**Morphology and genetic investigation of flatfish interspecies hybrids
(*Pleuronectes platessa* X *Platichthys flesus*) from the Baltic Sea**

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21 **Abstract:** Plaice (*Pleuronectes platessa*) and flounder (*Platichthys flesus*) are
22 economically valuable flatfishes found on the European shelf that are known to
23 naturally and artificially hybridize. Putative hybrids have intermediate coloration which
24 suggest the interspecies status between plaice and flounder. To further investigate this
25 hybridization process, genetic testing was performed on individuals from
26 morphologically identified hybrid samples from the Baltic Sea. Morphological
27 examinations indicate that putative hybrids are morphologically more similar to plaice
28 than flounder. Putative hybrids are difficult to differentiate from plaice without
29 thorough morphological examination. However, the number of vertebrae and scale
30 thrones can morphologically differentiate plaice, flounder, and putative hybrids.
31 Purebred plaice and flounder samples were well-discriminated by nuclear markers
32 TMO-4c4, pthrP, and microsatellites. The analysis results showing the genetic status of
33 some putative hybrids were in the intermediate position between their parent species
34 populations which confirmed their hybrid identities. Mitochondrial marker COI results
35 suggest uni-directional maternal material contributions from plaice for this
36 hybridization process. Despite consistent sampling effort at all study sites, putative
37 hybrids were only observed along the western edge of the Baltic Sea and not found in
38 the further west or northern sampling locations.

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40 **Keywords:** flatfish, hybridization, molecular identification

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INTRODUCTION

Recent studies have demonstrated that hybridization among marine fishes is not rare on coral reefs (Richards and Hobbs 2015) with almost 70% of marine fish hybrids reported from tropical waters (Montanari et al. 2016). This is logical due to higher species diversity in the tropics but contrasts with reports finding 90% of freshwater hybrids in temperate or subtropical waters (Scribner et al. 2000). There are, however, documented marine fish hybrids in temperate or subtropical waters, for example, within the flatfish family Pleuronectidae. In addition to naturally occurring hybrids, artificial Pleuronectidae hybrids were bred in attempts to produce new strains of fish with interesting traits for aquaculture (Riley and Thacker 1969; Purdom and Lincoln 1974; Lincoln 1981a,b). These previous studies indicate the potential for hybridization in this group of fishes.

Flounder *Platichthys flesus* (Linnaeus, 1758) and plaice *Pleuronectes platessa* (Linnaeus, 1758) are commercially valuable species that live on the European continental shelf. Both plaice and flounder have life history aspects that could result in hybridization, including pelagic spawning, larvae with high dispersal abilities, and adult migration (Kijewska et al. 2009). However, adults also display fidelity to specific, separate spawning grounds, which could play a role in isolating interspecific populations (van der Veer 1986; Rijnsdorp and Pastoors 1995). Plaice and flounder are both capable of tolerating low salinity and occupy differently, but partially overlapping, habitats due to the salinity range within the Baltic Sea (Eggens et al. 1995). They are known to coexist with freshwater species in the brackish waters of the Baltic Sea but still require certain salinity conditions for egg development (Nissling et al. 2002). The Baltic Sea receives high riverine input from the eastern coast, lowering the surface water salinity in that region, with salinity rising in deeper waters (Kijewska et al. 2009).

This limits suitable breeding grounds for plaice and flounder to the deepest areas of the southern Baltic Sea, for example in the Bornholm and Gdansk Basins (Nissling et al. 2002). The shared spawning grounds in these areas could contribute to hybridization (Nissling et al. 2002; Kijewska et al. 2009). That is why a group of flatfishes found in the Baltic Sea, with an intermediate appearance, known by the Danish name “leps”, has been suggested as interspecies hybrids between plaice and flounder (Norman 1934) .

Pleuronectidae systematics is traditionally based on morphology (Berendzen et al. 2002). Some flatfishes species belonging to different genera within the family have been known to hybridize (Norman 1934; Riley and Thacker 1969; Purdom and Lincoln 1974; Lincoln 1981a,b) and the presence of hybrids can lead to misidentification of specimens when exclusively identified using morphological characteristics (Kijewska et al. 2009). Previous systematic studies of the Pleuronectidae using molecular markers did not show reciprocal monophyly between plaice and flounder (Pardo et al. 2005; Kartavtsev et al. 2007; Betancur-R et al. 2013). Given the occurrence of intergeneric crosses within the Pleuronectidae family, their phylogenetic relationships, and the possibility of reverting some of those naturally hybridizing species into the common genus *Pleuronectes* need to be critically re-examined (Riley and Thacker 1969).

Various molecular analyses have been used to study plaice and flounder in the Baltic Sea. Haemoglobin (Sick et al. 1963), allozymes (He and Mork 2015), mtDNA, and nuclear markers (Kijewska et al. 2009) were used to try to identify plaice X flounder hybrids. Additionally, microsatellites were used to investigate the population genetic structures of both species (Hoarau et al. 2002b; Hemmer-Hansen et al. 2007).

In studies of hybridization, markers that can clearly and reliably separate two purebred lineages are useful as diagnostic markers (Boecklen and Howard 1997). In principle,

ideal markers will show a fixed interspecific difference (He et al. 2017; He et al. 2018a; He et al. 2018b). Diagnostic markers are important to extend further studies of interspecific hybrids, but also for stock assessment and recruitment studies of these important fishery species. The identification of such diagnostic markers requires assessing several potential markers in a suite of samples from both species. The restriction fragment length polymorphism (RFLP) patterns of the nuclear pthrP marker (the first intron of the parathyroid hormone-related protein gene) and rDNA marker internal transcribed spacer (ITS1) were found to be suitable for identification of plaice, flounder, and their hybrids (Kijewska et al. 2009). However, due to the potential sequencing difficulties caused by length polymorphism patterns of these markers, fixed interspecific differences in the sequences of these markers were not properly addressed. The sequences for hybrids with these markers, which could be used for comparisons of results between studies, are still lacking.

The specific aim of this study was to check the genetic status of putative hybrids between plaice and flounder for a better understanding of this marine fish hybridization process. After morphological investigations, molecular methods were used to assess the taxonomic status of putative hybrids. Sixteen putative hybrids were identified and collected from flatfishes' samples based on intermediate appearance and morphological characteristics. A suite of nuclear markers and mitochondrial gene markers, which were frequently used in other marine fish hybridization cases (He et al. 2017; He et al. 2018a; He et al. 2018b; He et al. 2019), were tested on *P. flesus*, *P. platessa*, and their putative hybrids. By comparing the results from the different analyses, the genetic status of putative hybrids was revealed. Sequences of the hybrid individuals from different diagnostic markers were provided to potentially benefit future studies exploring the associated evolutionary mechanisms involved.

MATERIAL AND METHODS

Sample collection and morphological examination

Archived samples, from the Natural History Museum of Denmark, of *P. platessa* (n=22), *P. flesus* (n= 25), and putative hybrids (n=16) from eight different sampling locations were obtained for analysis. The sampling locations were spread from the southern Kattegat strait (Grenå Havn, Polderev, Glatved Strand, Fønsskov, Knivlær Strand, Korsør Lystskov, Nøddebohuse and Hornbæk Plantage) to the western edge of Baltic Sea (Bellevue, Mosede, and Rødvig Fiskerihavn) (Fig. 1). Additional *P. platessa* (n= 50) and *P. flesus* (n= 41) samples were collected from the Norwegian fjords using multiple collecting methods, as described in He et al. (2015). A subset of these samples was used as purebred references due to the apparent low hybridization rate within that area (He et al. 2015). Fin clips of 31 plaice (20 Baltic Sea, 11 Norwegian fjords), 37 flounder (21 Baltic Sea, 16 Norwegian fjords), and 16 putative hybrids (all Baltic Sea) were taken from fresh specimens at the time of collection, preserved in 80% ethanol, and stored at -20°C. For the current study, a subsample (approximately two mm²) of each fin clip was retrieved and used for DNA extraction. The samples were extracted using a “hotshot” DNA extraction protocol (Truett et al. 2000).

In addition to the intermediate appearance of putative hybrids, detailed morphological examinations were performed according to Eschmeyer (1990) and (von Ubisch 1950). This included measurements of vertebrae, anal fin rays, dorsal fin rays, caudal fin rays, pectoral fin rays, scale thrones, and other potentially informative morphological characteristics.

DNA sequence analysis

To investigate potential diagnostic markers for clear species discrimination between plaice and flounder, nuclear single copy marker titin-like protein coding gene TMO-4c4 and ETS proto-oncogene 1 were amplified by primers TMO-4C4F/TMO-4C4R (Streelman et al. 2002) and ETS1F/ETS1R (Lyons et al. 1997). To cross reference the results from previous studies two nuclear markers, known diagnostic markers for plaice and flounder (Kijewska et al. 2009), nuclear intron marker pthrP (APTHF2/APTHR1) were also used. Finally, to investigate maternal contributions of the hybrids, mitochondrial COI fragments were amplified by FishF2/FishR2 (Ward et al. 2005). Additional nuclear markers were assessed including; RAG1, RAG2, S7, and BMP4, none of which were suitable for diagnostic purposes and therefore not discussed further (Appendix Table 2).

The QIAGEN Multiplex PCR Kit (Qiagen, Hilden, Germany) was used for the polymerase chain reaction. PCR cycling parameters were as follows: initial 95°C denaturation for 15 min., followed by 35 cycles of 94°C for 45 sec., annealing for 60 sec. (TMO-4C4F/TMO-4C4R: 58°C; ETS1F/ETS1R: 54°C; APTHF2/APTHR1: 56°C; FishF2/FishR2: 50°C), and 72 °C for 60 sec., with a final elongation step of 72 °C for 10 min. The PCR products were checked under UV light after running in 1% agarose gel at 90V for 45 minutes. All PCR products were cleaned by incubating with exonuclease I and FastAP™ Thermosensitive Alkaline Phosphatase (ExoFAP; USB, Cleveland, OH, USA) at 37 °C for 60 min., followed by 85 °C for 15 min. The final products were sequenced in forward and reverse direction with fluorescently labeled dye terminators following the manufacturer's protocols (BigDye, Applied Biosystems Inc., Foster City, CA, USA), and analyzed using an ABI 3130XL Genetic Analyzer (Applied Biosystems).

The sequences were aligned using the program Geneious R8 (Biomatters Ltd., Auckland, New Zealand) and were uploaded to GenBank (COI: MG253424 - MG253507; TMO-4c4: MG253340 - MG253423; ETS1: MG253285 - MG253339; pthrP: MH288000 - MH288074). COI fragments from each species were blasted on GenBank and were 98%-100% identical with voucher sequences from their own species (Accessions: KM654275, JN859194, JN859191, KM654278, EU513682, KJ128581). Only two other pthrP fragment sequences are available in GenBank from a previous study for our study species (EU075180 and EU07581) (Kijewska et al. 2009). Both were downloaded and included in subsequent analyses.

Haplotypes were inferred using the Bayesian analysis PHASE 2.1 (Stephens and Donnelly 2003) implemented in DnaSP 5.0 (Librado and Rozas 2009). Each run was performed with a burn-in of 10,000 generations, followed by 100,000 generations. All runs except the pthrP region fragments returned consistent results and were able to phase each haplotype pair with > 90% posterior probability. The samples with double peaks in sequence chromatograms were recognized to be heterozygotes (Flot and Tillier 2006). Two haplotypes of each sequence were separated using SeqPHASE (Flot 2010) when both haplotype alleles were the same length. Alternatively, when alleles had different lengths, sequences were separated using CHAMPURU (Flot 2010). The latter case was found in ETS1 and pthrP regions.

After aligning the forward and reverse sequence of pthrP fragment for each sample, around 1200bp alignment was extracted. Due to the observed fragment length polymorphism pattern of pthrP fragments, (Kijewska et al. 2009), 48% plaice pthrP fragment sequences showed intensive heterozygous patterns where different length alleles overlapped and created a consistently low-quality region (around 300 bp) within

the pthrP alignments. In addition to the possible hybridization process that could generate more heterozygous sites, the same region was observed in 50% of hybrids samples. Under this situation, it is difficult to acquire high posterior probability from Bayesian analysis using PHASE 2.1, and CHAMPURU analysis could not infer haplotypes confidently. Therefore, this low-quality region was removed from all sequences extracted from pthrP fragments.

After delineation, heterozygote specimens were represented by their two haplotypes sequences in the alignments used for network construction. Median-joining networks showing relationships among the haplotypes were generated in NETWORK v4.6.1.3 (Bandelt et al. 1999). Haplowebs (Flot et al. 2010) were derived from the median-joining networks by drawing curved lines to connect haplotypes that were co-occurring in heterozygous hybrids.

Microsatellite analysis

Microsatellites for all samples were amplified using the following primers: PL06, PL09, PL52, PL92, PL115, PL142, and PL167 (Hoarau et al. 2002a). QIAGEN Multiplex PCR Kit (Qiagen, Hilden, Germany) was used for PCR. Cycling parameters were as follows: initial 95°C denaturation for 15 min., followed by 40 cycles of touch-down 94 °C for 45 sec., 60 sec. annealing at 60 °C, and 72 °C for 60 sec. A final elongation step was performed at 72 °C for 20 min.

The entire microsatellite marker genotypes were successfully amplified and scored with Geneious R8 (Biomatters Ltd., Auckland, New Zealand). The presence of null alleles, the probabilities of departure from Hardy-Weinberg equilibrium (Schweitzer et al. 2002), and linkage disequilibrium (LD) were estimated using GenePop V4.3 (Rousset 2008) and Micro-Checker 2.2.3 (van Oosterhout et al. 2004) (Appendix Table 3 & 4).

The genetic structure among these tree flatfish groups and the level of admixture in hybrids were assessed using STRUCTURE *vers.* 2.2.3 with location prior for the purebred individuals (flounders and plaice) and without the location prior when the hybrids included. For both settings, 1,000,000 generations were analysed after 10,000 generations burn-in. Potential genotype cluster numbers were set from K=1 to K=10. For each cluster number was analysed with ten irritations. The running results were processed with Structure Harvester *vers.* 0.6.94 (Earl and von Holdt 2012) to determine the best genotype cluster value. The consolidated bar plot from 10 irritations for each K were generated by online software CLUMPAK (Kopelman et al. 2015).

To investigate the genetic status of putative hybrids, principal components analysis (PCA) (Mackiewicz and Ratajczak 1993) was performed on the dataset contains microsatellite markers and nuclear gene marker TMO-4c4 and pthrP. The analysis was carried out by using implemented add-in packages Factoextra, and FactoMineR in R v2.12 (R Core Team 2015) using functions *empca*. The results were visualized in a scatterplot generated using adegenet (Jombart 2008; Jombart et al. 2010).

RESULTS

Morphometric

Several morphological traits show a clear separation between plaice and flounder. Significant differences between the two species can be found in the number of anal fin rays, dorsal fin rays, caudal fin rays, vertebrae, and scale thrones (Table 1). Plaice and putative hybrids were found to have similar morphology (no significant difference in the numbers of dorsal fin rays and caudal fin rays) but could be discriminated by the number of anal fin rays, vertebrae, and scale thrones. No significant morphological relatedness was found between flounder and putative hybrids (Table 1).

Flounder had significantly fewer vertebrae than putative hybrids. Putative hybrids and plaice had a small range of overlap in vertebrae counts, however putative hybrids still had significantly fewer than plaice. Additionally, the number of thorns on each scale was shown to have diagnostic capabilities, due to large differences in the number of scale thorns between the three groups. For this characteristic, no overlap between the range of measurements for all three groups was found (Table 1). Flounder were observed to have significantly more thorns on their scales than both plaice and putative hybrids. Plaice exhibited thorns that were thinner than those found on flounder and had fewer thorns than putative hybrids. The range of the number of thorns for putative hybrids was intermediate to those found for the other two species (Table 1).

In summary, the number of vertebrae and scale thrones could serve as diagnostic morphology traits for plaice, flounder, and putative hybrids. Putative hybrids were more morphologically similar to plaice, and it should be noted that the two were difficult to differentiate without a thorough morphological examination, which is further indicated by morphological measurement results (Appendix Table 1).

Nuclear DNA

The 473 bp TMO-4c4 fragments from all samples were aligned and six variable sites were identified. Overall of the fragments there were four haplotypes (h) and haplotype diversity was 0.553.

The two flatfish lineages were well separated by four nucleotide substitutions in the TMO-4c4 marker across the sampled geographic distribution, identifying four diagnostic sites, which could be used for hybrid detection (Fig. 2a). Following analysis using SeqPHASE, 81% of putative hybrids haplotypes were grouped with *P. platessa*, while 19 % putative hybrids (3 individuals; hybrids 2, hybrids 4, and hybrids 5) were

heterozygous exhibiting species-specific haplotypes from both plaice and flounder populations (Fig. 2b). These three heterozygous hybrids represent an exception to the mutual exclusivity generated (if the hybrid specimens were excluded) by this marker which indicates these individuals are hybrid offspring of *P. platessa* and *P. flesus*.

There are 18 flounder, six putative hybrids, and five plaice with length heterozygous patterns on ETS1 region. Only 13 out of 29 of the length heterozygous individuals (flounder: 10 out of 18; hybrids: 1 out of 6; plaice: 2 out of 5) were successfully delineated using CHAMPURU (Flot 2007), while the remaining individuals could not be delineated due to alignment failure caused by low-quality sequences. This problem remained despite the application of internal primers specifically designed for this region (Appendix Fig. 1 & 2). Therefore, ETS1 marker is not well-suited for the diagnostic purpose for plaice, flounder, and putative hybrids.

After excluding the low-quality region and sites with gaps in each sample, the pthrP marker had a final fragment length of 929 bp. Samples were aligned with 97 variable sites, identifying 80 haplotypes (h), and haplotype diversity of 0.969. Plaice and flounder lineages were well separated by 35 nucleotide substitutions in the pthrP marker across the geographic range sampled, allowing for 35 diagnostic sites, which could be used for hybrid detection (Fig. 2c). After analyses using SeqPHASE, 75% of the putative hybrids were grouped with *P. platessa*, this excluded four putative hybrid individuals which were in unique positions on the haploweb. One individual (hybrids 1) was a heterozygote exhibiting species-specific haplotypes from both plaice and flounder populations (Fig. 2d). One hybrid individual (hybrids 5) was heterozygous exhibiting one plaice haplotype, while the other haplotype was closer to flounder haplotypes (Fig. 2d). The four haplotypes of the remaining two hybrids individuals (hybrids 2 and

hybrids 4) were located in the center of the pthrP haploweb, but were close to different species lineages (Fig. 2d). These four heterozygous hybrids individuals represent an exception to the mutual exclusivity generated (if the hybrid specimens were excluded) by this marker which indicates these individuals are hybrid offspring of *P. platessa* and *P. flesus*.

In PCA plots, the clear mutually exclusive pattern between plaice and flounder gene pools was shown (Fig. 3). The putative hybrids were genetically more related to plaice populations rather than flounder populations. However, four hybrids individuals (hybrids 1, hybrids 2, hybrids 4, and hybrids 5) could be discriminated from the plaice gene pool and were distributed in intermediate positions between plaice and flounder populations. The rest of the putative hybrids were not distinct from *P. platessa* populations.

Mitochondrial DNA

The 632 bp COI fragments from all samples were aligned and 54 polymorphic sites were identified. The total number of haplotypes (h) is 22 and haplotype diversity is 0.775. The two presumptive purebred flatfish lineages were well-separated by 34 nucleotide substitutions (Fig. 2e) and no well-supported phylogeographic structures were detected within clades. The haplotypes of the putative hybrid individuals were grouped with plaice, indicating that the maternal contribution of these individuals came from the *Pleuronectes platessa* clade (Fig. 2f).

DISCUSSION

Morphology examinations indicate that putative hybrids were more morphologically similar to plaice than flounders. Without thorough morphological examinations, plaice

and putative hybrids were difficult to differentiate. Although the number of vertebrae and scale thrones could morphologically separate plaice, flounder, and putative hybrids, these are not practical characters to assess in large numbers of samples

Purebred plaice and flounder samples were discriminated well by nuclear markers TMO-4c4, pthrP, and microsatellites, with no signs of incomplete lineage sorting between these two species observed. These nuclear markers can, therefore, serve as diagnostic methods to identify purebred lineages and detect hybrids (at least first-generation hybrids) between the two species. The heterozygous patterns found in diagnostic sites could provide direct genetic evidence about the hybridization. There were four hybrids (hybrids 1, hybrids 2, hybrids 4, and hybrids 5) that have genetic support of their hybrid identities from different nuclear markers. Three hybrid individuals (hybrids 2, hybrids 4, and hybrids 5) have hybridization support from almost all nuclear markers, while hybrids one was only supported by one nuclear fragment marker and microsatellites. The genetic evidence from hybrids confirmed that hybridization is ongoing between plaice and flounder, and “leps” are their hybrid offspring. The mitochondrial marker COI results suggest that maternal contributions of this hybridization process were only from plaice.

Hybrids were only observed at the sampling locations on the western boundary of the Baltic Sea. There were no hybrids observed in the sampling sites further to the west or in the Norwegian fjord locations. We, therefore, hypothesize that a western range boundary for hybridization process exists, restricting majorities of hybrids to the Baltic Sea. This distribution boundary for the flatfish hybridization process in the southwestern Baltic Sea was very similar to the bottom salinity boundary of 11-18 psu (Practical Salinity Unit) (Momigliano et al. 2018). Another flounder and plaice

hybridization spot in the Baltic Sea, Bornholm Basin (Kijewska et al. 2009), has very similar bottom salinity background too (Momigliano et al. 2017; Momigliano et al. 2018). Therefore, western hybridization boundary may be supports the hypothesis that the unique salinity gradient in the Baltic Sea forced plaice and flounder to share spawning grounds, resulting in hybridization (Nissling et al. 2002; Kijewska et al. 2009). It seems that the once the bottom water salinity rise up to 18 – 30 psu (sampling site Knivlær Strand, and Hornbæk Plantage), the hybridization between these two flatfish species was suddenly stopped. The hybrids are not found even if the sampling location is only a few kilometers away from the potential western boundary (Fig. 1). The north-eastern hybridization boundary of flounder and plaice hybrids could be shaped by the allopatric area of these two species and the salinity together. According to Momigliano et al. (2018), no *Platichthys flesus* sample was found in flatfish samples which were collected from Åland Archipelago and Öland. Furthermore, only a minority part of flatfish samples consisted of *Platichthys flesus* in Gotland and Irbe Strait, the rest of samples actually belong to a cryptic new species *Platichthys solemdali*. Furthermore, the Gotland Basin seems to be the only area in the north Baltic which has bottom salinity higher than 11psu. It is reasonable to deduce the hybridization process between plaice and flounder was not happening beyond the line of Öland - Gotland Basin - Irbe Strait (Fig. 1). These two proposed boundary lines only restrict the hybridization process between these two species, but not necessarily limit the distribution of the hybrid offsprings. It is totally possible and had been observed that hybrid offsprings between these two species spill over the aforementioned boundary and move to the vicinity areas (Sick et al. 1963).

The confirmed hybrid origin of some putative hybrids indicates that the natural environmental factors, which could affect inter-species reproduction (physical

environment, chemical environment, behavior etc.), were suitable enough to produce hybrids. However, the uni-directional backcross patterns observed during this study suggest that the reproductive process between the male plaice and female flounders was not successful in the natural environment. Previous studies suggested that a low rate of egg fertilization and severely deformed hatchlings were reasons contributing to the failure of hybridization between male plaice and female flounder in experimental conditions (Kandler 1935; Riley and Thacker 1969). To further explore what affects this inter-species reproduction process and led to the aforementioned phenomenon, additional artificial breeding experiments should be undertaken. Specifically, experiments should be designed to test hybrids' abilities to reproduce with plaice and flounder populations from the same sampling area under all gender combinations and what factors may facilitate or prevent reproductive success between the three groups.

Hybrids are usually sold by fishermen as plaice (Prof. Jarle Mork per.com). Reliance on only coarse morphological traits can lead to misidentifications and under-representation of hybrids in records (Kijewska et al. 2009). The misidentification of hybrids as the purebred stock could potentially propagate errors in studies of flatfish stocks or when making fisheries management considerations like establishing fishing quotas. The two diagnostic morphological traits (vertebrae number and scale throne number) along with diagnostic sites in different nuclear markers' sequence fragments described here provide useful tools for more accurate species identification and stock assessment. Confirmation of hybridization events and identification of tools to study these processes make valuable contributions to our general understanding of the role hybridization plays in evolutionary processes and biodiversity.

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TABLES

Table 1 Morphological trait counts for flounder *Platichthys flesus* (N=25), plaice *Pleuronectes platessa* (N=22), and putative hybrids (N=21). All counts were conducted following Eschmeyer (1990) and (von Ubisch 1950), p-values from t-tests of count comparisons between the three groups.

Morphological traits (mean value±SD) Samples (N)	Anal fin rays	Dorsal fin rays	Caudal fin rays	Pectoral fin rays	Vertebrae	Scale thrones
<i>P. platessa</i> (22)	51.41±2.12	68.23±3.27	19.00±0.60	11.20±0.54	42.36±1.71	1.36±0.48
Putative hybrids (21)	49.24±2.60	66.48±3.76	18.52±0.73	11.60±0.71	40.62±1.29	6.14±2.34
<i>P. flesus</i> (25)	40.76±1.86	58.56±2.32	17.68±0.55	10.87±0.62	34.56±0.64	18.84±4.98
Comparison categories	P-values					
<i>P. platessa</i> vs. Putative hybrids	0.0055**	0.1191	0.0280*	0.1054	0.0000**	0.0000**
Putative hybrids vs. <i>P. flesus</i>	0.0000**	0.0000**	0.0001**	0.0070**	0.0000**	0.0000**
<i>P. platessa</i> vs. <i>P. flesus</i>	0.0000**	0.0000**	0.0000**	0.1404	0.0000**	0.0000**

*P<0.05; **P<0.01