GENETIC RELATIONSHIPS AMONG STURGEONS REVEALED BY RAPD ANALYSIS

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RAPD analysis was utilised to differentiate six sturgeon species. Amplifications with different primers gave reproducible electrophoretic patterns which could be regarded as a data-set consisting of monomorphic and polymorphic characters. Our results provided evidence that RAPD markers can be used to discriminate between species of sturgeons and indicate new possible systematic considerations within the examined taxa. Some of the species-specific markers were isolated and cloned in order to provide effective molecular probes to distinguish the different sturgeons species. The present study investigated molecular markers to identify the following species of sturgeons and to study their systematic relationships: *Huso huso* Linnaeus, *Acipenser naccarii* Bonaparte, *A. baerii* Brandt, *A.r gueldenstaedtii* Brandt, *A. transmontanus* Richardson and *A ruthenus* Linnaeus. To this end, we applied a molecular technique based on the Polymerase Chain Reaction (PCR) known as Random Amplified Polymorphic DNA analysis. This technique provides genetic markers, derived from priming sites randomly distributed throughout the genome, inherited in a mendelian fashion. These markers allow analysis of complex genomes, like eukaryotes, without prior knowledge of the DNA sequence. From a previous screening of a wide set of primer-template combinations, we chose eight primers that gave a high number of polymorphic markers and a highly significant percentage of comigrating bands, assumed to be monomorphic characters. The selected primers were then employed to verify their reproducibility in independent experiments, the most important requirement for any RAPD analysis. The method has proven valuable as it is able to distinguish the different species These markers were isolated, cloned and sequenced, providing molecular data to

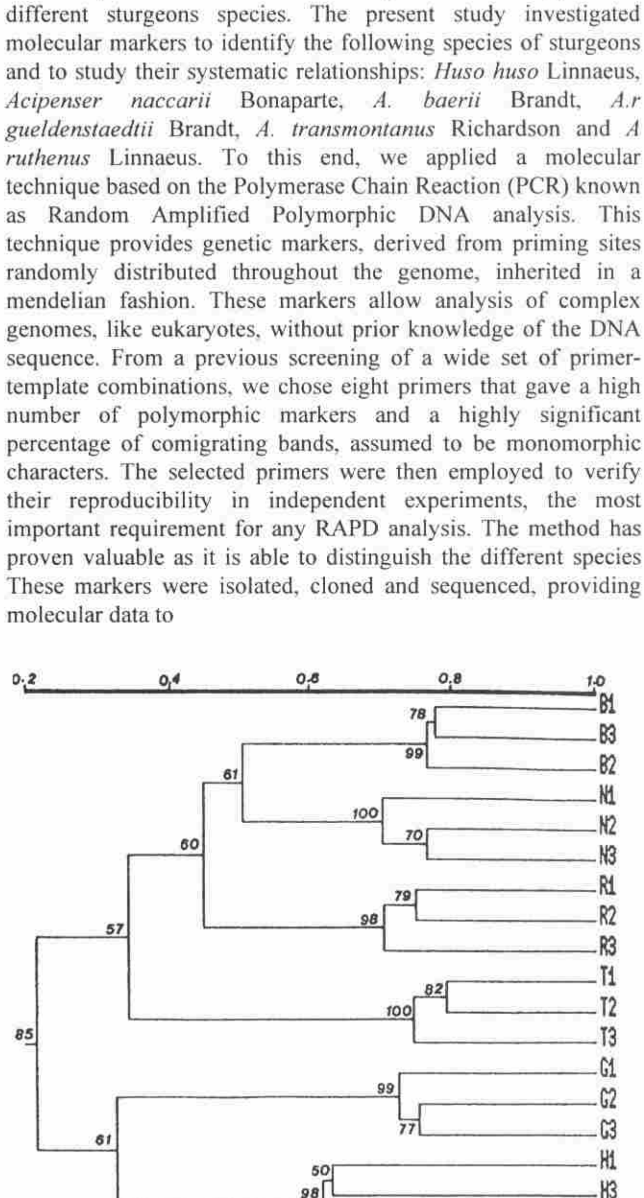

Fig. UPGMA dendrogram based on Dice similarity index. The number at the nodes are the bootstrap confidence values. The symbols referred to B, N, R, T, G, H (numbered from 1 to 3) indicated respectively *A.baerii*, *A.naccarii*, *A.ruthenus*, *A.transmontanus*, *A.gueldenstaedtii* and *H.huso*.

design species-specific probes. DNA markers at an intraspecies level were also produced. These polymorphisms reflect different degrees of genetic variability in the genome. The species relationships obtained by RAPD method, revealed by the topology and the different values of similarity of the species in the dendrogram (Fig) are in good agreement with their geographical distribution and their ecology, where environmental salinity is a primary factor. The RAPD analysis provides an efficient and sensitive method to discriminate among sturgeon species and may contribute our knowledge on sturgeon taxonomic relationships.

A MORPHOLOGICAL STUDY OF NATIVE STURGEON *Acipenser sturio* IN SPAIN, AND RECENT RECORDS OF EXOTIC SIBERIAN STURGEON *A. baerii*

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A morphological study of sturgeons kept in Spanish collections was performed to confirm the taxonomic status of native populations of sturgeon, and to describe their variability.

Specimens from twelve museums in Barcelona, Cabra (Córdoba), Granada, Madrid, Puerto de Santa María (Cádiz), Santander, Santiago de Compostela (A Coruña) and Sevilla were researched. This sample consists of 26 preserved (18 stuffed and 8 in alcohol) sturgeons. In a former review, Elvira *et al.* (1991) only reported 10 specimens housed in Spanish collections. These sturgeons were fished between the 19th century and 1988. 15 specimens have a known locality (Bay of Biscay, Cantabria, 1 ex.; San Vicente de la Barquera, Cantabria, 1 ex.; River Guadalquivir, 3 ex.; Cádiz, 3 ex.; River Ebro, 6 ex., and Mediterranean Sea, Spain, 1 ex.), while the others are only presumed to have come from Spanish rivers or seas.

20 specimens (standard length range=66.5-1640 mm) were measured in detail. The remainder were broken or in very bad condition. Comparative material included: one specimen of *A. sturio* (Sl=380 mm) from central Europe, four specimens of *A. naccarii* (Sl range=177-690 mm) from a fish farm, and 24 specimens of *A. baerii* (Sl range=148-620 mm).

39 morphometric and 13 meristic characters, mainly standardized according to Holcík *et al.* (1989a), were taken. The data matrix of metric and meristic features of different samples was studied using standard taxonomic numerical techniques. The correlation and distance matrices of samples were clustered by UPGMA, and the results presented in the form of phenograms. A minimum spanning tree was also developed from the distance matrix using the NTSYS software package. PCA was computed from the correlation matrix among characters. This numerical analysis indicated the existence of three groups corresponding to the three species.

Metric and meristic features of the Spanish sturgeons fall within the formerly described range of variation of *A. sturio* (Holcík *et al.* 1989b, and others). Bernini & Nardi (1989) considered the best diagnostic morphometric character for Mediterranean *Acipenser* species the index: distance between tip of snout and cartilaginous arch of mouth / internal width of mouth. This ratio also proved to be valid in determining all the native Spanish sturgeons as *A. sturio*. Nevertheless, one of the best diagnostic external characters for *A. sturio* is to have the skin between scute rows armored with rhombic plates arranged in oblique rows. This feature was first described by Berg (1948), and it is a synapomorphy for the Atlantic Sea sturgeons *A. sturio* and *A. oxyrhynchus* (Magnin 1962, Artyukhin 1995).

In conclusion, every native sturgeon from Spain was unequivocally determined as *A. sturio*. The adult female sturgeon fished in the Gulf of Cádiz in 1992, for which the only record is some colour photographs, also bears the skin pattern of *A. sturio* confirming our former identification (Elvira & Almodóvar 1993).

On the other hand, several specimens of the exotic Siberian sturgeon *A. baerii* were recently found in the river basins of Douro, Ebro and Guadalquivir. More than 50 specimens were fished in the River Ebro, between Flix (Lleida) and the Ebro Delta, from June 1995. Likewise, a solitary specimen was caught by a purse seiner in the River Guadalquivir at Coria del Río (Sevilla) in September 1995. Finally, several specimens were angled in the River Duratón at Fuentidueña (Segovia) near the confluence with the River Douro, from June 1996. The origin of these exotic introductions is not always clear, but *A. baerii* (brought from France) is commonly sold in Spanish pet shops, and there are also some fish farms which keep it in ponds.

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THE TEETH OF THE PADDLEFISH, *Polyodon spathula*

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The American paddlefish, *Polyodon spathula*, is a chondrostean fish that has numerous teeth when very young. Its generic name means "many toothed," but probably was named based on the large, numerous gillrakers. *Polyodon* starts filter feeding at age 26 days (Ballard, 1964), feeding mainly on zooplankton such as *cladocera*. Studies of a stained and cleared 35-day-old fish show obvious and numerous teeth visible on the dentary and maxilla. Teeth are found on other structures but are not visible here.

Teeth of *Polyodon spathula* develop early in their life. Yeager and Wallus (1982) report "tooth buds on the jaws, tongue and palate by 13mm total length (2½ days post hatching). Ballard and Needham (1964) state that *Polyodon* exhibits "tooth germs on both jaws beginning at stage 42 (15 days post hatching). In their paper, they illustrate a stage 43 fish (16 days post hatching) with clearly drawn teeth. Grande and Bemis (1991) clearly show teeth on a whole, stained and cleared 29mm total length specimen. (Approximately 35-40 days of age. Note that there is great variation in paddlefish growth rates, depending on the amount of available food) Visible in their specimen are maxillary and dentary teeth. Fink (1981) also reports only jaw teeth. Belyayeva (1991) reports teeth on the jaws and palatine (bone) in two to three rows. Our studies found paddlefish teeth in a variety of locations, some of which are in two rows at each location. One location, the roof of the mouth of a 35-day-old paddlefish, shows teeth on the maxillas and vomerine teeth patches. These vomerine teeth may previously have been reported as pointed tubercles. The floor of the mouth has teeth on the dentary, hypobranchial I and hypobranchial II. There are no teeth on the tongue. This tooth distribution parallels larval acipenserid distribution (Jollie, 1980).

Fink (1986) reports *Polyodon* teeth are of his type I, with complete ankylosis with the attachment bone, typical of actinopterygians. Studies of a cross section of a paddlefish dentary bone, show teeth and the ossified portion of the dentary. Attachment is complete, without any collagen or cartilage between tooth and bone. Cross sections of two hypobranchial arches with teeth reveal no supporting bone, only cartilage and perichondrium. Fink also states primitive actinopterygian teeth have a enameloid or acrodin cap laid down on collagen fibers, supported by denture, connected to the supporting bone by attachment bone. Paddlefish teeth appear to be of homogeneous composition, continuous with supporting bone or by cartilage. SEM studies also reveal a tooth without separate areas such as enameloid cap, dentine or predentine.

Teeth are lost in *Polyodon* in a gradual process (like sturgeon, Jollie, 1980) and are usually completely gone by 180 days. The teeth appear to be incorporated in the developing bones of the skull, as in sturgeon (Jollie, 1980). *Psepherus*, however, keeps its small teeth and is piscivorous throughout life. Mims, *et al* (1993) reports an intact 7.3 kg grass carp (cyprinidae) in the stomach of a 146 kg *Psepherus*. The small teeth (approximately 100 total on the maxilla and dentary) are used for catching and holding prey. Larval *Psepherus* are voracious feeders, feeding first on cladocerans and small decapods, then small fish, and finally larger fish.

In summary, young *Polyodon* has numerous teeth (up to several hundred, depending on the age) in various locations of its oral cavity, similar to other chondrosts. Like sturgeon, these teeth are lost with age. Young paddlefish, and perhaps adults, exhibit piscivorous behavior like *Psepherus*. Thus, *Polyodon* is anatomically and behaviorally linked to other chondrosts.

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