N	N	N	T	N	N	T	-	G	N	N	•	•	G	N	•
008C_ITS		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	•	•	•	•	•	•
008A_ITS		-	-	-	T	A	T	T	T	A	G	C	T	-	G	C	•	•	•	•	•	•
008D_ITS		C	T	T	T	A	T	T	T	A	G	C	T	-	G	C	•	•	•	•	٠	•
008E_ITS		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161_ITS		-	-	-	-	A	T	T	T	A	G	C	T	T	G	C	•	•	•	•	•	•
172_ITS		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	•	•	•	•
157B_ITS		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	•
015_ITS		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
034_ITS		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
166B_ITS		C	A	T	C	G	A	C	C	T	C	A	C	G	A	G	C	C	G	A	G	C
166A_ITS		-	-	-	-																	
008B_Fonly_ITS					N		T		G			N	T			N	G	A				N
008C_ITS																						
008A_ITS																						
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008E_ITS		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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172_ITS																						
157B_ITS																						
015_ITS		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
034_ITS		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
166B_ITS		G	A	T	C	C	A	C	C	G	C	T	A	G	A	A	G	T	T	G	T	G
166A_ITS																						
008B_Fonly_ITS						A	T												A			
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166B_ITS		A	A	T	-	-	G	G	T	T	A	G	G	C	C	C	T	A	G	T	T	G
166A_ITS					-	-																
008B_Fonly_ITS				G	-	-	A			N			N		N	N					A	
008C_ITS					-	-														C		
008A_ITS					-	-														C		
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161_ITS				-	-			•			•	•		•			•	•	C		
172_ITS				-	-							Α									
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166B_ITS	C	G	_	G	G	C	C	A	A	C	C	T	G	C	T	С	A	Α	T	A	A
166A_ITS	·	Ü	_	Ü	Ü	·	·			Č	·	•		Ü	•	Ü			•		
008B_Fonly_ITS	•	•		•	N	•	•	•	N	•	N	•	С	•	•	•	•	•	•	•	•
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008C_ITS	•	•	-	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
008A_ITS	•	•	-	•	•	•	•	•	•	•		•	•	•	•	•	٠	•	•	•	•
008D_ITS	•		-		•									•				•			•
008E_ITS			N																		
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031_115	•	•		•	•	•	•	•	•	•	•	Ü	••	•	•						
166B_ITS	A	G	A	G	C	A	T	A	A	T	T	G	G	T	T	T	T	G	T	C	G
166A_ITS		Ü		Ü	·		-			-	-			-	-	-	-		-		Ū
008B_Fonly_ITS	•	•	•	N	•	•	•	•	•	•	А		N	•	N	N	•	•	•	N	•
008C_ITS	•	•	•	14	•	•	•	•	•	•	А	•	14	•	11	14	•	•	•	11	•
	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
008A_ITS	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
008D_ITS	•	٠	٠	•	٠	•	•	•	•	٠	•	•	٠	٠	٠	•	٠	٠	٠	٠	٠
008E_ITS	•		•	•	٠	•	•	•	•		•	•	•	٠	•	•	•	•	•	•	•
161_ITS	•				•		C							•				•			•
172_ITS								-		C											
157B_ITS								-		C											
015_ITS	-	-	-	-	-	G											Α		A		
034_ITS	-	-	-	-	-	G											A		A		
166B_ITS	G	G	-	C	Α	T	A	C	A	C	C	C	-	-	-	C	T	G	G	G	T
166A_ITS			-										-	-	-						
008B_Fonly_ITS	T	N	-	N			C		N	N			N	N	N	N	N	N	N		
008C_ITS			_										_	_	_						
008A_ITS			_										_	_	_						
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161_ITS	•	•	_	•	•	•	•	•	•	•	•	•	_	_	_	•	•	•	•	•	•
172_ITS	•	•		•	•	•	•	•	•	•	•	•				•	C	•	•	•	•
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157B_ITS	•	•	-	•	•	•	•	•			•	•	-	-	-	•	C	•	•	•	•
015_ITS	•	•	C	•	•	•	•	•	G	G		•	-	-	-	•	C	A	•	•	•
034_ITS	•	•	C	•	•	•	•	•	G	G	•	•	-	-	-	•	C	A	•		•
166D ITC	т	т	٨	C	~	<i>C</i>	~	~	~	~	~	~	т	~	~	٨	~	~	٨	~	A
166B_ITS	T	T	A	C	G	C	C	G	C	G	G	G	T	C	G	A	G	G	A	G	A
166A_ITS	•		•	•	•	•	•	•	•			•				•	•	•	•	•	•
008B_Fonly_ITS	C				-	-	-						N	N				•			
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008A_ITS					A		T														
008D_ITS					A		T														

008E_ITS 161_ITS 172_ITS 157B_ITS 015_ITS 034_ITS					A A A A A		T T T			A A	A A								T T	T T	G G
166B_ITS	G	C	C	C	C	C	C	C	C	G	G	C	C	C	G	C	A	G	G	A	G
166A_ITS																					
008B_Fonly_ITS		-	G	A	N					N				N				N			N
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166A_ITS	_	_	_	_	_	_	_	_	_	_	_	_	_	_	-	_	_	_	_	-	_
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015_ITS	G	A	A	A	A	A	G	T	G	G	G	C	T	T	C	T	C	T	A	C	C
034_ITS	G	A	A	A	A	A	G	T	G	G	G	C	T	T	C	T	C	T	A	C	C
166B_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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008B_Fonly_ITS	_	_	_	_	_	_	_	_	_	_	_	-	_	_	_	-	_	_	_	_	_
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157B_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
015_ITS	A	G	C	G	C	A	C	T	G	G	A	A	A	A	A	G	G	G	G	G	C
034_ITS	A	G	C	G	С	A	С	T	G	G	A	A	A	A	A	G	G	G	G	G	C
166B_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
166A_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
008B_Fonly_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
008C_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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166B_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
166A_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
008B_Fonly_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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008D_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
008E_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
172_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
157B_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
015_ITS	A	A	A	G	G	C	G	G	N	N	N	C	T	C	T	Α	C	C	Α	G	C
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166B_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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008B_Fonly_ITS	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
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161_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
172_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
157B_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
015_ITS	G	C	A	C	T	G	G	A	A	A	A	A	G	G	A	G	G	C	T	T	C
034_ITS	G	C	A	C	T	G	G	A	A	A	A	A	G	G	A	G	G	C	T	T	C
166B_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
166A_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
008B_Fonly_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
008C_ITS	_	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
008A_ITS	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
008D_ITS	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
008E_ITS	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
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172_ITS																					
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034_ITS	T	C	T	A	C	C	A	G	C	G	C	A	C	T	G	G	A	A	A	A	A
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008B_Fonly_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
008C_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
008A_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
008D_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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172_ITS	_	-	_	_	_	_	-	_	_	_	_	_	_	_	_	_	_	_	-	-	_
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034_ITS	G	G	A	G	G	C	T	T	C	T	C	T	A	C	C	A	G	C	G	C	A
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034_ITS	A	C	C	A	G	C	G	C	A	C	T	G	G	A	A	A	A	A	G	G	G
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166B_ITS	_	_	_	_	_	_	_	_	_	_	C	G	A	G	C	G	C	A	_	_	_
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008B_Fonly_ITS	_	_	_	_	_	_	_	_	_	_		C						G	_	_	_
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008E_ITS	_	_	_	_	_	_	_	_	_	_									_	_	_
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015_ITS	G	G	C	T	T	C	T	C	T	A		C							C	T	G
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166B_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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008E_ITS	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
161_ITS	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
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157B_ITS	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
015_ITS	G	A	A	A	A	A	G	G	G	A	G	C	T	T	C	T	C	T	A	C	C
034_ITS	G	A	A	A	A	A	G	G	G	A	G	C	T	T	C	T	C	T	A	C	C
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008B_Fonly_ITS 008C_ITS 008A_ITS 008D_ITS 008E_ITS 161_ITS 172_ITS 157B_ITS 015_ITS 034_ITS 166B_ITS 166A_ITS												A	A	A	A	G	T T	G G	G G	G	C
008B_Fonly_ITS 008C_ITS 008A_ITS 008B_ITS 008E_ITS 161_ITS 172_ITS 157B_ITS 015_ITS 034_ITS 166B_ITS 166A_ITS 008B_Fonly_ITS												A	A	A	A C	G	T T	G G	G G	G	C A
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008B_Fonly_ITS 008C_ITS 008A_ITS 008A_ITS 008B_ITS 161_ITS 172_ITS 157B_ITS 015_ITS 034_ITS  166B_ITS 166A_ITS 008B_Fonly_ITS 008C_ITS 008D_ITS 008E_ITS												A	A	A C	A C . G G G G G	G	T T	G G	G G	G	C A

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166A_ITS		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
008B_Fonly_ITS	C	-	_	-	-	-	-	-	_	-	-	-	-	_	-	_	-	-	-	-	-
008C_ITS	G	-	_	-	-	-	-	-	_	-	-	-	-	_	-	_	-	-	-	-	-
008A_ITS	G	_	-	_	_	_	_	_	_	-	_	_	_	-	_	_	-	_	_	_	_
008D_ITS	G	_	-	-	-	-	-	-	-	-	-	_	_	-	_	-	-	_	-	_	_
008E_ITS	G	_	-	_	_	_	_	_	_	-	_	_	_	-	_	_	-	_	_	_	_
161_ITS	G	_	_	_	-	_	_	-	_	_	_	_	_	_	_	_	_	_	-	_	_
172_ITS	G	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
157B_ITS	G	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
015_ITS	T	C	C	A	G	C	A	G	G	A	G	G	A	C	G	G	C	C	G	C	C
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034_ITS	•	C	C	A	G	C	Т	G	G	A	C	G	A	C	Т	G	C	C	G	C	С
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034_ITS	-				G - -	C - -	T - -	G - -	G - -	A - -											
034_ITS 166B_ITS	-				G - -	C	T - -	G - -	G - -	A - -											
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034_ITS  166B_ITS 166A_ITS 008B_Fonly_ITS 008C_ITS 008A_ITS 008B_ITS 161_ITS 172_ITS 157B_ITS 015_ITS 034_ITS  166B_ITS 166A_ITS 008B_Fonly_ITS 008C_ITS 008A_ITS 008D_ITS 008B_ITS 161_ITS	G	C C C	C A A	A G A T .	- - - - - - C C	G G T .	G G G .	- - - - - - - A	G G T .	T T T	C	C	C	A	G	T	C	G . A	T . G	G	G
034_ITS  166B_ITS 166A_ITS 008B_Fonly_ITS 008C_ITS 008A_ITS 008D_ITS 008E_ITS 161_ITS 172_ITS 157B_ITS 015_ITS 034_ITS  166B_ITS 166A_ITS 008B_Fonly_ITS 008C_ITS 008A_ITS 008D_ITS 008E_ITS	G	C C C	C A A	A G A T .	- - - - - - C C	G G T .	G G G .	- - - - - - - A	G G T .	T T T	C	C	C	A	G	T	C	G	T	G	G

015_ITS 034_ITS								G		G G						C	G G		G G		
166B_ITS	C	A	C	T	T	C	C	G	C	C	G	C	T	G	C	T	G	C	T	G	C
166A_ITS																					
008B_Fonly_ITS								T	T		C	G		C		N		G			
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008E_ITS							T														
161_ITS							T														
172_ITS				A		G												A			
157B_ITS				A		G												A			
015_ITS		G		C			T	T			Α		C								
034_ITS		G					T				A										•
166B_ITS	C	C	C	C	A	G	C	T	T	T	C	T	G	T	C	A	-	-	-	-	-
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008B_Fonly_ITS								C	Α								A	T	C	T	A
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172_ITS	•	•				•	•	•		•	•				•		-	-	-	-	-
157B_ITS			•		•	•	•	•					•		•		-	-	-	-	-
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034_ITS	T	G	•	T	-	•	•	•	G	C	T	C	•	C	•	C	-	-	-	-	-
166B_ITS	-	-	-	-	-	-	-	A	T	C	T	C	T	C	T	A	T	C	T	T	T
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008B_Fonly_ITS	T	C	T	A	T	C	T					A									
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034_ITS	-	-	-	-	-	-	-	G	C	•	•		C	•	C	T	C	T	•	C	•
166B_ITS	C	T	C	T	G	G	T	T	T	T	T	G	C	T	T	T	A	T	G	T	T
166A_ITS																					
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008A_ITS					T		G														
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008E_ITS					T		G														
161_ITS					T		G														
172_ITS				C	C		G			C				A			T	G		-	-

157B_ITS 015_ITS 034_ITS			- -	C - -	C - -	· -	G - -	-	- -	C - -	· - -	· -	- -	A			T G G	G		-	- ·
166B_ITS 166A_ITS	C	C	Т	T	C	A	C	C	T	C	T	G	C	T	A	C	-	-	-	-	-
008B_Fonly_ITS	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	_	_	_	_	_
008C_ITS	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	_	_	_	_	_
008A_ITS			•	•				T	•				•	•	•	•	_	_	_	_	_
008D_ITS	Ō	į		į.	·		·	T	·	·	·	·	·	·	·		_	_	_	_	_
008E_ITS	Ō	į		į.	·		·	T	·	·	·	·	·	·	·		_	_	_	_	_
161_ITS	•	•	•	•	•	•	•	T	•	•	•	•	•	•	•	•	_	_	_	_	_
172_ITS	-	-	•	•	•	•	•	•	•	•	•	•	•	•	•	T	A	T	T	A	G
157B_ITS	_	_	•	•	•	•	•	•	•	•	•	•	•	•	•	T	A	T	T	A	G
015_ITS	A		•	•	•	•	•	•	•	•	-	-	-	-	•	T	-	-	-	-	-
034_ITS	A	•	•	•	•	•	•	•	•	•	_	_	_	_	•	T	_	_	_	_	_
034_115	11	•	•	•	•	•	•	•	•	•					•	1					
166B_ITS	_	C	A	G	A	G	A	A	G	A	G	A	G	A	A	T	A	G	G	T	G
166A_ITS	_																				
008B_Fonly_ITS	_																				
008C_ITS	_																				
008A_ITS	_																				
008D_ITS	_																				
008E_ITS	_																				
161_ITS	_																				
172_ITS	Α																			G	
157B_ITS	Α																			G	
015_ITS	_	T						G	A	G	C		T	G	G	G				C	
034_ITS	-	T						G	A	G	C		T	G	G	G				C	
166B_ITS	G	C	A	C	C	A	G	C	A	G	C	G	G	C	-	-	-	-	T	G	T
166A_ITS															-	-	-	-			
008B_Fonly_ITS															-	-	-	-			
008C_ITS															-	-	-	-			
008A_ITS															-	-	-	-			
008D_ITS															-	-	-	-			
008E_ITS															-	-	-	-			
161_ITS															-	-	-	-			
172_ITS						G						A			A	G	T	T			C
157B_ITS						G						A			A	G	T	T			C
015_ITS									G						-	-	-	-	G		C
034_ITS					•				G						-	-	-	-	G		C
166D ITC	т	~	~	т	~	C	т	~	т	~	<i>C</i>	C	т	т	т						
166B_ITS	T	G	C	T	G	C	T	G	T	G	G	C	T	T	T	-	-	-	-	-	-
166A_ITS	•	٠	٠	٠	•	•	•	٠	٠	٠	•	•	٠	٠	•	-	-	-	-	-	-
008B_Fonly_ITS	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	-	-	-	-	-	-
008C_ITS	•	•	•	•	•	•	-	-	-	-	-	-	-	•	•	-	-	-	-	-	-
008A_ITS	•	•	•	•	•	•	-	-	-	-	-	-	-	•	•	-	-	-	-	-	-
008D_ITS	•	•	•	•	•	•	-	-	-	-	-	-	-	•	•	-	-	-	-	-	-
008E_ITS	•	٠	•	•	•	•	-	-	-	-	-	-	-	•	•	-	-	-	-	-	-
161_ITS	•			•	•		-	-	-	-	-	-	-	•		-	-	-	-	-	-

170 ITC				~												_					
172_ITS	•	•	٠	C	•	•	-	-	-	-	-	-	-	٠	•	C	-	-	-	-	-
157B_ITS	•		•	C	•			-		_	-	-	-	•	•	Y		-	_	-	_
015_ITS	G		•	A			G	•	C	T	•	-	-	•	•	A	C	A	T	A	T
034_ITS	G			A			G	•	C	T		-	-			A	C	A	T	A	T
166B_ITS	-	-	-	-	-	-	-	-	A	A	Α	G	C	A	-	C	C	T	T	G	C
166A_ITS	-	-	-	-	-	-	-	-							-						
008B_Fonly_ITS	-	-	-	-	-	-	-	-							-						
008C_ITS	_	_	_	_	_	_	_	_							_						
008A_ITS	_	_	_	_	_	_	_	_							_						
008D_ITS	_	_	_	_	_	_	_	_	•	-	-	-	-	-	_	-	-	•	Ī	-	•
008E_ITS	_	_	_	_	_	_	_	_	•	•	•	•	•	•	_	•	•	•	•	•	•
161_ITS									•	•	•	•	•	•		•	•	•	•	•	•
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172_ITS	-	-	-	-	-	-	-	-	•	•	•	•	•	•	-	•	٠	•	•	•	•
157B_ITS	-	-	-	-	-	-	-	-	•		•	•	•	•	-	٠	•	•	•	•	•
015_ITS	A	A	T	A	C	T	G	G		C	•	•	•	•	C	•	•	•	•	•	•
034_ITS	Α	A	T	A	C	T	G	G		C					C						
166B_ITS	C	T	C	G	G	T	C	T	C	C	-	Α	C	C	G	G	C	Α	Α	G	Α
166A_ITS											-										
008B_Fonly_ITS											-										
008C_ITS											-			T							
008A_ITS											_			T							
008D_ITS											_			T							
008E_ITS	•	•	•	•	•	•	•	•	•	•	_	•		T	•	•	•	•	•	•	•
161_ITS	•	•	•	•	•	•	•	•	•	•	_	•		T	•	•	•	•	•	•	•
172_ITS	•	۰.	٠	•	•	•	T	•	•	•	-	•		T	•	•	•	•	•	•	•
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157B_ITS	•	A	A	•	•	•	T	•	•	•	-	•	•	T	•	•	٠	•	•	•	•
015_ITS	•	٠	•	٠	•	٠	T	•	•	•	A	•	•	A	•	•	•	٠	٠	•	•
034_ITS	•	•	•		•		T	•	•	•	A	•	•	A	•	•	•	•	•	•	•
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166B_ITS	G	C	A	A	C	A	A	A	C	T	G	G	T	A	A	T	G	A	T	C	C
166A_ITS							•	•	•		•	•	•		•		•	•		•	
008B_Fonly_ITS																					
008C_ITS							G														
008A_ITS							G														
008D_ITS							G														
008E_ITS							G														
161_ITS							G														
172_ITS																					
157B_ITS	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
015_ITS	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
013_ITS 034_ITS	•	•	•	-	-	-	-	•	•	•	•	•	•	•	•	•	•	•	•	•	•
034_113	•	•	•	-	-	-	-	•	•	•	•	•	•	•	•	•	•	•	•	•	•
166D ITC		æ	С	C	G	C	٨	G	C	T	T	C	A	C	C	т	٨	C	C	C	٨
166B_ITS	т				U	C	Α	U	U	1	1	C	Α	C	C	T	A		U	U	A
1.CCA ITTO	T	T	C	C																	
166A_ITS	T					•			•			•	•		•	•	•			•	
008B_Fonly_ITS	T																				
008B_Fonly_ITS 008C_ITS	T								· ·				· ·		· ·		•				
008B_Fonly_ITS 008C_ITS 008A_ITS	T	1		·						·		· · ·			· · ·	· · ·		· · ·			·
008B_Fonly_ITS 008C_ITS 008A_ITS 008D_ITS	T					· · · ·									· · · · ·					·	
008B_Fonly_ITS 008C_ITS 008A_ITS	T					· · · · · ·	·	· · · · ·	·		· · · · ·		· · · · ·		· · · · ·				· · · · ·	· · · · · ·	· · · · · ·

161_ITS 172_ITS		•	•				•	•	•	•	٠		•	•						•	•
157B_ITS	•	•	•		•		•	•	•	•	•		•	•			•	•	•	•	
015_ITS																					
034_ITS																					
166B_ITS	A	A	C	C	T	T	G	T	T	A	C	G	A	C	T	T	T	T	A	C	T
166A_ITS																					
008B_Fonly_ITS																					
008C_ITS																					
008A_ITS																					
008D_ITS																					
008E_ITS												•									
161_ITS																					
172_ITS				•	•	•						•									•
157B_ITS					•							•									•
015_ITS				•	•	•				•		•					•	•	•		•
034_ITS			•	•	•	•			•	•	•	•		•	•	•	٠	٠	•		•
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166B_ITS	T	C	C	T	C	T	A	A	A	T	G	A	T	C	A	A	G	T	T	T	G
166A_ITS	•	•	•	٠	•	٠	•	٠	•	٠	٠	•	٠	•	•	•	٠	٠	•	٠	•
008B_Fonly_ITS	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	٠	•	•	•
008C_ITS 008A_ITS	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
008A_ITS 008D_ITS	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
008E_ITS	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
161_ITS	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
172_ITS	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
157B_ITS	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
015_ITS	•	•	•				•	•	•	•	•	•	•	•	•		•	•		•	
034_ITS																					
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166B_ITS	G	A	C	A	T	C	T	T	T	C	C	G	G	C	G	C	G	T	C	G	A
166A_ITS																					
008B_Fonly_ITS																					
008C_ITS																					
008A_ITS																					
008D_ITS				•	•	•						•									•
008E_ITS					•						•	•			•	•		•	•		•
161_ITS					•							•									•
172_ITS			•	•	•	•	•		•	•	•	•		•	•	•	٠	٠	•		•
157B_ITS	•		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
015_ITS	•		•	•	•	•	•	٠	•	•		•	٠	•	•	•	•	٠	•	•	•
034_ITS		•	•	•	٠	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
166D ITS	C	C	C	C	A	C	C	C	C	٨	C	C	C	C	C	C	C	C	Α	C	C
166B_ITS	G	G	C	G	A	G	C	C	C	A	C	C	G	G	C	C	C	C	A	G	C
166A_ITS 008B_Fonly_ITS	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
008E_F0IIIy_11S 008C_ITS	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
008C_ITS 008A_ITS	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
008A_ITS 008D_ITS	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
000D_113	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•

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008E_ITS	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
161_ITS	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
172_ITS	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
157B_ITS	•	•	•	٠	•	٠	•	•	•	٠	•	•	•	•		•	٠	•	•	٠.	٠
015_ITS	•	•	•	•	•		•	•			•	•	•	•	T	•	•	•	•	A	•
034_ITS	٠	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•	•	•	A	•
166B_ITS	G	C	G	C	A	C	A	A	G	G	C	G	Т	G	A	A	_	G	G	G	G
166A_ITS													_				_				
008B_Fonly_ITS	•	•	•	•	•	•	•	•	•	•	•		•	•	•	•	_	•	•	•	•
008C_ITS	А	•	•	•	•	•	•	•	•	•		А		•		•	_	•	•	•	•
008A_ITS	A		•	•	•	•	•	•	•	•		A				•	N	•	•	•	•
008D_ITS	A	•	•	•	•	•	•	•				A				•	-	•	•	•	•
008E_ITS	A	•	•	•	•	•	•	•	•			A	•			•	_	•	•	•	•
161_ITS	A	•	•	•	•	•	•	•	•	•		A	•	٠	•	•	-	•	•	•	•
172_ITS	A	•	•	•	•	•	•	•	•	•	•	A	•	•	•	•	-	•	•	•	•
	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	-	•	•	•	•
157B_ITS	•	•		•		•			•	•	•	•		•	•	•	-	•	•	•	•
015_ITS	•	٠	A	•	G	•	G	G	•	•	•	•	C	•	٠	•	-	•	•	٠	•
034_ITS	•	•	A	•	G	•	G	G	•	•	•	•	C	•	•	•	-	•	•	•	٠
166B_ITS	T	G	A	C	A	A	G	C	G	G	A	G	С	C	C	A	G	_	T	C	C
166A_ITS																		_			
008B_Fonly_ITS																		_			
008C_ITS																		_			
008A_ITS	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	_	•	•	•
008D_ITS	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	_	•	•	•
008E_ITS	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	T	•	•	•
161_ITS	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	1	•	•	•
172_ITS	•	•	•	•	G	•	•	•	•	•	•	•	•	•	•	•	•	_	•	•	•
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157B_ITS	•	•	C	•	G	•	•	•	•	•	•	•	•	•	•	•	•	-	•	•	•
015_ITS	•	٠	C	٠	G	•	•	•	•	•	•	•	•	•	•	•	•	-	•	•	•
034_ITS	•	•	C	٠	G	•	•	•	•	•	•	•	•	•	•	•	•	-	•	•	•
166B_ITS	A	A	G	C	C	G	C	C	A	G	T	C	C	G	A	A	N	G	C	C	T
166A_ITS																	A				
008B_Fonly_ITS	N	N	N	N			N		N	N	N	N	N	N	N	N			N	N	N
008C_ITS																	G				
008A_ITS																	G				
008D_ITS																	G				
008E_ITS	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•
161_ITS	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•
172_ITS	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	G	•	•	•	•
172_ITS 157B_ITS	•	•	•	•	•	•	•	•	•	N	•	•	•	•	•	•	G	•	•	•	•
015_ITS	•	•	•	•	•	•	•	•	•	11	•	•	•	•	•	•	U	•	•	•	•
	•	•	•	•	•	•	•	•	•	- N	-	-	_	_	-	-	-	_	_	-	-
034_ITS	•	٠	•	•	•	•	•	•	•	N	•	٠	•	•	•	•	-	-	-	-	-
166B_ITS	C	A	C	T	-	A	A	A	T	C	A	T	T	C	A	A	T	C	-	G	G
166A_ITS					-														-		
008B_Fonly_ITS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	-	-
008C_ITS					-														-		
008A_ITS					A														G		

008D_ITS					-											N			-		
008E_ITS				N	-														G		
161_ITS					-														-		
172_ITS					-														-		
157B_ITS					-														-		
015_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
034_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
166B_ITS	T	A	G	T	A	G	C	G	A	N	G	G	G	G	-	-	-	-	-	-	-
166A_ITS						N		N		C					C	G	G	T	G	T	G
008B_Fonly_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
008C_ITS										C				C	G	G	G	-	-	-	-
008A_ITS										C				-	-	-	-	-	-	-	-
008D_ITS										C				C	G	G	T	G	T	G	A
008E_ITS										C	-	-	-	-	-	-	-	-	-	-	-
161_ITS										C					C	G	G	T	-	-	-
172_ITS										C				-	-	-	-	-	-	-	-
157B_ITS										C				C	G	G	T	G	T	G	-
015_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
034_ITS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-