# First Records of the Night Smelt, *Spirinchus starksi*, in the Salish Sea, Washington

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# FIRST RECORDS OF THE NIGHT SMELT, *SPIRINCHUS STARKSI*, IN THE SALISH SEA, WASHINGTON

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Key words: COI, distribution, genetics, Longfin Smelt, Night Smelt, Osmeridae, *Spirinchus starksi*, *Spirinchus thaleichthys*, Washington

Ten species of smelts (Osmeridae), including 1 introduced species, have been found in the eastern North Pacific (Love and others 2005). Of these 10 species, 5 have been recorded from the inland marine waters of the Salish Sea, including Puget Sound and the Straits of Juan de Fuca and Georgia (Hart 1973): Whitebait Smelt (*Allosmerus elongatus*), Surf Smelt (*Hypomesus pretiosus*), Capelin (*Mallotus villosus*), Longfin Smelt (*Spirinchus thaleichthys*), and Eulachon (*Thaleichthys pacificus*). Results from a study using molecular markers to distinguish smelt remains found in the stomach contents of fishes (Paquin and others 2014) indicated that a specimen collected from Discovery Bay, Washington, an embayment at the eastern terminus of the Strait of Juan de Fuca, was misidentified as *S. thaleichthys* in the online Barcode of Life Database (BOLD; Ratnasingham and Hebert 2007). Subsequent examination of the preserved specimen has confirmed its identity as Night Smelt (*Spirinchus starksi*) and constitutes a new record for this species in the Salish Sea. Since this discovery, additional specimens of *S. starksi* have been collected in the area for inclusion in this study.

To confirm the identification of the Discovery Bay specimen, both morphological and nucleotide sequence data from *S. starksi* were compared with data from the closely related species *S. thaleichthys*. Whole specimens and tissue samples (frozen or preserved in ethanol) from 11 *S. starksi* and 5 *S. thaleichthys* were obtained from the Northwest Fisheries Science Center (NWFSC) and the University of Washington Fish Collection (UW). Specimens of *S. starksi* were collected from Discovery Bay, Washington ($n$ = 1), Green Point, Washington ($n$ = 5), and north of Monterey Bay off the California coast ($n$ = 5). Specimens of *S. thaleichthys* were collected from the Gulf of Alaska ($n$ = 1), San Juan Islands ($n$ = 1), and Puget Sound ($n$ = 3) (Table 1).

DNA extractions were performed on fin clips using a QIAGEN[1] DNeasy kit (QIAGEN, Valencia, CA) and the manufacturer's animal tissue protocol. A 750 base-pair region of the mitochondrial genome, cytochrome *c* oxidase I (COI), was amplified using PCR with universal fish primer cocktail C_FishF1t1-C_FishR1t1 (Ivanova and others 2007). The COI gene region has been used as the barcode for biodiversity to distinguish among species, whether they are distantly related species such as mammals and insects, or closely related congenerics (Ward and others 2005, 2009; Holloway 2006; Ward and others 2009). Nucleotide sequence data were collected for 16 samples (including the misidentified specimen of *S. starksi* from Discovery Bay) in both forward and reverse directions with Big Dye chemistry using EXOSAP purified PCR products. Sequences were read using an ABI 3730 automated sequencer

[1] Reference to trade names does not indicate endorsement by NOAA-Fisheries or the US Government

TABLE 1. Specimen collection information, UW Fish Collection specimen catalog numbers, and results from analysis of nucleotide sequence data. Letter designations of DNA haplotypes correspond with those used in the text.

| Taxon | Number of individuals | Collection date | Location | Mitochondrial DNA haplotype (frequency) | Specimen catalog number |
|---|---|---|---|---|---|
| *Spirinchus starksi* | 1 | 23 May 2003 | Discovery Bay, WA | A | UW 048781 |
| | 5 | 29 Sep 2010 | North of Monterey Bay, CA | A (3), B (1), C (1) | UW 150968 |
| | 5 | 27 July 2011 | Green Point, WA | A (4), D (1) | UW 150964, 150965 |
| *S. thaleichthys* | 1 | 23 July 2005 | Gulf of Alaska, AK | b | UW 116237 |
| | 1 | 21 May 2010 | San Juan Islands, WA | c | UW 150984 |
| | 2 | 25 May 2010 | Hood Canal, Puget Sound, WA | a (2) | UW 150987 |
| | 1 | 03 June 2010 | Central Puget Sound, WA | a | UW 150985 |

(Life Technologies). All sequences were aligned using Sequencher 5.0 (Gene Codes Corp.) and analyzed for nucleotide differences in BioEdit (Hall 1999). To ensure that the COI gene region was sequenced, sequences were aligned and compared to the complete mitochondrial genome of another fish species, Walleye Pollock (*Theragra chalcogramma*). Sequence divergence estimation using Kimura 2-parameter (K2P) distance analysis (Kimura 1980) was performed in Arlequin Suite v. 3.5 (Excoffier and Lischer 2010).

We sequenced a 668 base-pair fragment corresponding to base positions 5490 to 6157 of the mitochondrial genome of Walleye Pollock (Yanagimoto and others 2004, GenBank Accession No. NC004449). This fragment contained a portion of the COI coding region. We analyzed nucleotide sequence data from 11 specimens of *S. starksi* and 5 specimens of *S. thaleichthys* (GenBank Accession Nos. KF196151–KF196157). These analyses revealed 4 unique haplotypes (nucleotide sequence that differs by at least 1 base pair), A, B, C, and D, among the *S. starksi* individuals and 3 haplotypes, a, b, and c, among the *S. thaleichthys* specimens (Table 1). For *S. thaleichthys*, a minimum spanning network connected all haplotypes by single mutational changes (Fig. 1). The network for *S. starksi* connected all haplotypes by single mutational changes with the exception of haplotype (D), which contained 1 missing intermediate (Fig. 1). The most common haplotype (A) is shared by 8 of 11 *S. starksi* specimens, including the Discovery Bay specimen (Table 1). Greater than 7.5% sequence divergence exists between species in this data set, whereas less than 1% sequence divergence exists between the most divergent of the haplotypes within species, corroborating our conclusions from morphological data.

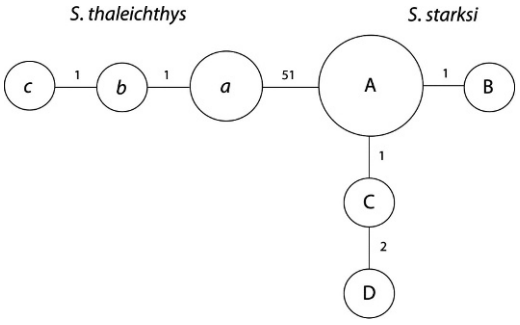


FIGURE 1. A minimum spanning network connected haplotypes (circles) within and between *Spirinchus thaleichthys* (a, b, and c) and *S. starksi* (A, B, C, and D). Numbers adjacent to lines indicate the number of mutational changes between haplotypes, and the sizes of circles are proportional to haplotype frequencies.

*Spirinchus starksi* is closely related to and morphologically similar to its only eastern Pacific congener, *S. thaleichthys*. The 2 species can be distinguished by a combination of morphological characters (Mecklenburg and others 2002): differences in pectoral-fin length (typically shorter in *S. starksi*, extending ≤ 84% of distance from pectoral-fin to pelvic-fin origin vs. ≥ 84%); snout shape (pointed, forming 54–65° angle to forehead in *S. starksi* vs. blunt, forming 68–90° angle); anal-fin ray length (shorter in *S. starksi*, 32–45% HL vs. 45–71% HL in *S. thaleichthys*); and lateral line scale count (higher in *S. starksi*, 60–66 vs. 54–63). Of these, pectoral-fin length relative to the distance to the pelvic fin is the most useful field character.

However, the pectoral fin of *S. starksi* is not short compared to other osmerids, and the overlap in the proportional measurement of pectoral to pelvic fin distance between the 2 species (for example, 69 to 94% in our *S. starksi* material vs. 83 to 118% in our *S. thaleichthys* material), likely leads to some misidentifications. With experience, differences in snout shape will distinguish the 2 species, but the differences are subtle for those unfamiliar with the species.

The specimen of *S. starksi* from Discovery Bay represents the 1st record of this species from the Salish Sea, and 5 additional specimens were subsequently collected farther west in the Strait of Juan de Fuca (UW 150964 and 150965; Table 1). Catch records from surveys conducted in the Strait by the NWFSC indicate the presence of *S. starksi* (ANK, unpubl. data), although none have been supported by vouchers. *Spirinchus starksi* is known from 2 records in Southeast Alaska, off Sitka, and in Shelikof Bay (Dryfoos 1961, McAllister 1963; Mecklenburg and others 2002), and a few records from Hecate Strait and the Queen Charlotte Islands (Hart 1973). Farther south, from La Push, Washington (Schultz 1936; Hart 1973) to central California (Love and others 2005), *S. starksi* is common (Miller and Lee 1972). While *S. starksi* may simply be rare in the Salish Sea and present only in the Strait of Juan de Fuca, its sympatric distribution pattern and morphological resemblance to *S. thaleichthys* raises the possibility that it might be more widespread, though not abundant, in isolated locations of Puget Sound proper or in the Strait of Georgia. Current collections might represent a relatively recent or periodic colonization event, a result of the species being advected from the more open coastal waters of the Pacific Ocean into the Strait of Juan de Fuca. A closer examination of specimens from Puget Sound and other inland waters is warranted.

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