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First photographic and genetic records of the genus *Martella* (Araneae: Salticidae)

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ABSTRACT: Phylogenetic analysis of the 28S gene supports a close relationship between *Martella* Peckham & Peckham 1892, *Sarinda* Peckham & Peckham 1892, and *Zuniga* Peckham & Peckham 1892 within the Amycoida clade. The genus is recorded from Belize for the first time, with photographs of a single male specimen of a possibly undescribed species.

KEY WORDS: *Martella*, jumping spider, Salticidae, Amycoida, phylogeny

Introduction

Martella Peckham & Peckham 1892 is a genus of ant-like spiders in the family Salticidae, native to North and South America, with a range spanning from Mexico to northern Argentina. The genus is poorly known and currently consists of twelve recognized species, six of which are described only from a single sex (World Spider Catalog 2014). It was synonymized with *Sarinda* Peckham & Peckham 1892 by Simon in 1901, restored by Galiano in 1964, and most recently revised by Galiano in 1996.

In this paper, the first photographic and genetic records for the genus are presented, as well as a limited analysis of its relationship to other genera in the Amycoida clade.

Materials and Methods

Sampling and Photography

A single specimen was manually collected in Bullet Tree Falls, Belize. The specimen was photographed and then preserved in 100% ethanol and refrigerated. All photography was done with a Canon EOS 550D SLR camera. Habitus photos were shot with a Canon EF 100mm f/2.8L Macro IS USM lens, Canon Macro Twin Lite MT-24EX flash, and homemade flash diffuser. Pedipalp photos were shot with a Canon MP-E 65mm f/2.8 Macro lens and Canon Macro Twin Lite MT-24EX flash. All pedipalp photos were constructed by focus stacking multiple image in Zerene Stacker for MacOS X.

Sequencing

Genomic DNA was extracted from the legs using the Qiagen DNeasy Blood and Tissue Kit. Two gene regions were amplified by PCR and sequenced: the nuclear 28S ribosomal RNA gene and the mitochondrial cytochrome c oxidase subunit 1 (CO1) gene. The following primers were used:

Gene	Direction	Name and Reference	Sequence
28S	forward	28S "O" (Hedin & Maddison 2001)	5'-GAA ACT GCT CAA AGG TAA ACG G-3'
28S	reverse	28S "C" (Hedin & Maddison 2001)	5'-GGT TCG ATT AGT CTT TCG CC-3'
CO1	forward	LCO1490 (Folmer et al. 1994)	5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3'
CO1	reverse	HCO2198 (Folmer et al. 1994)	5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3'

The CO1 gene region was primarily sequenced to facilitate future species identification, as this region is commonly used as a species "barcode", and has been shown to be effective for identifying spider species (Barrett & Hebert 2005; Robinson *et al*. 2009). It is not very effective for determining higher-level relationships in salticids, however (Hedin & Maddison 2001). The 28S gene region was sequenced to analyze phylogenetic relationships between *Martella* and other genera. This gene has been shown to be useful for determining higher-level relationships in many taxa, including salticids (Hedin & Maddison 2001).

PCR and Sanger sequencing was done by Quintara Biosciences in Richmond, California. Sequences were trimmed by hand to exclude primer sequences and any ambiguous base reads. Most bases were confirmed by high quality reads in both directions. All base reads in the final sequences were above Phred quality score 20. The final sequences were submitted to GenBank and BOLD (accession numbers in Appendix 1).

Phylogenetic analysis

The 28S sequence from the *Martella* specimen was aligned with sequences from several other genera in the Amycoida clade (*Sarinda* Peckham & Peckham 1892, *Zuniga* Peckham & Peckham 1892, *Noegus* Simon 1900, *Hypaeus* Simon 1900, *Mago* O. P.-Cambridge 1882, *Scopocira* Simon 1900, and *Hurius* Simon 1901) and then statistically analyzed to determine the most likely phylogenetic relationships. The GenBank sequences used for comparison are given below:

Taxon	Locality	Accession #	Length	Authors
Sarinda sp. MCH-2003	Ecuador: Sucumbíos	AY297244.1	750 bp	Maddison & Hedin
Sarinda cutleri (Richman)	USA: Arizona, Prescott	JX145744.1	1063 bp	Bodner & Maddison
Zuniga aff. magna Peckham & Peckham	Ecuador: Manabi	AY297247.1	748 bp	Maddison & Hedin
Scopocira aff. tenella Simon	Ecuador: Sucumbíos	AY297245.1	742 bp	Maddison & Hedin
Hurius vulpinus Simon	Ecuador: Pichincha	AY297239.1	743 bp	Maddison & Hedin
Noegus aff. rufus Simon	Ecuador: Sucumbíos	AY297243.1	752 bp	Maddison & Hedin
Hypaeus mystacalis (Taczanowski)	Ecuador: Manabi	AY297240.1	745 bp	Maddison & Hedin
Mago steindachneri (Taczanowski)	Ecuador: Sucumbíos	AY297242.1	747 bp	Maddison & Hedin

Alignment and analysis were conducted in MEGA for MacOS X (release #6140220). Alignment was done by Clustal method. Multiple alignments were executed with gap opening/gap extension costs set to 24/6 following Maddison & Hedin (2003). The alignments were visually inspected and no manual changes were made. Phylogenetic trees were generated using maximum likelihood and maximum parsimony analysis. Both were configured with full gap deletion and 1000 bootstrap replications.

Specimen notes and photographs

An adult male *Martella* was collected on foliage near the Mopan River in Bullet Tree Falls, Belize (17.172°N, 89.112°W). It was collected on April 13, 2014, around midday. This is the first record of the genus from Belize.

The specimen was keyed to *Martella* by the following characters: ant-like appearance, lack of abdomen constriction, and a proximal retrolateral apophysis on the cymbium (Figures 1 and 2). The specimen may belong to an undescribed species close to *Martella pottsi* Peckham & Peckham 1892, the type species for the genus. The pedipalp is similar to *M. pottsi*, but features a long, pointed retrolateral tibial apophysis (Figure 2). The specimen could also belong to one of the four *Martella* species described only from females. In particular, *M. lineatipes* was first collected in Teapa, Mexico, which is at roughly the same latitude. The species has been given the provisional designation *Martella sp. RK-2014* pending identification of a conspecific female.

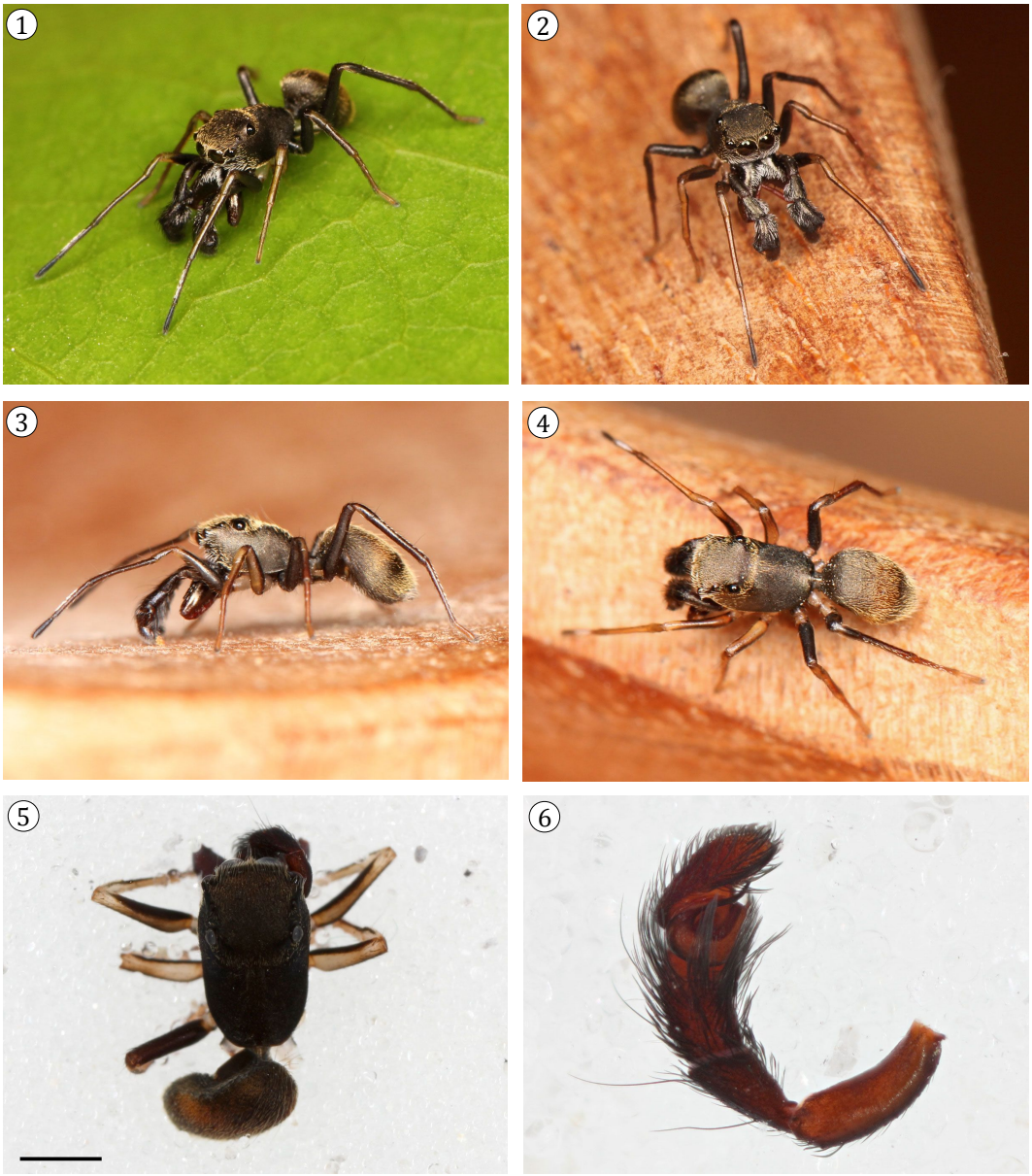

Figure 1. Male *Martella* from Bullet Tree Falls, Belize. **1,** Oblique view. **2,** Anterior view. **3,** Lateral view. **4,** Dorsal view. **5,** Dorsal view under alcohol (scale = 1 mm). **6,** Left pedipalp under alcohol.

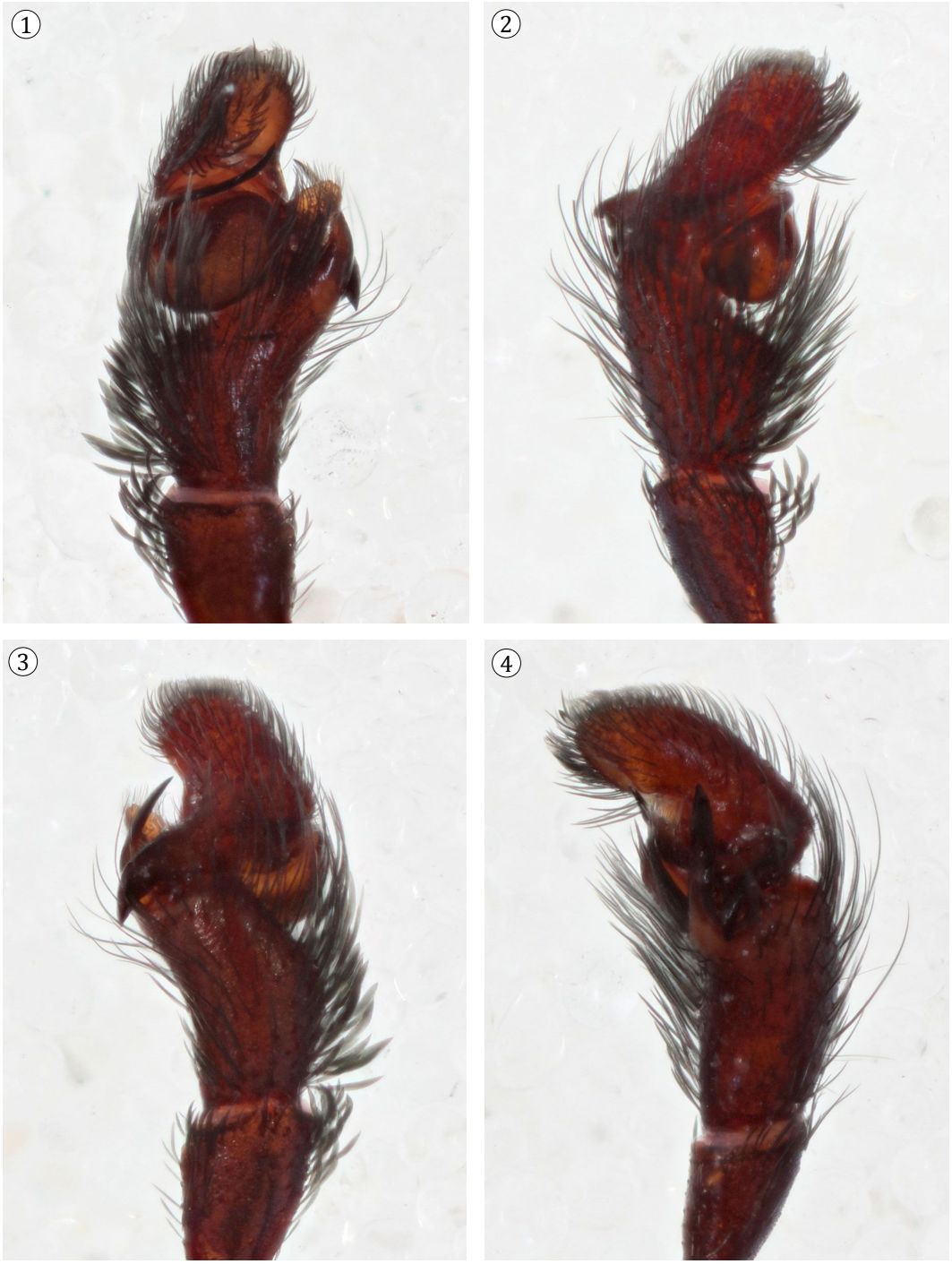

Figure 2. Palpal organ and tibia (left pedipalp). **1,** Ventral view. **2,** Prolateral view. **3,** Dorsal view. **4,** Retrolateral view.

Results of molecular analysis

CO1

BLAST searches on GenBank and BOLD failed to identify any CO1 sequence matches within 10% divergence. This was not surprising since no prior sequences in either database had been identified as *Martella*. Conspecific spider CO1 sequences are typically less than 4% divergent (Barrett & Hebert 2005).

The CO1 sequence was added to the BOLD database, and because it did not cluster with any existing sequences, it was assigned a new Barcode Index Number (BIN): ACM4146. BINs are a unique feature of the BOLD database in which barcode sequences are clustered algorithmically. These clusters show high concordance with species, and thus can be used to verify species identification, or in this case, to document undescribed species. The Nearest Neighbor to this new BIN at the time of publication was AAX9354, consisting of a single sequence from *Sarinda sp. MCH-2003* (GenBank accession no. AY297373.1). The distance between the two BINs was 12.02%.

28S

A BLAST search against the 28S sequence on GenBank also failed to yield any close matches. The closest match was from *Sarinda sp. MCH-2003* (accession no. AY297244.1), the same species as the BIN Nearest Neighbor. This sequence was 7% divergent from the *Martella* sequence.

Results of phylogenetic analysis are presented in Figures 3 and 4. The Maximum Likelihood Tree (Figure 3) shows moderate support for a clade composed of *Sarinda*, *Martella*, and *Zuniga* (71% bootstrap score). Maximum parsimony analysis produced two equally parsimonious trees. Both also support a clade composed of those three genera, but with one showing *Martella* as the basal genus and the other showing *Zuniga*. A consensus of the two most parsimonious trees is presented in Figure 4. Both the Maximum Likelihood Tree and the Maximum Parsimony Consensus Tree are in agreement with the All-Genes Bayesian Tree for Amycoida proposed by Maddison, Bodner, & Needham (2008).

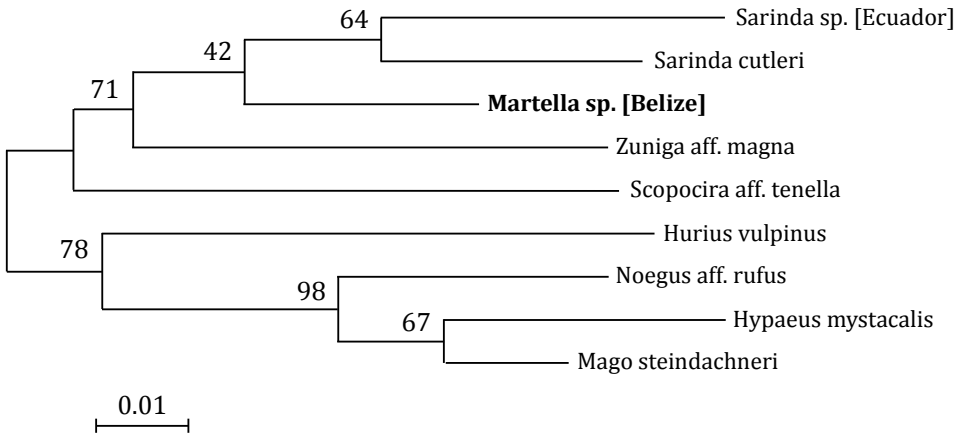

Figure 3. Phylogeny from 28S: Maximum Likelihood Tree, bootstrap values shown (1000 replications).

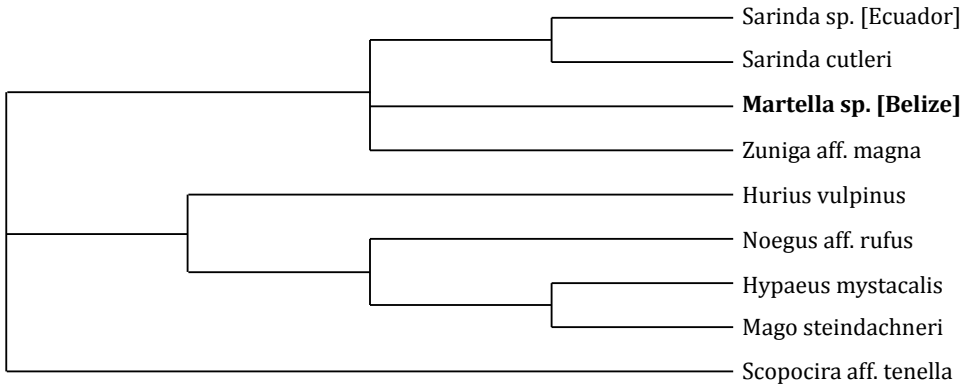

Figure 4. Phylogeny from 28S: Maximum Parsimony Consensus Tree.

Discussion

The results of the phylogenetic analysis support a close relationship between the genera *Sarinda*, *Zuniga*, and *Martella*. These results must be presented with two caveats, however. First, only one gene was used for the phylogenetic analysis. As different genes are subject to different evolutionary forces, it is hazardous to make any conclusions about evolutionary relationships without examining multiple genes. It should also be noted that none of the sequences used in the phylogenetic analysis were from the type species of their respective genera, with the exception of *Hurius vulpinus*. With that said, the results appear to be reasonable as *Sarinda*, *Zuniga*, and *Martella* have often exchanged species, and all three exhibit similar morphology. Further studies of these genera are necessary in order to establish their proper delineation and phylogeny. Some may turn out to be polyphyletic or warrant synonymy, but it is difficult to make any conclusions given the current lack of data.

Acknowledgements

This article is the first result of my foray into genetic sequencing. I am pursuing this work purely as an amateur with no formal training or institutional backing. The fact that this is possible is a testament both to the rapid advance of technology and to the valuable guidance I've received along the way. I would like to extend my thanks to Ken-ichi Ueda and Damon Tighe for helping me figure out the ins and outs of genetic sequencing, especially how to get around the hurdles that typically prevent amateurs from undertaking such an endeavor. I would like to thank Bernhard Suter and Christine Carle at Quintara Biosciences for doing all the real work and answering my numerous questions. I would also like to thank Wayne Maddison, Marshal Hedin, and Melissa Bodner for contributing the other sequences used in my phylogenetic analysis. Finally, I would like to thank David Hill, G. B. Edwards, and all the members of the Peckham Society for creating a welcoming environment for amateur naturalists to collaborate with experts and professionals.

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Appendix 1. Gene sequences

Cytochrome c oxidase subunit 1 (CO1) gene

```
GACTTTATAT TTGATTTTTG GAGCTTGATC TGCTATGGTG GGTACAGCGA TGAGTGTTTT
GATTCGAATA GAATTAGGTC AAATTGGTAG TTTATTAGGA AGAGATCATT TATACAATGT
TATTGTTACA GCTCATGCTT TTGTTATGAT TTTTTTTATA GTTATACCAA TTTTAATTGG
CGGATTTGGA AATTGATTGG TACCTTTAAT GTTGGGAGCG CCTGATATGG CTTTCCCTCG
AATAAATAAT TTAAGATTTT GATTACTACC TCCTTCTTTG TTTTTATTAT TTATTTCTTC
TATATCTGAG ATAGGAGTTG GAGCTGGATG AACTGTATAT CCACCTTTAG CTTCTACTGT
AGGGCACAGA GGTAGATCAG TTGATTTTGC TATTTTTTCT TTGCATTTGG CTGGGGCTTC
TTCTATTATA GGAGCTATTA ATTTTATTTC TACAGTAATT AACATACGCT CTATTGGGAT
ATCTATAGAT AAAATTCCTT TGTTTGTGTG ATCGGTTGTG ATTACTGCTG TGCTTTTATT
ATTATCATTG CCTGTATTAG CAGGTGCTAT TACTATATTG CTAACTGATC GAAATTTTAA
TACATCTTTT TTTGATCCTG CTGGTGGAGG TGATCCTATT TTGTTTCAAC ATTTATTT
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BOLD Sequence: SDNA005-14
BOLD BIN: ACM4146
GenBank Accession: KM612269

28S ribosomal RNA gene

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GTGGGCCCTC GAAATCCTGT GGCGAGAGGA TTCAGTCTGG TGCGGCGGAC TCGGAGCCGG
AAGAGTCGGC AGGGCTTCCC GAGACGGGGC GCCGTCCGGA ACCGAGGCCT CCGACGTACC
AGACGCATTT GTCTCTCGTC CGAAGGACGC TGCAGCCGGT CGGGCAGTGC AAGCGCGTCG
GCCTGAAGGC GGGGAGCCGG CAGGTGGCCG GTGGCGCGCC TCGTGCGCGT CGCCGGTTGT
TAGCCTTCTC CGCAGTGGCT CGACGCCCGA CCGTGGTGTC GCGAGGCCCT CCAGGGCCTC
GACGTCTCCT CCCTGCGTCG CGGGACGGAC GGTCGCAGGC GAACTCTGCT CCTTCGTCGC
ACTCCCTCGG AGTGGACGAG AAAGCAGAGG GCGCCGCTGG TGGCCGCGGA CCCGCGGGGG
ACCGGAGGCT CGCAGCGAGT AGGTCGGTCA CCCACCCGAC CCGTCTTGAA ACACGGACCA
AGGAGTCTAA CATGTGCGCG AGTCAATGGG TCTTGAACAG GCCCAGGGGC GCAATGAAAG
CGAAGGTCGG CCTCGCGTCG ACCGAGTCGG GATCTCCCCC CCAGGGGGGG CGCACCGACG
ACCCGTCCTA TTCGGCATGC CGTTTGGGCG GAGTTTGAGC GTACACGTTG GGACCCGAAA
GATGGTGAAC TATGCCCGGA CAGGACGAGG CCAGAGGAAA CTCTGGTGGA GGTCCGCAGC
GGTTCTGACG TGCAAATCGA TCGTCAGATC CGGGTCTAGG
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GenBank Accession: KM594522