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Crabs, scallops, fish, and more: barcoding the marine fauna of the North Sea

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Background: During the last years, the effectiveness of DNA barcoding for animal species identification has been proven in many studies, analyzing both vertebrate and invertebrate taxa. In terms of marine organisms, however, most barcoding studies typically focus on economically relevant species, for example, fish, as well as on the documentation of hotspots of species diversity, for example, tropical coral reefs or regions of the almost unexplored deep sea regions. In contrast to this, species diversity of “well-known” habitats is nearly neglected. As part of our running project we started to build up a comprehensive DNA barcode library for the metazoan taxa of the North Sea, one of the most extensively studied ecosystems of the world. The North Sea is characterized by a high amount of anthropogenic pressure such as intensive fishing and ship traffic as well as offshore installations. Environmental parameters (e.g., depth, sediment characteristics, temperature, and salinity) of this semi-enclosed shelf sea follow a distinct pattern: high seasonal fluctuations can be observed in southern areas, but low fluctuations are given in the northern regions. This heterogeneity is also displayed in macrobenthic community structures, with a lower number of species in the shallow southern parts (i.e., the German Bight) and more species in the central and northern North Sea. In addition to this, species with a typical Mediterranean-Lusitanian distribution are also known to occur in parts of the North Sea where oceanic influences prevail. **Results:** Our barcode library includes a broad variety of taxa, including typical taxa of marine barcoding studies, for example, fish or decapod crustaceans. Our on-growing library also includes groups that are often ignored as cnidarians, parasitic crustaceans, echinoderms, mollusks, pantopods, polychaets, and others. In total, our library includes more than 4200 DNA barcodes of more than 600 species at the moment. By using the Barcode of Life Data Systems (BOLD), unique BINs were identified for more than 90% of the analyzed species. **Significance:** Our data represent a first step towards the establishment of a comprehensive DNA barcode library of the Metazoa of the North Sea. Despite the fact that various taxa are still missing or are currently underrepresented, our results clearly underline the usefulness of DNA barcodes to discriminate the vast majority of the analyzed species. It should be also kept in mind that the benefits of DNA barcoding are not restricted to taxonomic or systematic research only. The rise of modern high-throughput sequencing technologies will change biomonitoring applications and surveys significantly in the coming years. Following this, reference datasets such as ours will become essential for a correct identification of specimens sequenced as part of a metabarcoding study. This is especially true for the North Sea, a marine region that has been massively affected by cargo ship traffic, the exploitation of oil and gas resources, offshore wind parks, and in particular extensive long-term fisheries.

DNA barcoding of *Pteris* species by *psbA-trnH* intergenic spacer: taxonomically complex and polyploid ferns

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The fern genus *Pteris* is taxonomically complex to identify based on morphological characters in wild ecosystems. *Pteris* species were proved to

exhibit bio-accumulation and antioxidant activities in previous studies. The DNA was isolated from the plant samples using a modified CTAB method (cetyl trimethyl ammonium bromide). PCR amplification of the *psbA-trnH* intergenic spacer was carried out using following gene-specific primers: F 5'-GTTATGCATGAACGTAAGCTC-3'; R 5'-CGCGCATGGTGGATTCAAATCC-3' in an Eppendorf thermal cycler (Eppendorf, Germany). The PCR conditions were as follows: an initial denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation for 1 minute at 94°C, annealing for 1 minute at 55°C, and extension for 1 minute at 72°C; the final extension was at 72°C for 10 minutes. Then the PCR product was separated in 1% agarose gel with ethidium bromide, and the bands were visualized in a gel documentation unit (Alpha Digidoc, USA). The evolutionary relationships of six *Pteris* species—namely *P. vittata* L. (NCBI Accession No: JX987795), *P. multiaurita* Ag (JX987792), *P. confusa* T.G.Walker (JX987791), *P. otaria* Bedd (JX987793), *P. tripartita* Sw. (JX987794), and *P. argyrea* T.Moore (JX987790)—were studied using DNA sequence data, and the sequences were submitted to NCBI. The sequence alignments were carried out using Clustal X software for multiple sequence alignment, with a final alignment length of 402 bp. We found variability in the sequence lengths among species: 355 bp (*P. argyrea*), 161 bp (*P. confusa*), 352 bp (*P. multiaurita*), 357 bp (*P. otaria*), and 300 bp (*P. vittata*), suggesting that *P. multiaurita* was very closely related to *P. otaria*. Furthermore, while comparing *P. tripartita* with other species of *Pteris* by pairwise analysis, *P. tripartita* is the most similar to *P. argyrea* (9 bp exhibited nucleotide differences between species), *P. multiaurita* (7 bp), and *P. otaria* (8 bp), with 38%, 43%, and 37% gaps, respectively, between their sequences, while the *P. vittata* (77 bp) and *P. confusa* (183 bp) showed more genetic divergence along with 25% and 58% of gaps, respectively. Among six *Pteris* species, significant distinct genetic variation was observed between *P. tripartita* and *P. confusa*. The evolutionary history was inferred by the maximum parsimony method. The present study revealed that DNA sequence data of the chloroplast *psbA-trnH* region was useful for resolving inter-specific relationships and performing identification among six taxonomically complex *Pteris* species.

“Ethnoichthyogenomics”: identification, phylogeny and documentation of ethnomedicinally important fishes using DNA barcodes

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Background: Fishes play a vital role as traditional medicine in healing practices of many coastal nations. They are used in the treatment of various ailments such as wound healing, joint pain (arthritis), and asthma in Pondicherry and Tamil Nadu coastal villages, aside from being a very important part of the cultural and religious life. We used DNA barcoding as a tool for species identification to confirm authenticity and unravel adulteration. **Results:** Ethnomedicinally important fishes (6) have been collected based on traditional knowledge of practitioners and local fishermen of the coastal villages of Pondicherry and Tamil Nadu, India. Based on various classical morphometric characters, the fish were identified as *Himantura imbricata*, *Naso annulatus*, *Siganus javus*, *S. luridus*, *Trichiurus lepturus*, and *Leiognathus bindus*. These were authenticated by DNA barcoding the mitochondrial COI gene. We also attempted genetic documentation and assessed the phylogenetic status of these fishes. **Significance:** The goal of the study was to create Biological Reference Material (BRM) for ethnomedicinally important fishes from the east and west coast of India under the title “ethnoichthyogenomics” in order to understand traditional use, active compounds, and disease cure. Furthermore, this study explored the biodiversity and evolutionary relationships using DNA sequences to determine potential cryptic species, the distribution of rare species, their ecological needs, including traditional ecological knowledge so that management and conservation strategies can be implemented.