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A study on teleost phylogeny using specific antisera

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The phylogenetic position of 26 teleost species has been studied by the use of a dot blot test based on eight rabbit antisera generated against serum components from representatives from various teleost orders. The isolated positions of Anguilliformes, Cypriniformes, Salmoniformes and Clupeiformes were confirmed. Lophiiformes showed some affinities for Pleuronectiformes and less for Gadiformes.

Key words: rabbit antisera; fish phylogeny; dot blot test; fish serum proteins; fish immunoglobulin.

Molecular sequences (in nucleic acids and proteins) have been evaluated for establishing phylogenetic relationships between various teleost groups (Stock *et al.*, 1991). Likewise, the degree of cross-reactivity of protein antigens reflects evolutionary time (Wilson *et al.*, 1977). Thus the evolutionary distance between various fish species has been elucidated by investigating the cross-reactivity of specific antibodies raised against chum salmon *Oncorhynchus keta* (Walbaum) (Kobayashi *et al.*, 1981), Atlantic salmon *Salmo salar* (L.) (Håvarstein *et al.*, 1988), rainbow trout *Oncorhynchus mykiss* (Walbaum) (Thuvander *et al.*, 1990) and cod *Gadus morhua* (L.) (Israelsson *et al.*, 1991). The present study was implemented to contribute phylogenetic evidence from detecting cross-reactivity of eight antisera raised against serum components from teleost species representing major phylogenetic groups.

Rabbit antisera used: anti-European eel *Anguilla anguilla* (L.) immunoglobulin (Ig) (Buchmann *et al.*, 1992), anti-Japanese eel *Anguilla japonica* (Temminck & Schlegel) Ig (Iida *et al.*, 1991), anti-turbot *Scophthalmus maximus* (L.) Ig (Kofod *et al.*, 1994). Antisera against total serum from *G. morhua*, carp *Cyprinus carpio* (L.), *O. mykiss*, herring *Clupea harengus* (L.) and immunoglobulin (Ig) from *S. salar* were generated in individual rabbits, by at least three subcutaneous immunizations by approximately 100–200 µg antigen in 200 µl phosphate buffered saline (PBS) dissolved in equal volumes of Freund's complete adjuvant (first immunization) or Freund's incomplete adjuvant (following immunizations).

Fish sera used: blood samples were drawn from living fish by caudal vein puncture. After clotting serum was recovered and frozen at -20°C .

Dot blot test: all steps were conducted at room temperature. Three µl serum diluted in PBS (1 : 3) from each of a total of 26 fish species (Table I) was applied to nine nitrocellulose membranes (Sartorius), whereafter additional protein binding sites were blocked for 30 min in blocking buffer [PBS containing 2% bovine serum albumin (BSA) (Sigma)]. Membranes were then incubated for 1 h with the appropriate antiserum diluted 1 : 2000 in blocking buffer (except for rabbit antisera against turbot Ig and salmon Ig which were diluted 1 : 5000) or blocking buffer alone (control). After washing in PBS with 0.05% Tween 80 (3 × 15 min) the membranes were incubated for 1 h with peroxidase conjugated Swine anti rabbit Ig (Dakopatt P217) diluted 1 : 2000 in blocking buffer. After final washing the reactive sites were developed with diamino benzidine tetrahydrochloride (DAB) (Kem-En-Tec cat. no. 4150, Copenhagen, Denmark) in the presence of hydrogen peroxide.

TABLE I. Reaction in dot blot test of eight rabbit antisera raised against serum proteins from the teleosts listed in upper row towards serum components from a total of 26 teleosts representing nine teleost orders

Antiserum against: Fish species	<i>Gadus morhua</i> serum	<i>Scophthalmus maximus</i> Ig	<i>Salmo salar</i> Ig	<i>Oncorhynchus mykiss</i> serum	<i>Cyprinus carpio</i> serum	<i>Clupea harengus</i> serum	<i>Anguilla anguilla</i> Ig	<i>Anguilla japonica</i> Ig	Control
Gadiformes:									
<i>Gadus morhua</i> (L.)	++								
<i>Merlangius merlangus</i> (L.)	+++								
<i>Melanogrammus aeglefinus</i> (L.)	+++								
<i>Pollachius virens</i> (L.)	+++								
<i>Pollachius pollachius</i> (L.)	+++								
<i>Merluccius merluccius</i> (L.)	+++								
<i>Trisopterus minutus</i> (L.)	+++								
<i>Molva molva</i> (L.)	+								
Pleuronectiformes:									
<i>Scophthalmus maximus</i> (L.)		+++	++						
<i>Platichthys flesus</i> (L.)		++							
<i>Pleuronectes platessa</i> (L.)	+	++							
<i>Limanda limanda</i> (L.)	+	+++							
<i>Solea solea</i> (L.)		+++	++						
<i>Microstomus kitt</i> (Walbaum)		++							
<i>Hippoglossoides platessoides</i> (Fabricius)		(+)							
Perciformes:									
<i>Trachinus draco</i> (L.)		++	++						
<i>Lumpenus lampretaeformis</i> (Walbaum)			++						
Scorpaeniformes:									
<i>Myoxocephalus scorpius</i> (L.)	+	++	++						
Salmoniformes:									
<i>Salmo salar</i> (L.)			+++	++					
<i>Oncorhynchus mykiss</i> (Walbaum)			+++	++					
Cypriniformes:									
<i>Cyprinus carpio</i> (L.)					+++				
Clupeiformes:									
<i>Clupea harengus</i> (L.)			(+)			++			
<i>Sprattus sprattus</i> (L.)			(+)			++			
Anguilliformes:									
<i>Anguilla anguilla</i> (L.)			(+)			++	++	++	

The results (Table I) indicate a close relation between even strongly geographically separated members of the genus *Anguilla*, but also the isolated position of the order Anguilliformes. Similarly *C. harengus* and sprat *Sprattus sprattus* (L.) are closely related but the order Clupeiformes occupy a more isolated position among the teleosts. Inside the Euteleostei the Cypriniformes is an isolated order like the Salmoniformes, which, however, show stronger resemblance to other euteleosts. The order Gadiformes has representatives with high similarity of serum proteins and some affinities to members of the Superorder Acanthopterygii.

The cross-reactivity data suggest the correct grouping of Pleuronectiformes together with Scorpaeniformes and Perciformes in the superorder Acanthopterygii. The reactions of serum from anglerfish *Lophius piscatorius* (L.) (Lophiiformes) with rabbit antiserum raised against *S. maximus* and *S. salar* but not with rabbit anti *G. morhua* serum question the location of this order near the gadiformes in the superorder Paracanthopterygii. Also Nelson (1984) mentions the questionable relationship between Gadiformes and Lophiiformes.

Besides giving a contribution to teleost phylogenetic studies the present study indicates that specific rabbit antisera against immunoglobulin from a given species may find satisfactory application in other species. Thus anti-*S. salar* Ig is applicable to *O. mykiss* immunological studies which is in accordance with cross-reactivity studies in salmonids conducted by Kobayashi *et al.* (1982), Håvarstein *et al.* (1988) and Thuvander *et al.* (1990). Anti-*S. maximus* Ig serum can be used in *S. solea* (L.) and *L. limanda* (L.) and the *A. anguilla* Ig serum is applicable at least in *A. japonica* as well.

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