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A survey of spiders (Arachnida: Araneae) of Prince of Wales Island, Alaska; combining morphological and DNA barcode identification techniques

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A survey of spiders (Arachnida: Araneae) of Prince of Wales Island, Alaska; combining morphological and DNA barcode identification techniques

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Abstract. Surveys during the summer of 2004 and August 2009 on Prince of Wales Island, Alaska, USA resulted in collection of 1064 adult spiders representing 84 species. Barcoding of spiders collected in 2009 resulted in DNA barcode data for 212 specimens representing 63 species. DNA barcode data were then used to facilitate the identification of otherwise unidentifiable juvenile and female specimens as well as to investigate phylogenetically four lineages with large branch lengths between specimens. Using morphological and DNA barcode identifications provided a more complete list of identified specimens than was possible using morphological data alone.

Introduction

Often overlooked and under-appreciated, baseline inventories provide valuable data as to where species occur, species habitat limitations, population numbers and occasionally newly discovered species (Slowik and Sikes 2011). Prince of Wales Island (POW), Alaska, is the third largest island in the United States and encompasses more than 5,800 km^2. The climate of the island is a temperate rainforest which may see as much as 300 cm of rain a year (Geophysical Institute, University of Alaska Fairbanks). Dominant trees in the forests include western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) and Sitka spruce (*Picea sitchensis* (Bong.) Carr.). The forests of Southeast Alaska have seen decades of clear cut logging, of which POW is no exception, and it is unclear what kind of impact this type of forest management has had on the environment (Mackovjak 2010). This area is also an amazing natural laboratory as islands often contain interesting island-specific species assemblages resulting from the geography, topology, and history particular to the island (Parmesan 2006).

Spiders make for an interesting survey animal, as they are significant predators of invertebrates, as well as a food source for many bird and mammal species (Foelix 1996). Being obligate predators they may be representative of a particular habitat not only because of the physical limitations of that habitat, but also due to the available prey (Foelix 1996, Wise 1993). Moreover, they provide a fairly easy animal to collect and many references are available for morphological identification. The use of DNA barcoding techniques has been established for spiders (Barrett and Hebert 2005, Robinson et al. 2009) allowing for the use of those techniques to provide identification data for specimens not identifiable morphologically, such as juveniles and females of some spider families. Spiders as a whole may constitute as many as a hundred thousand species, of which only around 42,000 have been described (Platnick 2012, Ubick et al. 2005). However, previous studies in Southeast Alaska put the expected number of species to be close to 100 (Slowik 2006) which make them taxonomically manageable.

Here we report on the spider species found during two survey periods on Prince of Wales Island, Alaska in 2004 and 2009, and provide DNA barcode data from the 2009 survey. We also demonstrate how DNA barcode data can be used to aid in the identification of specimens and result in a more complete survey.

Methods

Spiders were collected from 9-21 August 2009 into 100% EtOH and kept at -20ºC in an effort to preserve the DNA. Spiders were collected using a variety of methods and included sweep nets, beat sheets, hand collection both during the day and at night with a headlamp, pitfall traps, and moss sifting

(Fig 1). Habitats were categorized as in Slowik (2006) with the addition of closed canopy second growth stands as habitat 23. Spiders were sorted and morphologically identified at the University of Alaska Museum, Fairbanks, Alaska. Taxonomic names follow Platnick (2012). Additional specimens used to compile a species list but not used for DNA barcoding came from a general study from 10 April to 30 September 2004 by J. Slowik, from the personal collection of J. Slowik, and from the University of Alaska Insect Collection.

A representative set of 285 specimens representing 67 species was sent to the Canadian Centre for DNA Barcoding, University of Guelph, Guelph, Ontario for molecular barcoding. Specimens for barcoding had a single leg removed for DNA extraction and were photographed. DNA extraction and cytochrome oxidase I (COI) sequence generation followed Robinson et al. (2009) and produced an aligned fragment of 669 bases, 658 bp unaligned. Sequences generated were uploaded to the BOLD database (Ratnasingham and Hebert 2007) under the Spiders of Alaska project. All survey specimens are deposited as vouchers at the University of Alaska Insect Collection. Sequences have been uploaded to GenBank under the accession numbers HQ580516-HQ580724.

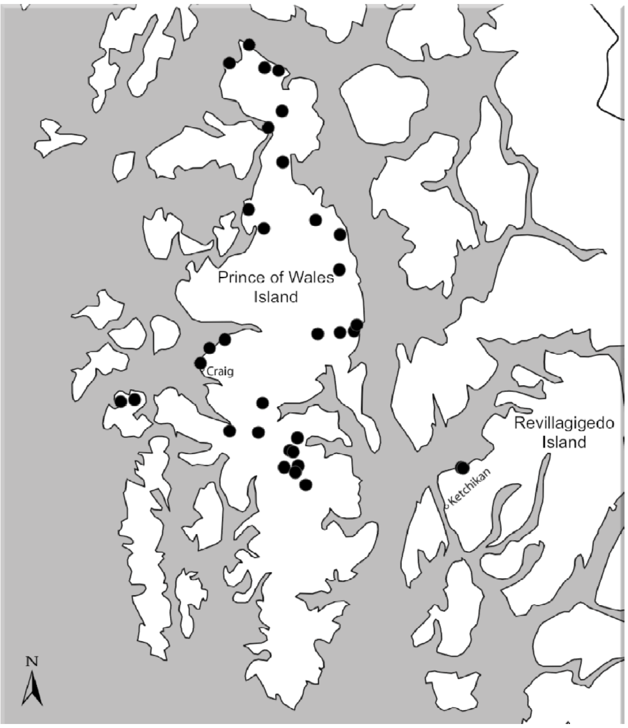

Figure 1. Map of collection locations on Prince of Wales Island (55.779° N, 132.817° W), Alaska.

DNA barcode identification was conducted by examining a Neighbor Joining (NJ) tree using Kimura 2-Parameter (K2P) distances (Kimura 1980, Barret and Hebert 2005, Robinson et al. 2009) generated by the BOLD website. Tree results were analyzed for incorrect species in clades of other species, and also for specimens showing long branches from other members of similarly identified species. Specimens showing incorrect tree placement or extended distances were re-examined and identifications were corrected where appropriate. Specimens in which the morphological identification was correct, but still showed incorrect tree placement or extended branches, were marked as a clade of interest for further Bayesian analyses with additional publicly available sequences to verify clade identification and branch length significance (Cognato 2006). Because of the low species diversity of comparison sequences in GenBank, comparison species were found by BLAST searching the sequence in GenBank (www.ncbi.nlm.nih.gov/genbank/) and BOLD, and incorporating the closest matches.

Bayesian analysis consisted of examining the aligned results from the barcode analysis using Modeltest 3.7 (Posada and Crandall 1998) implemented in PAUP* v 4.0b10 (Swofford 2002) to determine the correct phylogenetic model for each codon position. These results selected the GTR+I+G model for each codon position which was run using a three partitioned analysis in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). Analyses were run for each clade of interest for 2,000,000 generations, using four chains (one heated), and sampled every 1000 trees. Stationarity was determined based on ESS values using Tracer V1.4 (http://evolve.zoo.ox.ac.uk/software.htm), all runs reached stationarity. A burn in period of 50% of the produced trees was discarded to be conservative. A consensus tree was created for all branches showing posterior probabilities over 0.90 or 0.70 for *Parazygiella dispar* (Kulczyn'ski). These phylogenetic results were then compared to the results of the previous K2P distance identification analysis. Specimen names used in figures 2-5 include the BOLD sequence record (SPIAL163-10) followed by the GenBank sequence record (HQ580637) followed by the BOLD specimen number (ALASKA-02-F08) and then by the species name and gene used (*Hyptiotes_gertschi*_COI_5P). Figure 2 lists only the species name and BOLD specimen number and is representative of how results are shown from BOLD tree generation analyses.

Results

The 2009 DNA barcode survey combined with the 2004 general survey resulted in 1064 adult specimens representing 18 families, 70 genera, and 85 species out of 19 families and 95 species known to occur on the island (Appendix 1). Of these, we attempted DNA barcoding on 285 specimens representing 67 species. This effort resulted in COI data for 212 specimens representing 63 species, a 74% sequencing success rate. The total number of species collected on POW is comparable to another study that found 95 species on Chichagof Island, Alaska (Slowik 2006). However, that study indicated undersampling and that new species records will continue to be found.

Using the BOLD interface the Neighbor Joining tree indicated 21 specimens, located in ten clades, in which an error was made in the identification or data recording of specimens (Ratnasingham and Hebert 2007) (Table 1). Thirteen specimens, located on five clades, were examples of tentative identifications of females in which BOLD recommended the correct species (Fig. 2A, 2B, 2C, 2D, 2G). Two specimens, on two clades, were of examples in which juveniles were included with adults incorrectly (Fig. 2C and 2E; Table 1). Three specimens were of incorrectly labeled specimens in which labels were not updated prior to databasing - *Kaestneria anceps* (Kulzcyn'ski) for *Linyphantes orcinus* (Emerton) (Fig. 2F; Table 1). Additionally identification of three female specimens of *Erigone aletris* Crosby and Bishop was confirmed by BOLD data (Table 1).

An additional five clades contained a branch of one or more specimens of the same species noticeably longer than the branches connecting the other specimens, from here on referred to as a long branch, indicative of the possibility for a cryptic species or lineage (Fig. 2G, 2H, 2I, 2J, 2K; Table 1). The five clades were further analyzed using phylogenetic methods and a larger dataset of similar species (Table 2).

The *Hyptiotes gertschi* Chamberlin and Ivie (Fig. 2I) clade had a long distance analysis branch indicating the possibility of a cryptic species. Phylogenetic analysis with additional specimens strongly supports two separate clades (Fig. 3). However, the variability of the phylogenetic branch lengths between specimens within the two clades indicates an isolated gene line rather than a cryptic species.

A single *Parazygiella dispar* (Kulczynski) specimen was found to have a long divergent branch in the distance analysis (Fig 2J). Neighbor Joining analysis including additional specimens of *P. dispar* from Europe show a large branch length between continents, with the single divergent Alaska specimens somewhere in-between. Phylogenetic analysis strongly supports the separation of the one Alaska species (SPIAL275-10) from other conspecifics but shows only a 0.70 posterior probability for the separation of the continents (Fig. 4).

A single specimen each of *Bathyphantes orica* Ivie and *B. canadensis* (Emerton) (Fig. 2K) from POW show intermediate genetic placement between the two species (Fig. 5). Each specimen, SPIAL013-10 *B. orica* and SPIAL224-10 *B. canadensis*, morphologically identify as the listed species. The long distance analysis branch and high posterior probability in the Bayesian analysis identify the two specimens as belonging to a clade separate from both *B. orica* collected on POW, and *B. canadensis* collected from the mainland. Further analysis is needed to clarify what this clade represents.

Phylogenetic analysis of *Tachygyna ursina* (Bishop and Crosby) specimens (Fig. 6) showed strong support for the two clades identified in the distance analysis (Fig. 2G). However, interpretation of the phylogenetic branch lengths is unclear, the two clades may represent population structure or the presence of a second species, likely *Tachygyna vancouverana* (Chamberlin and Ivie). Although males of the two species are morphologically distinct, females lack definitive characters. At this time no male *T. vancouverana* has been found on POW, also no male *T. vancouverana* sequences are available to confirm the presence of the species on the island.

A long branch in the distance analysis of *Grammonota subarctica* Dondale specimens (Fig. 2H) turned out to be the result of one incorrectly identified specimen (*G. subarctica*, ALASKA-03-E08). Upon reexamination, all three making up the clade sister to *G. subarctica* were found to be female *Symmigma minimum* (Emerton).

Discussion

This paper highlights the utility of an enhanced survey technique in which specimens are initially identified using classical morphological characters followed by confirmation/correction through molecu-

Table 1. Examples of identification mistakes, broken into four categories, the correct identification, and corresponding figures, of specimens identified in the Neighbor Joining tree produced using the BOLD interface.

Clades in which species ID was tentative because of female Erigonine or Dictynidae:	Original ID	Correct ID	Figure
	Walckenaeria|ALASKA-03-D10	*Walckenaeria directa*	1A
	Walckenaeria spiralis|ALASKA-02-B04	*Pocadicnemis pumila*	1B
	Erigoninae|ALASKA-02-B11	*Pocadicnemis pumila*	1B
	Erigoninae|ALASKA-02-G11	*Meioneta simplex*	1C
	Erigoninae|ALASKA-02-F11	*Meioneta simplex*	1C
	Erigone|ALASKA-02-H11	*Tachygyna ursina*	1G
	Dictyna|ALASKA-01-H07	*Dictyna major*	1D
	Dictynidae|ALASKA-01-C07	*Dictyna major*	1D
	Emblyna peragrata|ALASKA-01-B07	*Dictyna major*	1D
	Emblyna peragrata|ALASKA-01-H06	*Dictyna major*	1D
	Emblyna peragrata|ALASKA-01-A07	*Dictyna major*	1D
	Sisicottus|ALASKA-02-C09	*Sisicottus nesides*	1C
	Sisicottus|ALASKA-02-D09	*Sisicottus nesides*	1C
	Erigone|ALASKA-02-D02	*Erigone aletris*	Not shown
	Erigone|ALASKA-02-E02	*Erigone aletris*	Not shown
	Erigone|ALASKA-02-F02	*Erigone aletris*	Not shown
Clade in which juveniles were incorrectly grouped with adults:	**Original ID**	**Correct ID**	**Figure**
	Bathyphantes orica|ALASKA-01-E01	*Sisicottus nesides*	1C
	Tetragnatha versicolor|ALASKA-01-H03	*Metellina curtisi*	1E
Clade with incorrectly labeled specimens:	**Original ID**	**Correct ID**	**Figure**
	Kaestneria anceps|ALASKA-03-D04	*Linyphantes orcinus*	1F
	Kaestneria anceps|ALASKA-03-C04	*Linyphantes orcinus*	1F
	Kaestneria anceps|ALASKA-03-B04	*Linyphantes orcinus*	1F
Clades with long branches	**Original ID**	**Correct ID**	**Figure**
	Hyptoites gertschi	*Hyptoites gertschi*	1I
	Parazygellia dispar	*Parazygellia dispar*	1J
	Bathyphantes orica	*Bathyphantes orica*	1K
	Tachygyna ursina	*Tachygyna ursina*	1G
	Erigoninae|ALASKA-02-A11	*Symmigma minimum*	1H
	Erigoninae|ALASKA-02-G02	*Symmigma minimum*	1H
	Grammonota subarctica|ALASKA-03-E08	*Symmigma minimum*	1H

lar analysis. Molecular analysis has the ability to identify or correct morphological identifications of specimens which otherwise could not be confidently identified. It also provides interesting preliminary data on molecular variation and gene histories of species or the possible existence of cryptic species. Admittedly, the 74% success rate was low, normal rates range around 90% (G. Blagoev pers. obs.). Of the 73 specimens which didn't sequence 47 were small Linyphids, in which a single leg may not have provided enough material for good amplification. Also some collections took place in remote areas in which specimens were placed on ice but could not be placed into a -20ºC freezer for several days after collection. It is likely these two factors played a role in the lower than normal success rate.

It should be noted that the short section of mitochondrial DNA used for the barcode analysis may not be representative of a species phylogenetic history and should not be inferred as such. Furthermore, the phenetic Neighbor Joining analysis done comparing K2P distances is not a phylogenetic analysis, and there is no evidence that a particular universal phenetic distance equates to species boundaries (Fitzhugh 2005, Cognato 2006). However, the short length of the DNA segment used for DNA barcoding sequences reliably provides enough information for species level identification in most spider groups (Barrett and Hebert 2005, Robinson et al. 2009).

The occurrence of long branches in several species collected during this survey may support deeply divergent lineages and the hypothesized existence of one or more refugia along the Pacific coast of North America during Pleistocene glaciation (Demboski et al. 1999; Byun et al. 1999). Additionally the long branch lengths found among specimens of *P. dispar* from Europe and North America may indicate long separation of the two populations. However, the single Alaskan specimen of *P. dispar* (ALASKA-03-H01) may simply represent a distinct lineage or a nuclear copy of the mtDNA. Obviously, a much larger sample of specimens and the addition of a larger section of mtDNA and some nuDNA are needed before a valid conclusion can be made about the phylogenetic history of the species with long branches.

From a practical aspect the additional effort required for this type of an enhanced survey is relatively minor. The additional effort required ensures that specimens are collected with concern for future mo-

Table 2. Additional sequences used for phylogenetic analysis. Table lists species of the sequence, the database in which the sequence was obtained from, the database identifier for the sequence, and the identification string used in Figures 2-5.

Species	Database	Database ID	Taxon ID
Hyptiotes gertschi	BOLD	BBCAN161	BBCAN161_09_HM435691_CCDB_04286_F06_*Hyptiotes_gertschi*_COI_5P
Hyptiotes gertschi	GenBank	FJ607566	gi_223403363_gb_FJ607566_1__*Hyptiotes_gertschi*
Waitkera waitakerensis	GenBank	FJ607587	gi_223403405_gb_FJ607587_1__*Waitkera_waitakerensis*
Uloborus diversus	GenBank	FJ525329	gi_220684135_gb_FJ525329_1__*Uloborus_diversus*
Uloborus glomosus	GenBank	EU003310	gi_157673974_gb_EU003310_1__*Uloborus_glomosus*
Parazygiella dispar	BOLD	TURAR1685	TURAR1685_11_BIOUG00608_C09_*Parazygiella_dispar*_COI_5P
Parazygiella dispar	BOLD	SPRMA201	SPRMA201_10_HQ580815_10_SKBC_0201_*Parazygiella_dispar*_COI_5P
Parazygiella dispar	BOLD	SPIRU644	SPIRU644_11_JF886866_BIOUG00605_H09_*Parazygiella_dispar*_COI_5P
Parazygiella dispar	BOLD	SPIRU645	SPIRU645_11_JF886867_BIOUG00605_H10_*Parazygiella_dispar*_COI_5P
Parazygiella dispar	BOLD	SPIRU646	SPIRU646_11_JF886868_BIOUG00605_H11_*Parazygiella_dispar*_COI_5P
Parazygiella dispar	BOLD	SPIRU617	SPIRU617_11_JF886843_BIOUG00605_F06_*Parazygiella_dispar*_COI_5P
Parazygiella dispar	BOLD	SPIRU848	SPIRU848_11_JF886968_BIOUG00608_C02_*Parazygiella_dispar*_COI_5P
Parazygiella dispar	BOLD	SPIRU853	SPIRU853_11_JF886973_BIOUG00608_C07_*Parazygiella_dispar*_COI_5P
Zygiella nearctica	BOLD	SPICH1126	SPICH1126_09_GU684584_09PROBE_905_*Zygiella_nearctica*_COI_5P
Zygiella nearctica	BOLD	SPICH1130	SPICH1130_09_GU684585_09PROBE_909_*Zygiella_nearctica*_COI_5P
Zygiella nearctica	BOLD	SPICH1133	SPICH1133_09_GU684586_09PROBE_912_*Zygiella_nearctica*_COI_5P
Zygiella nearctica	BOLD	SPICH123	SPICH123_09_GU684001_09PROBE_1687_01_*Zygiella_nearctica*_COI_5P
Zygiella nearctica	BOLD	SPICH146	SPICH146_09_GU683823_09PROBE_1700_01_*Zygiella_nearctica*_COI_5P
Zygiella nearctica	BOLD	SPISH046	SPISH046_09_GU684739_09PROBE_764_*Zygiella_nearctica*_COI_5P
Bathyphantes canadensis	BOLD	BBCAN388	BBCAN388_09_GU683434_CCDB_04552_A08_*Bathyphantes_canadensis*_COI_5P_
Bathyphantes canadensis	BOLD	SPICH292	SPICH292_09_GU684338_09PROBE_1978_49_*Bathyphantes_canadensis*_COI_5P_
Bathyphantes canadensis	BOLD	SPICH296	SPICH296_09_GU684339_09PROBE_1978_53_*Bathyphantes_canadensis*_COI_5P_
Bathyphantes brevis	BOLD	SPICH501	SPICH501_09_GU684037_09PROBE_1978_258_*Bathyphantes_brevis*_COI_5P_
Bathyphantes brevis	BOLD	SPICH533	SPICH533_09_GU684123_09PROBE_1978_290_*Bathyphantes_brevis*_COI_5P_
Bathyphantes brevis	BOLD	SPICH546	SPICH546_09_GU684120_09PROBE_1978_303_*Bathyphantes_brevis*_COI_5P_
Bathyphantes brevipes	BOLD	SPICH796	SPICH796_09_GU684389_09PROBE_1978_553_*Bathyphantes_brevipes*_COI_5P_
Bathyphantes brevipes	BOLD	SPICH797	SPICH797_09_GU684390_09PROBE_1978_554_*Bathyphantes_brevipes*_COI_5P_
Bathyphantes brevipes	BOLD	SPICH803	SPICH803_09_GU684352_09PROBE_1978_560_*Bathyphantes_brevipes*_COI_5P_
Bathyphantes gracilis	GenBank	FJ899797	gi_255688106_gb_FJ899797_1__*Bathyphantes_gracilis*_
Bathyphantes gracilis	GenBank	FJ899798	gi_255688108_gb_FJ899798_1__*Bathyphantes_gracilis*_
Bathyphantes gracilis	GenBank	FJ838650	gi_262068394_gb_FJ838650_1__*Bathyphantes_gracilis*_
Bathyphantes pallidus	BOLD	BBCAN109	BBCAN109_09_GU683269_CCDB_04286_B02_*Bathyphantes_pallidus*_COI_5P_
Bathyphantes pallidus	BOLD	BBCAN189	BBCAN189_09_GU683266_CCDB_04286_H10_*Bathyphantes_pallidus*_COI_5P_
Bathyphantes pallidus	BOLD	BBCAN738	BBCAN738_09_GU683478_CCDB_05296_G12_*Bathyphantes_pallidus*_COI_5P_
Bathyphantes pallidus	BOLD	BBCAN753	BBCAN753_09_GU683014_CCDB_05302_A04_*Bathyphantes_pallidus*_COI_5P_
Hybauchenidium gibbosum	BOLD	SPISH045	SPISH045_09_GU684473_09PROBE_763_*Hybauchenidium_gibbosum*_COI_5P
Hybauchenidium gibbosum	BOLD	SPISH042	SPISH042_09_GU683983_09PROBE_1977_01_*Hybauchenidium_gibbosum*_COI_5P
Hybauchenidium gibbosum	BOLD	SPISH041	SPISH041_09_GU683981_09PROBE_1976_01_*Hybauchenidium_gibbosum*_COI_5P
Hybauchenidium gibbosum	BOLD	SPISH040	SPISH040_09_GU683984_09PROBE_1975_01_*Hybauchenidium_gibbosum*_COI_5P
Hybauchenidium gibbosum	BOLD	SPISH039	SPISH039_09_GU683982_09PROBE_1974_01_*Hybauchenidium_gibbosum*_COI_5P
Grammonota angusta	BOLD	BBCAN699	BBCAN699_09_GU683498_CCDB_05296_D09_*Grammonota_angusta*_COI_5P
Grammonota angusta	BOLD	BBCAN592	BBCAN592_09_GU683538_CCDB_05269_C09_*Grammonota_angusta*_COI_5P
Grammonota angusta	BOLD	BBCAN736	BBCAN736_09_GU683496_CCDB_05296_G10_*Grammonota_angusta*_COI_5P
Grammonota angusta	BOLD	BBCAN319	BBCAN319_09_GU683084_CCDB_04551_C10_*Grammonota_angusta*_COI_5P
Grammonota angusta	BOLD	BBCAN692	BBCAN692_09_GU683495_CCDB_05296_D02_*Grammonota_angusta*_COI_5P
Grammonota angusta	BOLD	BBCAN812	BBCAN812_09_GU682966_CCDB_05302_F03_*Grammonota_angusta*_COI_5P
Grammonota angusta	BOLD	BBCAN596	BBCAN596_09_GU683587_CCDB_05269_D01_*Grammonota_angusta*_COI_5P
Grammonota angusta	BOLD	BBCAN371	BBCAN371_09_GU683085_CCDB_04551_H02_*Grammonota_angusta*_COI_5P
Grammonota maritima	BOLD	SPICH554	SPICH554_09_GU684180_09PROBE_1978_311_*Grammonota_maritima*_COI_5P
Grammonota maritima	BOLD	SPICH687	SPICH687_09_GU684554_09PROBE_1978_444_*Grammonota_maritima*_COI_5P
Grammonota maritima	BOLD	SPICH692	SPICH692_09_GU684553_09PROBE_1978_449_*Grammonota_maritima*_COI_5P
Grammonota maritima	BOLD	SPICH695	SPICH695_09_GU684552_09PROBE_1978_452_*Grammonota_maritima*_COI_5P
Grammonota maritima	BOLD	SPICH698	SPICH698_09_GU684555_09PROBE_1978_455_*Grammonota_maritima*_COI_5P
Grammonota maritima	BOLD	SPICH427	SPICH427_09_GU684205_09PROBE_1978_184_*Grammonota_maritima*_COI_5P
Grammonota maritima	BOLD	SPICH081	SPICH081_09_GU683860_09PROBE_1942_01_*Grammonota_maritima*_COI_5P
Grammonota gentilis	BOLD	SPICH679	SPICH679_09_GU684571_09PROBE_1978_436_*Grammonota_gentilis*_COI_5P
Grammonota gentilis	BOLD	SPICH691	SPICH691_09_GU684557_09PROBE_1978_448_*Grammonota_gentilis*_COI_5P
Grammonota gentilis	BOLD	SPICH671	SPICH671_09_GU684569_09PROBE_1978_428_*Grammonota_gentilis*_COI_5P
Grammonota gentilis	BOLD	SPICH663	SPICH663_09_GU684567_09PROBE_1978_420_*Grammonota_gentilis*_COI_5P
Grammonota gentilis	BOLD	SPICH668	SPICH668_09_GU684568_09PROBE_1978_425_*Grammonota_gentilis*_COI_5P
Grammonota gentilis	BOLD	SPICH678	SPICH678_09_GU684570_09PROBE_1978_435_*Grammonota_gentilis*_COI_5P
Grammonota gentilis	BOLD	SPICH681	SPICH681_09_GU684573_09PROBE_1978_438_*Grammonota_gentilis*_COI_5P
Grammonota gentilis	BOLD	SPICH688	SPICH688_09_GU684574_09PROBE_1978_445_*Grammonota_gentilis*_COI_5P
Grammonota gentilis	BOLD	SPICH082	SPICH082_09_GU683861_09PROBE_1943_01_*Grammonota_gentilis*_COI_5P

lecular analysis. Specimens should be transferred to 100% EtOH or 100% propylene glycol and stored on ice shortly after collecting. Using 100% EtOH does make specimens brittle, which may result in specimen damage during identification. However, once morphotypes are identified and a voucher set is created, or several legs are removed for future molecular analysis, specimens can be rehydrated, and some amount of flexibility returned, in 75% EtOH prior to identification. During our survey morphological identification of specimens stored in 100% EtOH did result in more legs breaking off than usual but did not hinder the identification, and specimens were not rehydrated to facilitate morphological identification.

Conclusion

This survey and identification methods used demonstrates the usefulness of DNA barcode data to aid in the identification of spider specimens. However, it also demonstrates that careful morphological and phylogenetic analysis should be done before conclusions about taxonomic novelties, such as new cryptic species or lineages, be made. Our DNA barcode data showed several interesting clades which were not evident in the morphological analysis of the animals and may provide preliminary data for future research. Therefore, future surveys should weigh the added time required to create a voucher set of specimens or their legs for DNA barcoding against the potential rewards of additional data.

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Appendix 1. Spider species found on Prince of Wales Island, Alaska. *** = Species known to occur on the island but not found in this survey.

Amaurobiidae
Callobius pictus (Simon, 1884)
Cybaeopsis wabritaska (Leech, 1972)

Antrodiaetidae
Antrodiaetus pacificus (Simon, 1884)

Araneidae
Araneus nordmanni (Thorell, 1870)
Araniella displicata (Hentz, 1847)
Cyclosa conica (Pallas, 1772)
Larinioides patagiatus (Clerck, 1757)
Parazygiella dispar (Kulczynski, 1885)

Clubionidae
Clubiona pacifica Banks, 1896

Cybaeidae
Cybaeota shastae Chamberlin and Ivie, 1937
Cybaeus morosus Simon, 1886
Cybaeus reticulatus Simon, 1886

Dictybnidae
Cicurina simplex Simon, 1886 ***
Dictyna brevitarsa Emerton, 1915
Dictyna major Menge, 1869
Emblyna peragrata (Bishop and Ruderman, 1946)

Gnaphosidae
Sergiolus montanus (Emerton, 1890)
Zelotes fratris Chamberlin, 1920

Hahniidae
Antistea brunnea (Emerton, 1909)
Cryphoeca exlineae Roth, 1988
Dirksia cinctipes (Banks, 1896)
Ethobuella tuonops Chamberlin and Ivie 1937

Linyphiidae
Agnyphantes arboreus (Emerton, 1915)
Agyneta sp1.
Agyneta sp2.
Bathyphantes alascensis (Banks, 1900)
Bathyphantes brevipes (Emerton, 1917)
Bathyphantes canadensis (Emerton, 1882)
Bathyphantes keeni (Emerton, 1917)
Bathyphantes orica Ivie, 1969
Centromerus sp.
Ceraticelus atriceps (O. P.-Cambridge, 1874)
Ceratinella acerea Chamberlin and Ivie, 1933
Ceratinella alaskae Chamberlin and Ivie, 1947
Ceratinella ornatula (Crosby and Bishop, 1925)
Ceratinella tigana Chamberlin, 1948
Ceratinops inflatus (Emerton, 1923)
Erigone aletris Crosby and Bishop, 1928
Erigone zographica Crosby and Bishop, 1928
Eulaira arctoa Holm, 1960 ***
Grammonota subarctica Dondale, 1959
Kaestneria rufula (Hackman)
Linyphantes orcinus (Emerton)
Linyphantes pualla Chamberlin and Ivie, 1942
Meioneta simplex (Emerton, 1926)
Mermessus trilobatus (Emerton)
Microlinyphia dana (Chamberlin and Ivie, 1943)
Mythoplastoides erectus (Emerton, 1915) ***
Neriene digna (Keyserling, 1886)
Oreonetides filicatus (Crosby, 1937) ***
Oreonetides rotundus (Emerton, 1913)
Pacifiphantes magnificus (Chamberlin and Ivie, 1943)
Pityohyphantes tacoma Chamberlin and Ivie, 1947
Pocadicnemis pumila (Blackwall, 1841)
Poeciloneta bihamata (Emerton, 1882) ***
Poeciloneta fructuosa (Keyserling, 1886)
Saaristoa sammamish (Levi and Levi, 1955)
Scotinotylus patellatus (Emerton, 1917
Sisicottus nesides (Chamberlin, 1921)
Sisis rotundus (Emerton, 1925)
Symmigma minimum (Emerton, 1923)
Tachygyna ursina (Bishop and Crosby, 1938)
Tapinocyba sp. 1
Tapinocyba dietrichi Crosby and Bishop 1933 ***
Tenuiphantes tenuis (Blackwall, 1852)
Tenuiphantes zibus (Zorsch, 1937)
Tenuiphantes zelatus (Zorsch, 1937)
Walckenaeria cornuella (Chamberin and Ivie, 1939) ***
Walckenaeria columbia Millidge, 1983

Walckenaeria occidentalis Millidge 1983 ***
Walckenaeria spiralis (Emerton, 1882)
Wubana pacifica (Banks, 1896)

Lycosidae
Arctosa alpigena (Doleschall, 1852)
Pardosa dorsuncata Lowrie and Dondale, 1981
Pardosa metlakatla Emerton, 1917
Pardosa moesta Banks, 1892
Pirata piraticus (Clerck, 1757)
Trochosa terricola Thorell, 1856

Nesticidae
Nesticus silvestrii Fage, 1929 ***

Philodromidae
Philodromus rufus pacificus Banks 1898 ***
Tibellus oblongus (Walckenaer, 1802)

Pimoidae
Pimoa altioculata (Keyserling, 1886)

Salticidae
Pelegrina aeneola (Curtis, 1892)
Salticidae gen. sp.

Telemidae
Usofila pacifica (Banks, 1894)

Tetragnathidae
Metellina curtisi (McCook, 1894)
Tetragnatha extensa (Linnaeus, 1758)
Tetragnatha laboriosa Hentz, 1850
Tetragnatha versicolor Walckenaer, 1842

Theridiidae
Robertus vigerens (Chamberlin and Ivie, 1933)
Rugathodes sexpunctatus (Emerton, 1882)
Theonoe stridula Crosby, 1906
Theridion saanichum Chamberlin and Ivie, 1947

Thomisidae
Xysticus pretiosus Gertsch, 1934

Uloboridae
Hyptiotes gertschi Chamberlin and Ivie, 1935

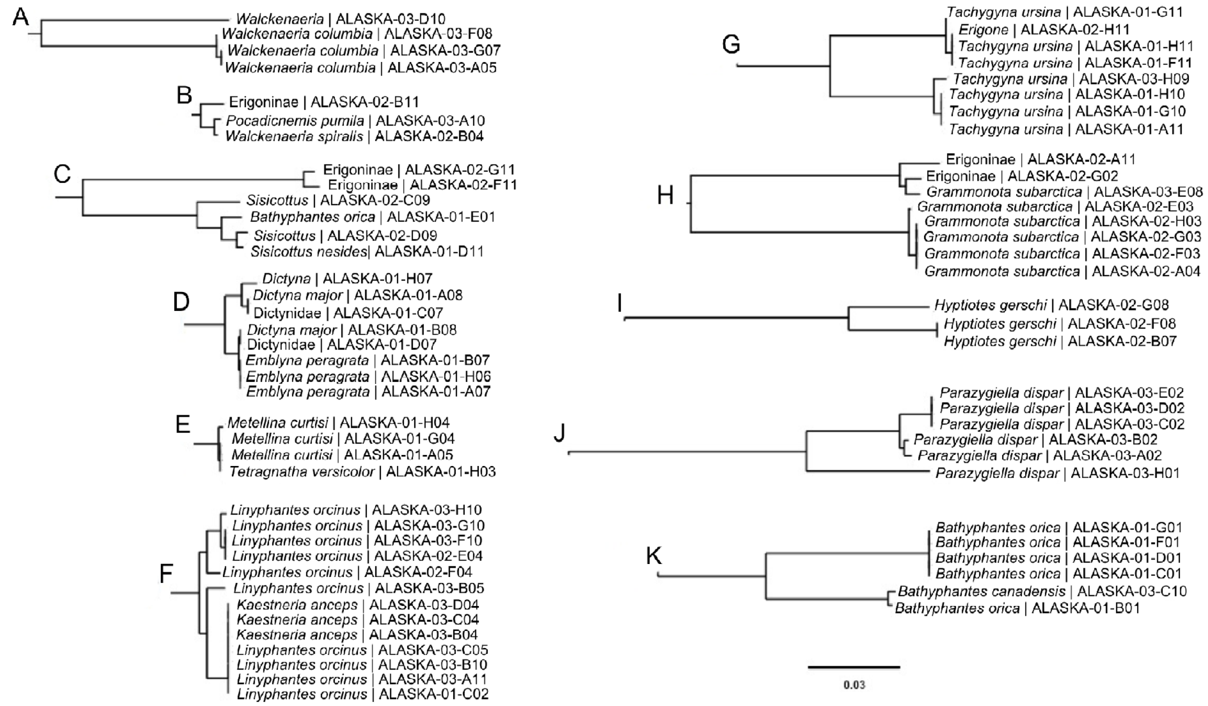

Figure 2. Examples of identification mistakes and long branches identified in the Neighbor Joining tree produced using the BOLD interface. Tree letters refer to clade specific identification mistakes listed in Table 1. ALASKA-##-## refers to the BOLD specimen record number.

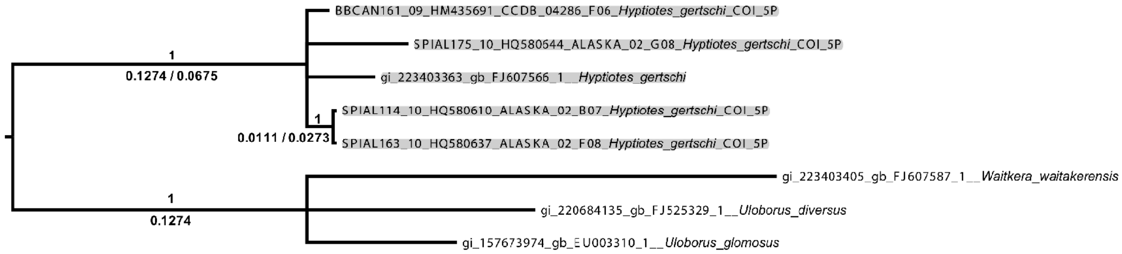

Figure 3. Bayesian 90% majority rule consensus phylogram for the species *Hyptiotes gertschi* Chamberlin and Ivie and outgroup using a three partitioned model (GTR+I+G for each codon position) of a 669 bp region of the COI gene. Survey specimens are highlighted. Posterior probabilities are recorded above branches. Branch lengths from the Bayesian analysis followed by the branch lengths from the Neighbor Joining analysis, where applicable, are recorded below branches. Specimen names include the BOLD sequence record (ex SPIAL163-10) followed by the GenBank sequence record (HQ580637) followed by the BOLD specimen number (ALASKA-02-F08) and then by the species and gene (*Hyptiotes_gertschi*_COI_5P)

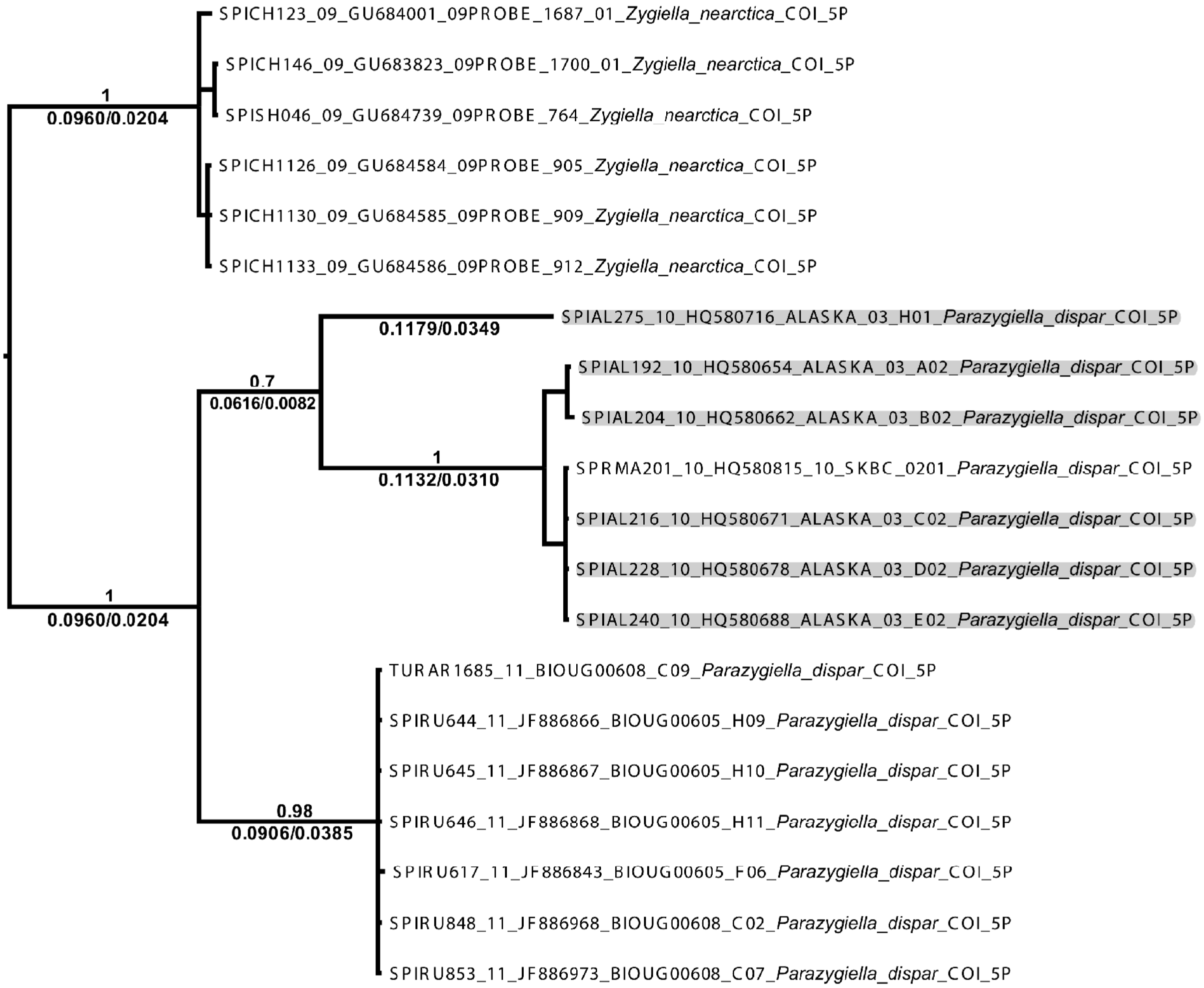

Figure 4. Bayesian 70% majority rule consensus phylogram for the species *Parazygiella dispar* (Kulczynski) and outgroup using a three partitioned model (GTR+I+G for each codon position) of a 669 bp region of the COI gene. Survey specimens are highlighted. Posterior probabilities are recorded above branches. Branch lengths from the Bayesian analysis followed by the branch lengths from the Neighbor Joining analysis, where applicable, are recorded below branches.

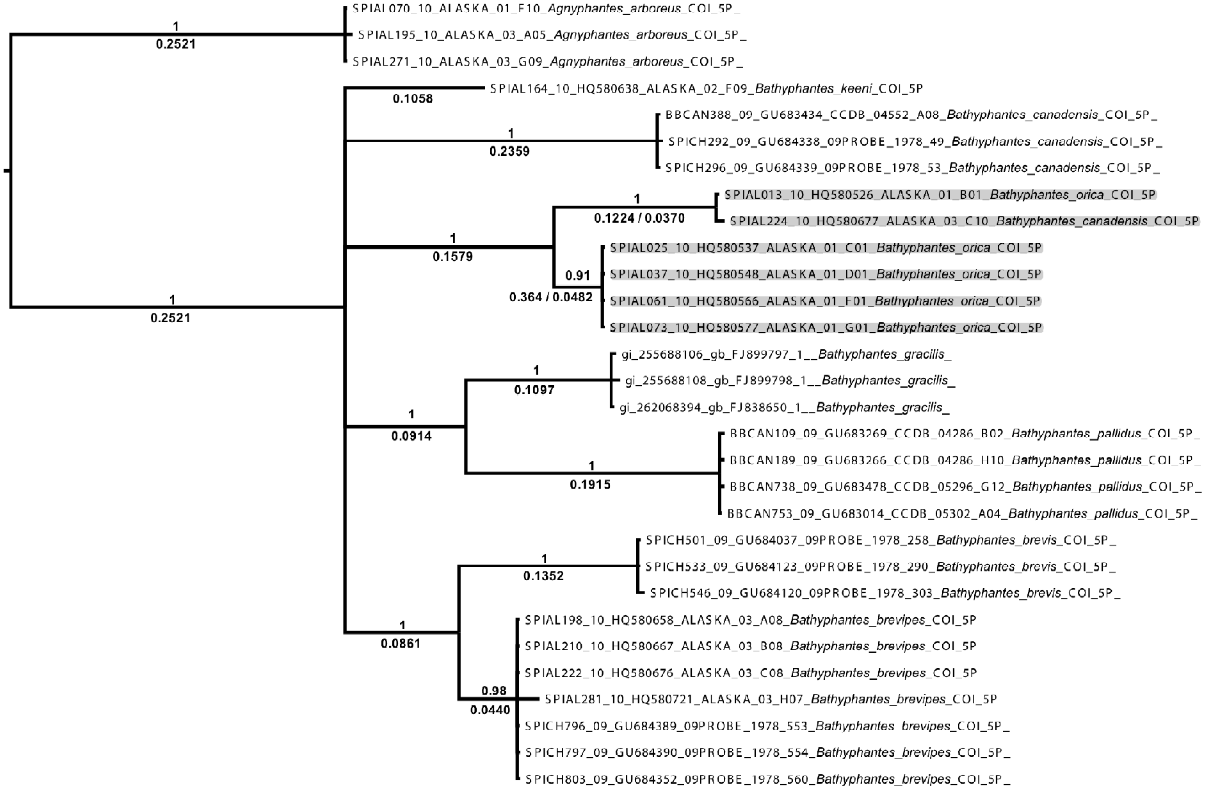

Figure 5. Bayesian 90% majority rule consensus phylogram for the species *Bathyphantes orica* Ivie and *B. canadensis* (Emerton) and outgroups using a three partitioned model (GTR+I+G for each codon position) of a 669 bp region of the COI gene. Survey specimens are highlighted. Posterior probabilities are recorded above branches. Branch lengths from the Bayesian analysis followed by the branch lengths from the Neighbor Joining analysis, where applicable, are recorded below branches.

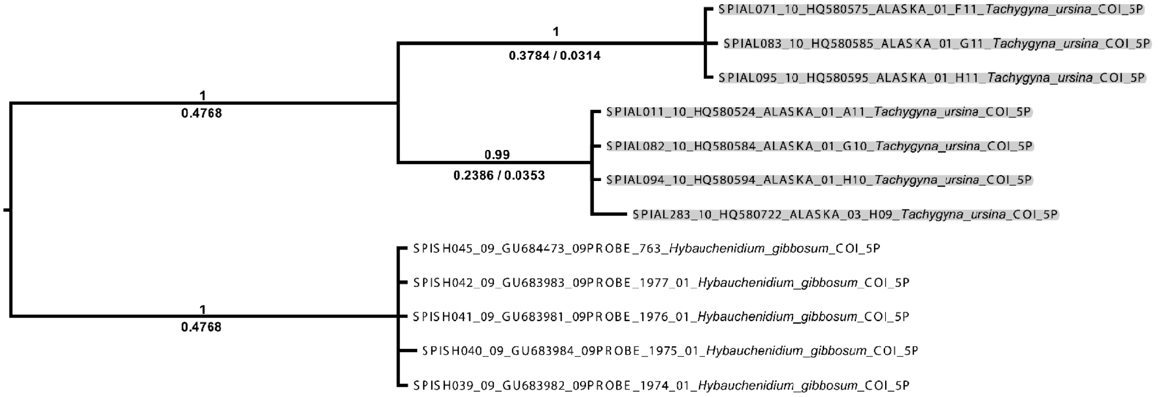

Figure 6. Bayesian 90% majority rule consensus phylogram for the species *Tachygyna ursina* (Bishop and Crosby) and outgroup using a three partitioned model (GTR+I+G for each codon position) of a 669 bp region of the COI gene. Survey specimens are highlighted. Posterior probabilities are recorded above branches. Branch lengths from the Bayesian analysis followed by the branch lengths from the Neighbor Joining analysis, where applicable, are recorded below branches.