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Inter- and intraspecific variation in the surface pattern of the dermal bones of two sturgeon species

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Summary

Archaeological bone remains of sturgeon (*Acipenser sturio*/*Acipenser oxyrinchus*) from northwestern Europe are often identified to species on the basis of their surface morphology and then used to reconstruct the spatial distribution of the two species through time. The dermal bones of *A. sturio* are said to have an exterior surface pattern consisting of tubercles, while those of *A. oxyrinchus* are said to display alveoli. In the present paper, the validity of the surface pattern as a species-specific characteristic is critically assessed. To this purpose, dermal plates from modern, genetically identified museum specimens were studied and the surface morphology observed in a series of archaeological remains was compared with the genetic identifications obtained on these same remains. The analyses show that the surface pattern of dermal bones is related to the size of the individual, with the pattern of small *A. oxyrinchus* being similar to that of *A. sturio*. In addition, variations in the surface pattern among the bones of a single individual and within the same bone have been observed. These findings explain previous conflicting results between morphological and genetic identifications and allow the formulation of some recommendations for more accurate morphological identification of isolated archaeological sturgeon dermal bones.

Introduction

Traditionally, the only sturgeon species considered to be indigenous to Western Europe was the common, or European, sturgeon, *Acipenser sturio* Linnaeus, 1758 (Bemis and Kynard, 1997). Since 2002, the historical presence of the Atlantic sturgeon, *A. oxyrinchus* Mitchill, 1815, previously thought to be restricted in its distribution to the east coast of North America, has been demonstrated in the Baltic Sea and along the Atlantic coast of France (e.g. Ludwig et al., 2002, 2008; Desse-Berset, 2009, 2011; Chassaing et al., 2013; Popović et al., 2014). Archaeological sturgeon remains from Belgium, the UK and the Netherlands used to be attributed to *A. sturio* by default, but the possibility that *A. oxyrinchus* may also have occurred in the North Sea is now acknowledged (E. Thieren, A. Ervynck, D. Brinkhuizen, A. Locker, W. Van Neer, in review).

Different morphological parameters for the identification of *A. oxyrinchus* and *A. sturio* have been described for complete modern specimens (see Table 1). Morphological species identification of archaeological sturgeon remains is less straightforward because only the criteria pertaining to individual ossified elements are potentially suitable for species-level identification.

For the ossified dermal bones, such as the scutes, the surface structure needs to be considered. The pattern on the exterior, exposed part of the bone consists of pits and ridges formed by the fusion of denticle- or tubercle-like elements to the underlying bone. Although this pattern is extremely variable amongst acipenserids (Hilton et al., 2011), it is said to be species-specific for *A. sturio* and *A. oxyrinchus*. According to Magnin (1964), scutes and other dermal bones of *A. sturio* have small, round tuberculae and feel smooth to the touch, while those of *A. oxyrinchus* have small, deep alveoli separated by thin, sharp septa and feel rough to the touch.

The diagnostic character of the exterior pattern of the dermal bones has been used in other studies (e.g. Ludwig et al., 2008; Desse-Berset, 2009; Chassaing et al., 2013), but its reliability has been questioned (e.g. Becker, 2009). The results of the studies of Ludwig et al. (2008) and Chassaing et al. (2013), in which morphological and genetic species identifications of archaeological and museum specimens were compared, indicate that the surface structure of dermal bones is not a foolproof species-specific trait; when researchers use only this criterion, hybrid individuals may remain undetected. In addition, some ambiguity may arise when dealing with different-sized individuals, since the surface pattern of both *A. oxyrinchus* and *A. sturio* changes as they grow to adult size (Wuertz et al., 2011).

Because the morphological species identification of *A. sturio* and *A. oxyrinchus* based on the surface pattern of the dermal bones does not always correspond with the genetic identification and because the surface pattern seems to be size-dependent, its diagnostic value is assessed in the present study. To this purpose, museum specimens and archaeological remains of *A. sturio* and *A. oxyrinchus* are examined using a combined morphological and genetic approach.

Table 1
Species-specific morphological characteristics of *Acipenser sturio* and *A. oxyrinchus* described in the literature

Characteristic	*A. oxyrinchus*	*A. sturio*	Reference
Fontanel	Present between frontals and parietals; closes in larger individuals	Absent; frontals rhombic-shaped, with one or more frontal bones in between	Magnin (1964)
	Present between frontals and parietals; closes in individuals larger than 900 mm TL	Absent	Kottelat and Freyhof (2007)
	Present between frontals and parietals, also in large specimens	Absent	Artyukhin and Vecsei (1999)
	Present between frontals and parietals in young individuals		Vladykov and Greeley (1963)
Size and morphology of scutes	Bigger scutes compared to *A. sturio;* small, deep alveoli separated by thin, sharp edges that feel rough to the touch	Smaller scutes compared to *A. oxyrinchus,* with small tubercles that feel smooth to the touch	Magnin (1964)
	Larger scutes compared to *A. sturio,* has a 'spiny' type ornamentation	Smaller scutes compared to *A. oxyrinchus,* with a smooth type of ornamentation	Wuertz et al. (2011)[a]
	Alveolar scute surface (Baltic Sea)[b]		Debus (1999)
	Alveolar scute surface (Baltic Sea[b] and east coast of North America)	Tubercular scute surface	Tichij, 1929 fide Debus (1999)
	Larger scutes compared to *A. sturio,* with sharp-edged alveoli	Smaller scutes compared to *A. oxyrinchus;* has a tubercular surface	Desse-Berset (2011)
		Tubercular scute surface	Ninua, 1976 fide Debus (1999)
	Alveolar-radial scute surface (St. Lawrence River and eastern Baltic Sea[b])	Tubercular-radial scute surface	Artyukhin and Vecsei (1999)
	Dorsal scutes with radiating rows of alveoli	Dorsal scutes with radiating rows of tubercles	Kottelat and Freyhof (2007)
Number of dorsal scutes	10 (9.76 ± 0.018)	12–13 (12.54 ± 0.126)	Magnin (1963)[c]
	9.8 (7–13)		Vladykov and Beaulieu (1946); Vladykov and Greeley (1963)[c]
		9–10	Jordan (1910)[d]
	10	13–14	Desse-Berset (2011)[e]
		10–13	Mohr (1952)[d]
	8–12 (median 10)	12–15 (median 14)	Wuertz et al. (2011)[a,d]
	7–16	9–16	Vecsei et al. (2001) [c]
	St. Lawrence River: 9.76 (taken from Magnin (1963)?)	12.74 (taken from Magnin (1963) ?; probably excludes basal dorsal fin fulcrum)	Artyukhin and Vecsei (1999)[c]
	Baltic Sea[b]: 10.25 (9–12) (probably excludes basal dorsal fin fulcrum)		
Number of lateral scutes	29 (28.67 ± 0.062)	35 (35.13 ± 0.201)	Magnin (1963)[f]
	24–35 (mean 28.3)		Vladykov and Beaulieu (1946); Vladykov and Greeley (1963)[f]
		26–31	Jordan (1910) [d]
		24–40	Mohr (1952) [d]
	18–27 (median 23)	26–34 (median 30)	Wuertz et al. (2011)[a, d]
	24–35	24–40	Vasil'eva (1999)[d]
	24–35	24–40	Vecsei et al. (2001)[g]
	24–40	24–40	Kottelat and Freyhof (2007)[g]
	St. Lawrence River: 28.67 (taken from Magnin (1963)?) Baltic Sea[b]: 26–33 (mean 27.38)	35.13 (taken from Magnin (1963) ?)	Artyukhin and Vecsei (1999)[d]
Number of ventral scutes	10 (9.64 ± 0.071)	11 (11.03 ± 0.097)	Magnin (1963)
	9.55 (8–12)		Vladykov and Beaulieu (1946)
		9–10	Jordan (1910)
	7–11 (median 8)	7–11 (median 9)	Wuertz et al. (2011)[a]
		11–13	Mohr (1952);
	6–14	8–15	Vecsei et al. (2001)

Table 1
(Continued)

Characteristic	*A. oxyrinchus*	*A. sturio*	Reference
Correlation between fork- (FL) and total length (TL)	FL = 0.867TL+1	FL = 0.923TL−1.95	Magnin (1964)
Pectoral–pelvic distance (PV)	PV = 0.346TL−0.116	PV = 0.416TL+0.4084	
Pelvic–anal distance (VA)	VA = 0.126TL−0.328	VA = 0.145TL−0.800	
Maximum body height (H)	H = 0.141TL−1.007	H = 0.116 TL+0.375	
Minimum body height (h)	h = 0.320TL+0.274	h = 0.033 TL−0.049	
Caudal pedunculus (pc)	pc = 0.147TL+1.299	pc=0.130TL+0.890	
Dorsal fin base (Db)	Db = 0.080TL+0.800	Db = 0.098TL−0.537	
Head length (T)	T = 0.201TL+2.586	T = 0.195TL+1.510	
Distance rostrum–nostril (RN)	RN = 0.276T+2.35	RN = 0.244T+2.22	
Distance rostrum–eye RO	RO = 0.413T+1.75	RO = 0.360T+1.57	
Distance Rostrum–barbels (Rb)	Rb = 0.171T+2.69	Rb = 0.160T+1.88	
Distance rostrum–mouth (RB)	RB = 0.381T+2.69	RB = 0.330T+2.66	
Distance anal fin–caudal fin fork	Larger	Smaller	

[a]Juveniles only.
[b]At the time of this publication, the presence of *A. oxyrinchus* in the Baltic Sea was not yet confirmed.
[c]Basal dorsal fin fulcrum not included; occipital included for scute number counts.
[d]No indications of the definition of 'first' or 'last' scute are given.
[e]Basal dorsal fin fulcrum and occipital included for scute number counts.
[f]Supracleithrum included.
[g]Supracleithrum not included.

Materials and methods

Morphological species identification

The first author carried out morphological analyses of 65 sturgeons (Table A1) ranging in size from 17.5 to 300 cm total length (TL) from different European museums and one private collection. Other researchers had previously studied the morphology of some of these sturgeons (see Table 2).

All specimens in this study were re-identified morphologically, using the previously published criteria summarized in Table 1. Since the purpose of this study was to evaluate the surface pattern of dermal bones as a diagnostic characteristic, this criterion was not used during the initial morphological species identification. Although some body ratios described by Magnin (1964) were considered, specimens were identified mainly by the number of scutes and the presence of a fontanel between the frontals and parietals. For the dorsal scutes, we considered the occipital or median nuchal, which is fully incorporated into the roof of the skull, to be the first dorsal scute, and the last regular-shaped scute prior to the basal dorsal fin fulcrum to be the terminal dorsal scute. For the lateral scutes, we excluded the supracleithrum and considered the last scute having a ridge or crest along its short axis to be the terminal lateral scute. For the ventral scutes, we included all scutes between the pectoral and pelvic fins. When the number of scutes in the paired rows differed within a single individual, we calculated the average of the left and right rows. In some specimens, the poor state of preservation hampered the observation of some of the characteristics.

After species identification of the museum specimens based on morphological criteria, the external surface pattern of the dermal bones was recorded, when permitted by their state of preservation. The sturgeons were then assigned again to species, this time based solely on the surface pattern of the dermal bones. In a final step, the identification obtained on the surface pattern was compared with that based on the other characteristics in Table 1.

Archaeological specimens were classified according to the surface pattern of their dermal bones. When possible, the corresponding body length of the fish was calculated using the equations in Thieren and Van Neer (2016).

Genetic species identification

DNA isolation and sequencing. Tissue samples were taken from 35 museum specimens to validate their morphological species identification. For 11 of those specimens, genetic species identification was available from Ludwig et al. (2002) and Chassaing et al. (2013). Sixty-nine archaeological samples from excavations in Belgium, the Netherlands and the

Table 2
Details of the examined *Acipenser oxyrinchus* (oxy) and *A. sturio* (stur) specimens in museum collections, ordered by the taxonomic identification based on genetics and, within that, by size (TL [cm]), or, in the case genetic identification is missing, by the new identification based on genetics and morphology and, within that, by size

						Taxonomic identification based on morphology					
Museum collection[a] and inventory number	TL (cm)	Fontanel	Dorsal scutes	Lateral scutes	Ventral scutes	As published in the literature[b]	Based on Table 1 excluding ornamentation	Based on ornamentation only	Based on Table 1 including ornamentation for TL >1 m	Taxonomic identification based on genetics	New taxonomic identification based on genetics or morphology
NRM 8950	19.5	Present	9	28	9		oxy	stur	oxy	oxy (5)[c]	oxy (5)
MNHN-IC-B-2598	60	Present	10	30	9	oxy (2)	oxy	stur	oxy	oxy (6)	oxy (6)
MNHN-IC-1869-0127	71	Present	11	29	9.5		oxy	oxy	oxy	oxy (6)	oxy (6)
RBINS 24792	74	Present	9	23	9		oxy	Indet.	oxy	oxy (6, 8)	oxy (6, 8)
NRM 21707	77	Absent	13	28.5	11		Indet.	stur	Indet.	oxy (4, 5)	oxy (4, 5)
NRM 49317	77.5	Present	10	31	11		stur	Indet.	stur	oxy (6)	oxy (6)
RBINS 1528	78	Present	10	29	9		oxy	stur	oxy	oxy (6)[c]	oxy (6)
MNHN-IC-0000-3494	86	Present	10	29.5	9	oxy (2, 3)	oxy	oxy	oxy	oxy (6)[c]	oxy (6)
NRM60821	99	Present	10	28	8		oxy	Indet.	oxy	oxy (7)	oxy (7)
NRM60292	102.5	Present	9	22.5	9		oxy	stur	oxy	oxy (5, 7)[c]	oxy (5, 7)
MNHN-IC-0000-3108	139	Absent	11	29	10.5	oxy (1), stur (2, 3)	oxy	oxy	oxy	oxy (1, 6)	oxy (1, 6)
NRM 61784	146	Absent	10	25	8		oxy	oxy	oxy	oxy (7)	oxy (7)
NRM 1709	146.7	Present	11	32	12		stur	oxy	oxy	oxy (4, 5)[c]	oxy (4, 5)
NRM 35438	154.5	Absent	10	30	11		stur	oxy	oxy	oxy (6)[c]	oxy (6)
MNHN-IC-0000-3113	158.5	Present	10	32	10	oxy (3)	Indet.	oxy	oxy	oxy (6)[c]	oxy (6)
MNHN-IC-0000-3110	186	Absent	9	27.5	9	oxy (3)	oxy	oxy	oxy	oxy (6)	oxy (6)
SML N/A	250	N/A	11	27	10		oxy	oxy	oxy	oxy (6)[c]	oxy (6)
MHNN Z19558	276	Present	10	27	9	oxy (1)	oxy	oxy	oxy	oxy (1)	oxy (1)
MNHN-IC-0000-3573	300	Absent	N/A	N/A	N/A	oxy (1), stur (2, 3)	N/A	oxy	oxy	oxy (1)	oxy (1)
NRM 8948	31.5	Absent	13	35.5	11.5		stur	stur	stur	stur (5)[c]	stur (5)
NRM 21708	38	Present	11	28.5	9.5	stur (4)	oxy	stur	oxy	stur (5)[c]	stur (5)
NRM 21710	47.2	Present	10	26	10	stur (4)	oxy	stur	oxy	stur (5)[c]	stur (5)
NRM 35435	52.3	Absent	12	32	12		stur	stur	stur	stur (5)[c]	stur (5)
MHNN Z19398	61	Absent	14	41	13	stur (1)	stur	stur	stur	stur (1)	stur (1)
MNHN-IC-0000-3493	66.7	Absent	12	31	10.5	oxy (2)	stur	stur	stur	stur (6)	stur(6)
MHNN Z58275	70	Present	11	33	12	stur (1)	stur	stur	stur	stur (1)[c]	stur (1)
NRM 13336	134	Absent	14	39	11	stur (4)	stur	stur	stur	stur (4, 5)	stur (4, 5)
MHNN Z58274	151	Absent	14	38	11	stur (1)	stur	stur	stur	stur (1)	stur (1)
MNHN-IC-0000-3119	153	Absent	14	36.5	12.5	stur (1, 2, 3)	stur	stur	stur	stur (1)	stur (1)
NRM 35439	154.5	Absent	11	32	10	oxy (4)	stur	oxy	oxy	stur (5)[c]	stur (5)
RBINS N/A (1)	223	Absent	13	38	10.5		stur	stur	stur	stur (6)[c]	stur (6)
NRM 36001	260	Absent	N/A	N/A	N/A	stur (4)	stur	Indet.	stur	stur (4, 5)[c]	stur (4, 5)
NRM 21712 (small)	81.3	Present	10	30	10.5		oxy	stur	oxy	Hybrid (6)	Hybrid (6)
NRM 21712 (medium)	96.2	Absent	12	29.5	11.5		stur	stur	stur	Hybrid (6)	Hybrid (6)
NRM 21712 (large)	110.5	Absent	11	26.5	9		Indet.	Indet.	Indet.	Hybrid (6)	Hybrid (6)
MNHN-IC-0000-3115	148	Absent	9	27	11	oxy (1), stur (2)	Indet.	oxy	oxy	Hybrid (1, 6)	Hybrid (1, 6)
RBINS N/A (2)	231.5	Absent	11	30	12		stur	oxy	oxy	Hybrid (6)	Hybrid (6)
NRM 21711	36	Present	10	29	11		oxy	stur	oxy		oxy

Table 2
(Continued)

Museum collection[a] and inventory number	TL (cm)	Fontanel	Dorsal scutes	Lateral scutes	Ventral scutes	Taxonomic identification based on morphology				Taxonomic identification based on genetics	New taxonomic identification based on genetics or morphology
						As published in the literature[b]	Based on Table 1 excluding ornamentation	Based on ornamentation only	Based on Table 1 including ornamentation for TL >1 m		
MNHN-IC-0000-9114	51	Present	9	23	8.5	oxy (2, 3)	oxy	Indet.	oxy		oxy
NRM 21705	57	Present	10	27.5	10.5	stur (4)	oxy	stur	oxy		oxy
NRM 55538	65.2	N/A	9	28	9		Indet., probably oxy	stur	Probably oxy		Probably oxy
NRM 36074	78.5	Present	10	28	10		oxy	Indet.	oxy		oxy
NHM 2005.6.22.6	80.2	Present	10	29	10		oxy	oxy	oxy		oxy
MNHN-IC-0000-4843	88.4	Absent	12	27.5	10	oxy (2); stur (3)	oxy	oxy	oxy		oxy
AML 8797	93	Present	9	29	9.5		oxy	oxy	oxy		oxy
NHM 1859.3.15.1	128	Present	10	33	13		stur	oxy	Probably oxy		Probably oxy
KUL MD N/A	160.5	Absent	10	27	10		oxy	oxy	oxy		oxy
MRSN N/A	210	Present	11	33	12		stur	oxy	Probably oxy		Probably oxy
NMB N/A	226.5	Present	11	N/A	11		oxy	oxy	oxy		oxy
NHM 1865.5.23.3	237.5	Present	12	31	10		stur	oxy	Probably oxy		Probably oxy
MNHN-IC-0000-3574	245	Absent	9	N/A	N/A	oxy (2, 3)	N/A	oxy	Probably oxy		Probably oxy
RBINS 4449	56	Absent	12	29	9.5		stur	stur	stur		stur
NRM 35442	69	Present	13	30.5	13		Indet., probably stur	Indet.	Probably stur		Probably stur
NRM 18265	73	Absent	13	32	10		Indet., probably stur	stur	stur		stur
DCB 721	90.6	Absent	12	33	9		stur	Indet.	stur		stur
BAI 1884	94	N/A	12	32	11		stur	stur	stur		stur
RBINS N/A (5)	118.5	Absent	14	36	12		stur	stur	stur		stur
NHM 1931.12.7.1	142	Absent	14	36	9		stur	stur	stur		stur
NHM 2015.2.18.1	160.5	Absent	11	40	11		stur	stur	stur		stur
NRM 94	17.7	Present	11	30	10		Indet.	stur	Indet.		Indet.
NHM 1986.5.21.1	123	Absent	14	36	11		stur	oxy	Indet.		Indet.
NRM 36002	140	N/A	12	36	11	oxy (4)	stur	oxy	Indet.		Indet.
UUZM UPSZTY 170	195.5	Absent	12	30	9.5		stur	oxy	Indet.		Indet.
NHM 1886.8.24.1	204.5	N/A	12	31	9		stur	oxy	Indet.		Indet.
UUZM UPSZTY N/A	222.5	Absent	11	33.5	13		Indet., probably stur	oxy	Indet.		Indet.

Indet.: indeterminate; N/A: no information available.

[a]BAI: Biologisch-Archaeologisch Instituut, now Groningen Institute of Archaeology, the Netherlands; DCB: D.C. Brinkhuizen personal collection, Groningen, the Netherlands; KUL MD: KULeuven Museum voor Dierkunde, Leuven, Belgium; MHNN Nantes: Muséum d'histoire naturelle de Nantes, France; MNHN: Muséum national d'histoire naturelle, Paris, France; MRSN: Muséum régional des sciences naturelles, Mons, Belgium; NHM: Natural History Museum, London, UK; NMB: Natuurmuseum Bruinenberg, Westereen, the Netherlands; NRM: Naturhistoriska riksmuseet, Stockholm, Sweden; RBINS: Royal Belgian Institute of Natural Sciences, Brussels, Belgium; SML: Stadsmuseum Lokeren, Belgium; UUZM: Evolutionsmuseet, Uppsala, Sweden.

[b]Numbers in parentheses indicate the source for the identification: (1) Chassaing et al. (2013), morphological identification based on scute surface structure and number of scutes; (2) Vasil'eva (1997), morphological identification criteria unknown; (3) Magnin (1964), morphological identification based on provenance; (4) Ludwig et al. (2002), morphological identification based on criteria in Magnin (1964) and Artyukhin and Vecsei (1999); (5) Institute of Genetics and Biotechnology (IGiB) of the University of Warsaw, Poland; (6) Joint Experimental Molecular Unit (JEMU) of the Royal Belgian Institute of Natural Sciences and the Laboratory of Forensic Genetics and Molecular Archaeology (ACLFOR) of the University of Leuven, Belgium; (7) Reintroduced tagged specimen of *A. oxyrinchus*; (8) Aquaculture specimen of Canadian *A. oxyrinchus* stock obtained from Joosen-Luyckx Aqua Bio, Turnhout, Belgium.

[c]identified by mtDNA only

UK, dating from the Neolithic to the 18th century AD, were also selected for genetic species identification (Table A2).

DNA analyses were carried out either in the laboratory of the Joint Experimental Molecular Unit (JEMU) of the Royal Belgian Institute of Natural Sciences and the Laboratory of Forensic Genetics and Molecular Archaeology (ACLFOR) of the University of Leuven, Belgium, or in the Institute of Genetics and Biotechnology (IGiB) of the University of Warsaw, Poland.

JEMU & ACLFOR. DNA extractions of museum specimens were conducted in the archive DNA laboratory of JEMU that was especially designed for working with museum samples. DNA from archaeological samples was extracted in the facilities of ACLFOR dedicated to the analysis of ancient DNA. Amplification by means of standard PCR and sequencing of DNA of both museum and archaeological samples took place at ACLFOR. Pre- and post-PCR procedures were carried out in laboratories that were physically separated, and both museum and archaeological samples were handled using standard contamination precautions (Gilbert et al., 2005). Separate pre-PCR rooms were used for storage and handling of DNA from the museum and archaeological samples. No sturgeon had ever been analysed in either of the facilities prior to this study.

Samples from museum specimens included both fluid-preserved soft skin tissue (approx. 0.25 cm^3) and fin clips from dry-mounted specimens. Prior to extraction, fluid-preserved samples were washed three times in 1.5 ml HPLC-grade bottled water and cut into small pieces with a sterile scalpel. DNA was extracted and purified with a silica-membrane kit (QIAamp Minikit Qiagen, Hilden, Germany) after overnight incubation at 56°C. DNA was eluted by two elution steps, each using 120 μl of elution buffer incubated for 5 min at room temperature. Tissue from dry specimens was first cleaned with a sterile blade and then UV-irradiated and ground with a mortar and pestle. Samples were incubated at room temperature for 24 h in 1.5 ml EDTA 0.5M pH 8.0 and washed three times with HPLC-grade bottled water; extraction continued thereafter as with the fluid-preserved samples.

Archaeological sturgeon samples consisted of dermal bones, which were cleaned with a sterile blade and UV-irradiated to remove surface contamination. The bone was ground in a 6770 Freezer/Mill (Spex CertiPrep, Metuchen, NJ), after which 200 mg of bone powder was incubated in 1.6 ml lysis buffer (0.5M EDTA, pH8.0 and 0.1 mg/mL proteinase K) for 24 h at 56°C, followed by 24 h at 37°C. DNA was purified with the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany) following the manufacturer's guidelines, producing a final elution of 100 μl of DNA extract for each sample. Whenever the amount of available tissue allowed, two DNA extractions per sample were carried out.

Two non-overlapping fragments of cytochrome *b* were amplified with the primers CytB-Acip-FWD 5′-CCGAAATATTCATGCAAACGGGGC-3′ and CytB-Acip-REV 5′-TGGAGGTATGAGCCGTAGTATATGCC-3′ (90 bp) and with H15392-Acip-FWD 5′-GACAAAGTAACATTCCACCC-3′ and H15497-Acip-REV 5′-TAAAGTTGTCTGGGTCGC-3′ (124 bp) (Teletchea et al., 2008). Carry-over prevention strategies based upon uracil-N glycosylase (UNG) and dUTP were followed (Pruvost et al., 2005). DNA was amplified using 5–10 μl extract and 2.5 U Taq Gold (Applied Biosystems, Foster City, CA) in a final reaction volume of 50 μl, containing 1× Buffer II and 2.5 mM $MgCl_2$ (Applied Biosystems), 1.25 mM dUTPs mix (dATP, dCTP, dGTP 0.25 mM each, dUTP 0.5 mM, Bioline, London, UK), 0.5U of UNG (ArcticZymes, Tromsø, Norway), 100 nM of each primer (IDT, Coralville, IA), and 1 mg/ml UV-BSA (Sigma-Aldrich, St Louis, MO). PCR was performed under the conditions: 1 cycle at 37°C for 15 min; 1 cycle at 94°C for 10 min; 50 cycles at 94°C for 45s, at 58°C for 1 min and at 72°C for 1 min; and a final polymerization of 5 min at 72°C. During preparation of the samples for pulverization, extraction and PCR set-up, strict precautions to minimize the risk of contamination were followed, as described elsewhere (Ottoni et al., 2013). At least two independent PCRs were carried out on each DNA extract.

Two microsatellite markers, *LS68* (136-172 bp) and *D161* (120-173 bp), were amplified with primers described in May et al. (1997) and Henderson-Arzapalo and King (2002). For *LS68*, the forward primer was labelled with a fluorochrome for size determination by capillary electrophoresis. PCRs were performed in a singleplex with the Qiagen Multiplex PCR kit, according to the manufacturer's guidelines. Amplification was performed as: 1 cycle at 94°C for 15 min; 40 cycles at 94°C for 30 s, at 58°C for 90 s, and at 72°C for 90 s; and a final polymerization of 10 min at 72°C.

Sequences were aligned manually using BioEdit Software v.7.2.5 (Hall, 1999) to reference sequences obtained from the GenBank of *A. sturio* (accession numbers for Cyt *b*: AJ245839, AJ428497, FJ974043, AF283742, AF217209, AF006134, FN256388 and FN256389; for *LS68*: FR689727 and FR689728; for *D161*: FR686860–FR686863) and *A. oxyrinchus* (accession numbers for Cyt *b*: AJ245838, L35111, AF308923 and AF006128; for *LS68* FR689729–FR689738; for *D161* FR686853–FR686859).

IGiB. Extractions of DNA from 14 ethanol-preserved museum specimens were performed in a sterile room dedicated to the analysis of ancient DNA at the Institute of Genetics and Biotechnology of the University of Warsaw. The room was over-pressurized and equipped with a system of airlocks and had never been used for work with contemporary DNA samples or PCR products. The room was UV-irradiated when not in use. Tissue fragments were washed with double-distilled water. DNA extraction was performed following the protocol described in Popović et al. (2014). A 185 bp fragment of the mitochondrial control region was amplified using the primers Hetero1 and Hetero2 (Ludwig and Jenneckens, 2000). A 150 bp fragment of cytochrome b was amplified using the primers CYTBACI1 and CYTBACI1REV (Popović et al., 2014). All PCR and sequencing reactions were conducted according to Popović et al. (2014). Two independent PCR reactions were performed and sequenced for each of the samples. Consensus sequences were

called in BIOEDIT Software v.7.0.5.3 (Hall, 1999) and aligned to reference sequences of *A. sturio* and *A. oxyrinchus* (GenBank accession numbers AF006145 and AF162716, respectively).

Results

Museum specimens

Of the 65 museum specimens identified to species on the basis of morphology using the criteria in Table 1 excluding the surface pattern, 25 specimens were morphologically identified as *A. oxyrinchus* and 29 as *A. sturio* (Table 2). A further nine morphological analyses were inconclusive, and for two other specimens the data were insufficient for an accurate species assignment (Indet. and N/A in Table 2). Of the 65 specimens, 23 were previously identified based on morphology by Magnin (1964), Vasil'eva (1997), Ludwig et al. (2002) and/or Chassaing et al. (2013). The identifications disagreed with each other in four cases, and with our own morphology-based identifications in eight cases (Table 2). Therefore, morphological identifications were genetically validated using the DNA data obtained by either Ludwig et al. (2002), Chassaing et al. (2013), or this study.

Genetic species identifications were obtained for 37 specimens, of which 23 had not been genetically analysed previously. Both mtDNA and nDNA could be retrieved from 20 of the 37 specimens; five were identified as *A. sturio*, 10 as *A. oxyrinchus* and five as hybrids. For the remaining 15 specimens, only mtDNA sequences were available, meaning that it was not possible to exclude hybridization.

One of the seven *A. oxyrinchus* identified only by mtDNA, was a tagged animal from the reintroductory program for *A. oxyrinchus* in the Baltic Sea. This specimen, as well as two other tagged animals from the same program that were not genetically tested, were considered to be *A. oxyrinchus*. In total, 19 *A. oxyrinchus*, 13 *A. sturio* and five hybrids were identified through our own or through previously published genetic analyses (Table 2).

Comparisons were made between the genetic and the morphological species identifications (Table 3); in most cases the molecular and morphological species identifications correspond. As expected, the fish genetically identified as *A. oxyrinchus* tend to have a lower number of scutes than the sturgeon identified as *A. sturio* (Fig. 1). In total, 15% of the genetically identified *A. sturio* was classified as *A. oxyrinchus* based on the morphological characteristics in Table 1 (excluding the surface pattern of dermal bones). For the genetically identified *A. oxyrinchus*, this percentage was similar (19%). However, because some of the genetic identifications are based solely on mtDNA sequences, we cannot exclude the possibility that these individuals might be hybrids.

Two of the five hybrid specimens were morphologically assigned to *A. sturio* and one to *A. oxyrinchus*. For the other two, the morphological species identification remained inconclusive. Because of the risks of misclassification, the validity of the surface pattern as a species-specific characteristic was studied on genetically identified specimens only.

The surfaces of the dermal bones of the genetically identified sturgeons were evaluated visually (Table 4). Although the described alveolar *A. oxyrinchus* type and tubercular *A. sturio* type are distinguishable, there appear to be noticeable intra- and interindividual variations within each type. Some sturgeons display an intermediate type or both types, sometimes within the same scute row or within the same scute.

Among the 19 genetically identifiable *A. oxyrinchus*, most of the smaller specimens (smaller than ~1 m TL) had an overall tubercular-like surface pattern. Two of the 10 sturgeons up to ~1 m TL displayed a surface pattern consisting mainly of spines or tubercles (Fig. 2a). Six specimens had some dermal bones with the tubercular pattern, and some bones had the alveolar or a tubercular-alveolar pattern (Fig. 2b). Only two of the 10 sturgeons up to ~1 m TL displayed a clear alveolar pattern on most of the dermal bones, albeit sometimes with tubercular characteristics (Fig. 2c). Larger specimens (n = 9), that is, those >1 m TL, all displayed the typical *A. oxyrinchus* pattern (Fig. 2d). This difference in the surface pattern between small and large individuals was also observed in the additional nine specimens that were only morphologically identified as *A. oxyrinchus*, and which ranged in size between 35 and 230 cm TL. In animals with an alveolar surface structure, the pattern can become more tubercular-like near the edges of a single bone in some individuals (Fig. 3a,b) or, in case of the scutes of the lateral row, in scutes positioned towards the end of that row (Fig. 3c,d).

The 13 genetically identified *A. sturio* range in size between 31 and 260 cm TL. One specimen, with a TL of 154.5 cm (NRM 35439), displayed the 'alveolar' surface pattern. Eleven specimens showed the expected 'tubercular' pattern (Fig. 4a,b), although sometimes with the tubercles fused to alveolar-like structures (Fig. 4c), which resulted in the more mixed 'alveolar-tubercular' pattern in one specimen. If we include the 14 specimens that were only morphologically identified, a total of seven out of 27 specimens displayed the 'alveolar' type. We must bear in mind, however, that morphologically identified specimens may be misclassified or may represent hybrids (cf. Table 3). As we note above, the latter concern also holds for specimens identified through mtDNA only.

When the genetically identified sturgeons were identified on the basis of their overall dermal bone surface, a total of 21% were misclassified (Table 3). Misclassifications mainly concerned small *A. oxyrinchus* (TL <1 m) that were morphologically classified as *A. sturio*. In all, 12% of the sturgeons (excluding hybrids) could not be identified based on this criterion because they displayed a mix of both surface pattern types. When small sturgeons (TL <1 m) were excluded from the calculations, misclassification decreased considerably, to 13%. When the identifications of sturgeons larger than 1 m TL were based on the characteristics listed in Table 1 including the surface pattern, plus those sturgeons smaller than 1 m based on the same characteristics excluding the surface pattern, 87% could be classified correctly on the basis of morphology. Two of the three genetically identified *A. sturio* that had been misclassified as *A. oxyrinchus* on the basis of

Table 3
Taxonomic identification of *Acipenser sturio* (stur) and *A. oxyrinchus* (oxy) museum specimens from Chassaing et al. (2013) and this study. Species identification by Chassaing et al. (2013) is based on scute morphology and scute counts, which both gave the same assignments

Museum specimens				Molecular identification				
				stur	oxy	Hybrid	Total	% Miscl.
Morphological identification	Chassaing et al. (2013)	Based on scute morphology and scute count	stur	14[a]	**1**		15	**7%**
			oxy		3	1	4	**0%**
			Total	14	4	1	19	
			% Miscl.	**0%**	**25%**			**6%**
	This study	Based on all characteristics in Table 1, excluding dermal bone surface pattern	stur	11[b]	**3**[c]	2	16	**21%**
			oxy	**2**[d]	13[e]	1	16	**13%**
			Indet.		3[f]	2	5	
			Total	13	19	5	37	
			% Miscl.	**15%**	**19%**			**17%**
		Based on scute ornamentation	stur	11[b]	**5**[f]	2	18	**31%**
			oxy	**1**[d]	11[e]	2	14	**8%**
			Indet.	1[d]	3[c]	1	5	
			Total	13	19	5	37	
			% Miscl.	**8%**	**31%**			**21%**
		Based on scute ornamentation, excluding specimens <1 m TL	stur	4[c]	**1**		5	**20%**
			oxy	**1**[d]	9[g]	2	12	**10%**
			Indet.	1[d]		1	2	
			Total	6	10	3	19	
			%Miscl.	**20%**	**10%**			**13%**
		Based on all characteristics in Table 1, including dermal bone surface pattern of specimens >1 m TL	stur	10[a]	**1**[d]	1	12	**10%**
			oxy	**3**[d]	17[b]	3	23	**15%**
			Indet.		1	1	2	
			Total	13	19	5	37	
			% Miscl.	**23%**	**6%**			**13%**

Morphological species identification in this study is based on the characteristics summarized in Table 1. Hybrids and unidentified specimens were not included for the calculation of the percentage of misclassifications. The number and proportion of misclassified specimens are indicated in boldface. Indet.: indeterminate; Miscl.: misclassified.
[a]Molecular species identification of 5 specimens based on mtDNA only.
[b]Molecular species identification of 6 specimens based on mtDNA only.
[c]Molecular species identification of 1 specimen based on mtDNA only.
[d]Molecular species identification based on mtDNA only.
[e]Molecular species identification of 4 specimens based on mtDNA only.
[f]Molecular species identification of 2 specimens based on mtDNA only.
[g]Molecular species identification of 3 specimens based on mtDNA only.

morphology are small individuals (TL < 50 cm) and with an uncharacteristically low number of scutes for *A. sturio*.

The specimens that had been only morphologically examined were once more assigned to species, now including the surface pattern as a criterion (Table 2). When they displayed an overall alveolar pattern, they were assigned to *A. oxyrinchus*, disregarding their size. When the specimens displayed a tubercular pattern, this was not taken into account as a diagnostic trait for small specimens (TL < 1 m); identification in that case was based on the number of scutes and the presence of the fontanel. Specimens larger than 1 m with a tubercular surface pattern were assigned to *A. sturio*. Fourteen specimens were identified as *A. oxyrinchus*, although three of them had a number of scutes more characteristic of *A. sturio*. Eight specimens were identified as *A. sturio,* and six specimens were not assigned to a species; these identifications have yet to be genetically validated.

As with other morphological characteristics, the effect of hybridization or the level of hybridization between *A. sturio* and *A. oxyrinchus* on the surface pattern is unknown. No consistent surface patterns could be discerned in the five identified hybrids.

Archaeological samples

To evaluate the surface structure as a species-specific criterion, 69 archaeological dermal bones were selected for genetic analysis. Results were obtained for 34 bones (Table 5). Of these 34 bones, 26 were assigned to *A. oxyrinchus* (10 identified based on both nDNA and mtDNA, and 16 on mtDNA only). A further six bones were identified as *A. sturio* (two identifications based on both nDNA and mtDNA, and four on mtDNA only). The remaining two bones were of hybrid origin.

For 30 of the 34 genetically identified specimens, we were able to establish whether the fish was smaller or larger than 1 m TL (Table 5). The back-calculated lengths, based on models in Thieren and Van Neer (2016), of 18 bones of the head or the pectoral girdle, ranged between approx. 1 and 2 m. In the case of 12 isolated scutes, a rather crude size

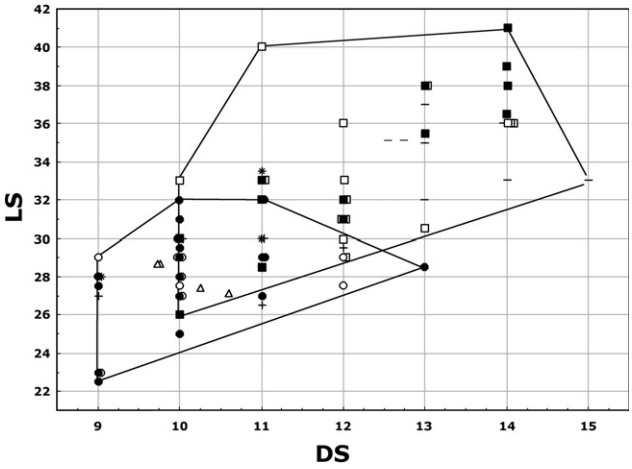

Fig. 1. Number of lateral scutes plotted against number of dorsal scutes, *Acipenser oxyrinchus* and *A. sturio,* identified based on characteristics summarized in Table 1 excluding the surface pattern of dermal bones and those identified both morphologically and genetically. Literature data (Table 1) and additional specimens from Chassaing et al. (2013) (not examined in this study) are also included. Chassaing et al. (2013) do not clearly define whether they consider the supracleithrum, basal fulcrum of the dorsal fin and occipital to be part of the lateral or dorsal row. ▲ Genetic identification hybrid (n = 5); △ Morphological identification indeterminate (n = 11); ● Genetic identification *A. oxyrinchus* (n = 17); ○ Morphological identification *A. oxyrinchus* (n=8); ◍ *A. oxyrinchus* identification from the literature (n = 5); ■Genetic identification *A. sturio* (n=12); □Morphological identification *A. sturio* (n = 7); ▨ *A. sturio* identification from the literature (n = 9).

reconstruction was carried out by visual comparison with sturgeons of known size.

The morphological identifications based on the surface patterns were compared with the genetic data (Table 5, Table 6). During the comparison, the tubercular pattern was not considered when it occurred only at the margin of the bone. Overall, 87% of the identifications based on morphology were borne out by the genetic analyses. In the case of *A. sturio,* two out of six morphological identifications proved to be wrong; in the case of *A. oxyrinchus,* misclassifications were markedly lower (two out of 26).

The two hybrid specimens could not be distinguished based on the surface pattern (Table 5, Table 6).

Discussion

Modern specimen identification

Although different criteria for the distinction between *A. sturio* and *A. oxyrinchus* have been described in the literature (Table 1), our results demonstrate that morphological species assignment of museum specimens, which are sometimes in poor condition, is not always straightforward or conclusive. The differences in species assignment by different authors (Table 2) and disagreements between the morphological and molecular species identifications of complete museum specimens (Table 3) indicate that the overall morphological difference between *A. oxyrinchus* and *A. sturio* is small. Although some authors (e.g. Vecsei et al., 2001) claim that the two species cannot be distinguished morphologically, we have demonstrated here that the two species can be distinguished on the number of scutes and the presence of a fontanel in more than 80% of the cases. When the surface pattern is also included for species identification, taking the size of sturgeons into account (see below), this proportion rises to 87%. Although an increase in the percentage of misclassifications (from 15% to 23%) was observed for *A. sturio* when the surface pattern was also included, a large decrease was observed for *A. oxyrinchus* (from 19% to 6%). Moreover, the number of unidentified specimens decreased, from five to two (Table 3).

Genetic analyses of museum specimens and archaeological samples revealed hybridization between *A. sturio* and *A. oxyrinchus*. Interspecific hybridization tends to occur naturally in sturgeons, and hybrid offspring can be fertile (Birstein et al., 1997). Our results (Table 2), and those of Chassaing et al. (2013) and Popović et al. (2014), indicate that *A. sturio* x *A. oxyrinchus* hybrids are indeed fertile and that introgression is possible. Not much is known about the phenotypic characteristics of *A. sturio* x *A. oxyrinchus* hybrids. Hybrid sturgeon phenotypes depend on the parental species and are often intermediate between the two (e.g. Szczepkowski et al., 2002; Szczepkowska et al., 2011). No firm conclusions about *A. sturio* x *A. oxyrinchus* hybrid morphology can be derived from the present study, perhaps due to the fact that the level of hybridization is not known.

Evaluation of the surface of dermal bones

The evaluation of the surface structure of dermal bones of museum specimens indicates that differentiating between *A. sturio* and *A. oxyrinchus* is not that straightforward. The alveolar pattern type described by Magnin (1964) is found in most cases in *A. oxyrinchus*, but it seems this species shows quite some variation in the surface pattern of its dermal bones. The tubercular pattern, which our results show is almost always displayed by *A. sturio*, also seems to occur in *A. oxyrinchus* smaller than 1 m TL.

Our dataset shows that the surface pattern of *A. oxyrinchus* is size-dependent. Large animals display the typical *A. oxyrinchus* surface pattern (rounded alveoli with smooth septa). Smaller specimens, on the other hand, have a more tubercular-like surface pattern, similar to that described for *A. sturio*, although sometimes some alveolar structures are present. This phenomenon was already suggested by Wuertz et al. (2011), who described the bone surface pattern of juvenile *A. oxyrinchus* as 'spiny'. Possibly, the pattern type observed in our smaller *A. oxyrinchus* and defined as tubercular corresponds to this 'spiny' type or to some transitional stage between the 'spiny' and alveolar types. The observations above may offer an explanation for the high proportion (68%) of archaeological remains morphologically identified as *A. sturio,* which turned out genetically to be *A. oxyrinchus* in the study of Ludwig et al. (2008) (Table 6), albeit no indications of fish length were given for those specimens. Chassaing et al. (2013) analysed only five samples (Table 6), and the fact that the single morphological identification of an *A. sturio* was genetically confirmed may be aleatory. Among

Table 4
Description of the surface pattern of the dermal bones of the analysed museum specimens, ordered by inventory number

Museum collection and inventory number	Surface pattern
AML 8797	Alveolar on subopercle, PG and scutes; tubercular on S, towards edges of LS and LS towards end of row
BAI 1884	Tubercular on S; tubercular-alveolar on scutes and subopercle
DCB 721	Tubercular on scutes and S; alveolar on subopercle and PG
KUL MD N/A	Alveolar
MHNN Z19398	Tubercular
MHNN Z19558	Alveolar
MHNN Z58274	Tubercular; some LS have alveolar characteristics
MHNN Z58275	Tubercular
MNHN-IC-B-2598	Tubercular on most bones; subopercle and some LS have some alveoli
MNHN-IC-0000-3108	Alveolar; pattern on LS more tubercular towards end of row
MNHN-IC-0000-3110	Alveolar
MNHN-IC-0000-3113	Alveolar
MNHN-IC-0000-3115	Alveolar
MNHN-IC-0000-3119	Tubercular
MNHN-IC-0000-3493	Tubercular
MNHN-IC-0000-3494	Alveolar
MNHN-IC-0000-3573	Alveolar on S and scutes
MNHN-IC-0000-3574	Alveolar
MNHN-IC-0000-4843	Tubercular on S and VS; alveolar on DS and LS
MNHN-IC-0000-9114	Tubercular on scutes and S; alveolar on supracleithrum
MNHN-IC-1869-0127	Alveolar; more tubercular towards edges of DS and LS at end of row
MRSN N/A	Alveolar
NHM 1859.3.15.1	Alveolar
NHM 1865.5.23.3	Alveolar
NHM 1886.8.24.1	Alveolar
NHM 1931.12.7.1	Tubercular
NHM 1986.5.21.1	Tubercular-alveolar on S and DS and VS; alveolar on LS
NHM 2005.6.22.6	Alveolar-tubercular on DS; alveolar on S, PG, VS and LS
NHM N/A	Tubercular
NMB N/A	Alveolar
NRM 94	Tubercular
NRM 1709	Alveolar
NRM 8948	Tubercular
NRM 8950	Tubercular
NRM 13336	Tubercular
NRM 18265	Tubercular
NRM 21705	Tubercular
NRM 21707	Tubercular; anal fin fulcrum and subopercle have some alveoli
NRM 21708	Tubercular
NRM 21710	Tubercular
NRM 21711	Tubercular
NRM 21712 (large)	Tubercular; some scutes with alveoli
NRM 21712 (medium)	Tubercular
NRM 21712 (small)	Tubercular
NRM 35435	Tubercular
NRM 35438	Alveolar
NRM 35439	Alveolar
NRM 35442	Tubercular on scutes and S; some alveoli on subopercle and supracleithrum
NRM 36001	Alveolar on LS1; tubercular-alveolar on bones of S and PG
NRM 36002	Alveolar on S, PG, VS and LS; tubercular on VS and LS towards end of row
NRM 36074	Tubercular on DS, VS and S; tubercular-alveolar on LS, bones of the PG and subopercle
NRM 49317	Tubercular on S, PG, DS and LS; mixed alveolar and tubercular on the VS
NRM 55538	Tubercular
NRM 60292	Tubercular on S; tubercular with alveolar characteristics on PG and scutes
NRM 60821	Tubercular on S and PG; alveolar with tubercular characteristics on DS; more tubercular towards edges of DS and on LS and VS
NRM 61784	Alveolar with tubercular characteristics; pattern on LS more tubercular towards end of row
RBINS 1528	Tubercular
RBINS 4449	Tubercular
RBINS 24792	Tubercular on most bones; alveolar on some scutes
RBINS N/A (1)	Tubercular
RBINS N/A (2)	Alveolar on S, PG, LS and VS; tubercular-alveolar on DS
RBINS N/A (5)	Tubercular
SML N/A	Alveolar on all scutes
UUZM UPSZTY 170	Alveolar
UUZM UPSZTY N/A	Alveolar-tubercular

S: bones of the skull; PG: bones of the pectoral girdle; DS: dorsal scutes, LS: lateral scutes; VS: ventral scutes. For abbreviations of institutions, see Table 2.

the 15 *A. sturio* museum specimens analysed by that team, only one was misidentified on the basis of scute morphology (Table 3), but unfortunately no size was indicated for this individual.

The authors who have thus far described the surface pattern type as a species-specific characteristic (Magnin, 1963; Artyukhin and Vecsei, 1999; Debus, 1999; Desse-Berset, 2009, 2011) did not report it being size-dependent, nor did they mention a 'spiny' or 'smooth' type in small *A. oxyrinchus* or *A. sturio*. Desse-Berset (2011) only studied the scute surface pattern on larger *A. oxyrinchus* [132–241 cm; lengths inferred by us from the figures and plots in Desse-Berset (2011)]; hence, any possible size-related change in scute surface pattern would go unnoticed. Although Debus (1999) and Artyukhin and Vecsei (1999) studied both small and large Baltic Sea specimens, they did not report any size-related change in surface patterns: the pattern of Baltic Sea sturgeon was described as 'radially alveolar', and no specimens with a 'tubercular' pattern were mentioned. Magnin (1964) also makes no comment on a deviant bone surface pattern in his study of 12 *A. oxyrinchus* (five of which were re-examined in the present study) and 12 *A. sturio* (four also

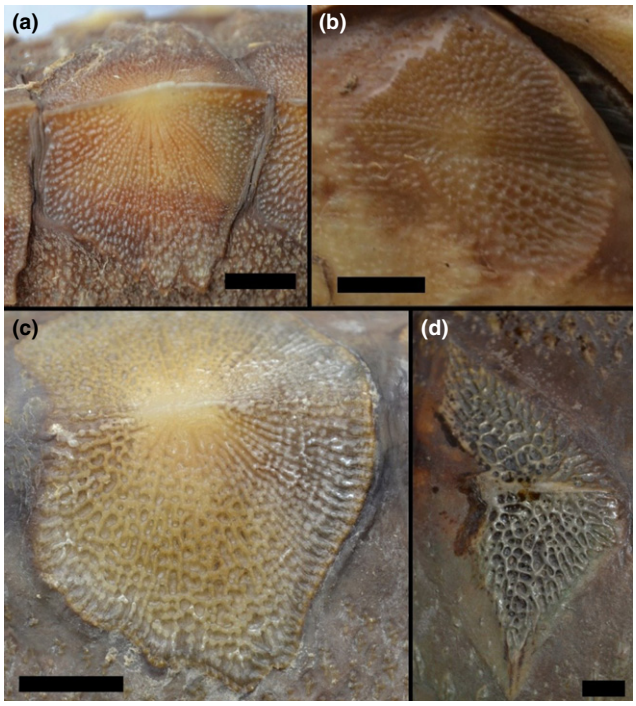

Fig. 2. Surface pattern types observed in *A. oxyrinchus* dermal bones: (a) tubercular pattern in a small individual (NRM 21707, 7th dorsal scute, 77 cm TL); (b) tubercular pattern with alveolar characteristics in that same individual (NRM21707, subopercle, 77 cm TL); (c) alveolar pattern with tubercular characteristics on a ~1m individual (NRM60821, 4th dorsal scute, 99 cm TL); (d) alveolar pattern in a large individual (MHNNZ19558, 6th left lateral scute, 276 cm TL). Scale bar = 1 cm.

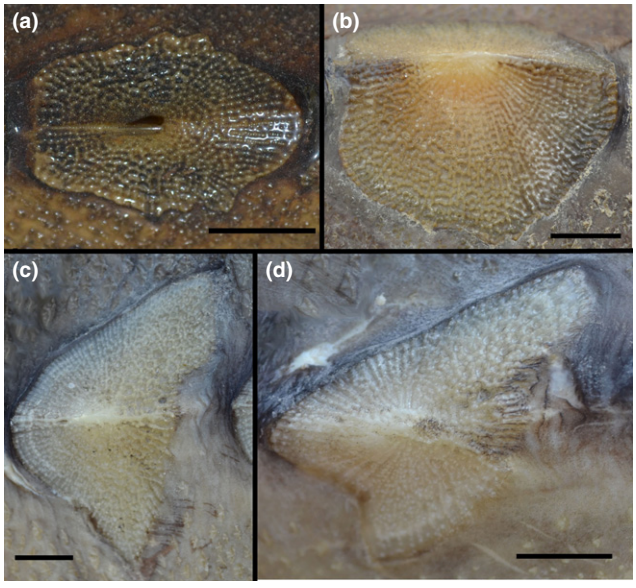

Fig. 3. Alveolar pattern on *A. oxyrinchus* dermal bones. The pattern becomes more tubercular towards the edges of the bone, as shown in (a) MNHN-IC-1869-0127 (71 cm TL), 11th dorsal scute, and (b) NRM60821 (99 cm TL), 6th dorsal scute; and towards the end of the row, as shown in (c) NRM61784 (146 cm TL), 7th right lateral scute, and (d) NRM61784 (146 cm TL), 19th right lateral scute. Scale bar = 1 cm.

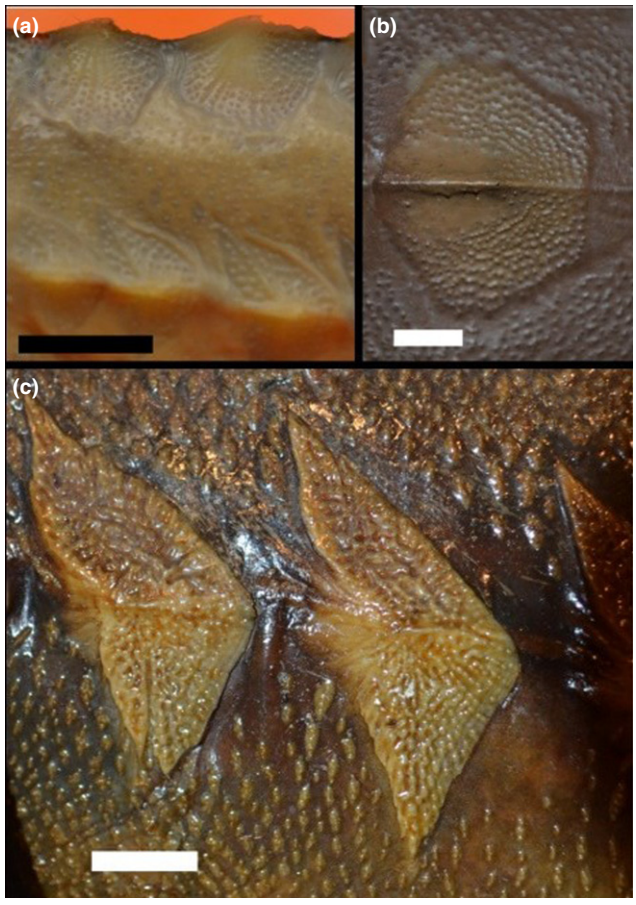

Fig. 4. Tubercular pattern on *A. sturio* dermal bones: (a) small specimen, NRM21708 (38 cm TL), 2nd and 3rd dorsal and 1st to 3rd left lateral scutes; (b) larger specimen, MNHN-IC-0000-3119 (153 cm TL), 4th dorsal scute, overpainted; (c) specimen with tubercular pattern (3rd left lateral scute) and more alveolar-like pattern (2nd left lateral scute), MHNNZ58274 (151 cm TL). Scale bar = 1 cm.

studied here) from the Muséum national d'histoire naturelle (MNHN) in France. Two of the 12 specimens that had previously been identified as *A. sturio* turned out to be *A. oxyrinchus* on the basis of recent genetic analysis (Chassaing et al., 2013; present study). We found that both of these specimens, as well as a third specimen that was not genetically identified, have a clear alveolar scute surface pattern, a feature that was not mentioned by Magnin (1964).

In addition to displaying this size-dependent variability in the surface pattern, *A. oxyrinchus* dermal bones with an alveolar pattern type sometimes display a more tubercular-like type of surface pattern toward their edges, and the surface pattern of lateral scutes in the posterior end of that row also becomes more tubercular-like.

Among the archaeological remains, the typical *A. oxyrinchus* alveolar pattern was found in 24 sturgeon bones genetically identified as *A. oxyrinchus*, in one hybrid and in two genetically identified as *A. sturio* (Table 6). Similar to Ludwig et al. (2008), the percentage of *A. oxyrinchus* morphologically misclassified as *A. sturio* is lower than that of *A. sturio* morphologically misclassified as *A. oxyrinchus* (two out of 26 vs. two out of six).

Table 5
Reconstructed length, date, genetic and morphological identification, and surface pattern of archaeological samples

Country	Locality	Sample identifier	Reconstructed TL	Date	Reference(s)	Surface pattern	Taxonomic identification based on morphology	Taxonomic identification based on genetics
BE	Antwerpen, Zwartzusterstraat	Put 17	/	16th–17th c. AD	Veeckman et al. (1992)	Tubercular with some alveoli (edge of bone)	Indet.	Hybrid
BE	Antwerpen, Zwartzusterstraat	Put 1	>1 m	16th–17th c. AD	Veeckman et al. (1992)	Alveolar with some tubercles (edge of bone)	oxy	oxy[a]
BE	Ename, Castrum	N/A	>1 m	First half 11th c. AD	Callebaut et al. (1997/1998)	Alveolar with spines on septa	oxy	oxy[a]
BE	Gent, E. Braunplein (EB'96)	1A II	181.5 cm	Second half 10th–11th c. AD	Laleman and Stoops (1996)	Alveolar	oxy	oxy[a]
BE	Londerzeel, Burcht (LM1/092)	N/A	min. 150 cm	Last quarter 13th–first half 14th c. AD	Dewilde et al. (1994)	Alveolar with some tubercles	oxy	oxy[a]
BE	Londerzeel, Burcht (LM1/092)	N/A	>1 m	Last quarter 13th–first half 14th c. AD	Dewilde et al. (1994)	Tubercles fused to ridges, with some alveoli	oxy	oxy[a]
NL	Barendrecht-Zuidpolder 20-58 Trace 1	328	>1 m	Early Bronze Age–Middle Bronze Age	Moree et al. (2002)	Alveolar	oxy	oxy[a]
NL	Brakel, Molenkampseweg	100054	/	Second half 1st–first quarter 3rd c. AD	Schrijer et al. (2005)	Alveolar	oxy	oxy[a]
NL	Den Haag, Oldenbarneveltlaan (JOB84)	32	<1 m	6th–7th c. AD	Magendans and Waasdorp (1989)	Alveolar	oxy	oxy[a]
NL	Den Haag, Oldenbarneveltlaan (JOB84)	531	115 cm	6th–7th c. AD	Magendans and Waasdorp (1989)	Alveolar	oxy	Hybrid
NL	Hekelingen III	23d 456 III	134 cm	3000–2600 BC	Louwe Kooijmans (1986)	Alveolar	oxy	Excluded
NL	Kapel-Avezaath, Stenen Kamer	98STKV2075	179 cm	Ottonic period to High Middle Ages	Verhoeven and Brinkkemper (2001)	Alveolar	oxy	oxy
NL	Kerk-Avezaath, Huis Malburg	97MALOBOT034A	min. 139 cm	AD 900–1049	Oudhof et al. (2000)	Alveolar	oxy	oxy[a]
NL	Rijswijk, Rijksweg A4 (RZO93)	173	/	3625–3400 BC	Hessing (1994)	Alveolar	oxy	oxy[a]
NL	Rijswijk, Rijksweg A4 (RZO93)	321	100 cm	3625–3400 BC	Hessing (1994)	Alveolar	oxy	oxy
NL	Rijswijk, Ypenburg (RYP4)	v21-5-256	~1 m	3900–3700 BC to 3200 BC	Koot et al. (2008)	Alveolar	oxy	oxy[a]
NL	Rijswijk, Ypenburg (RYP4)	V1-1-79	/	3900–3700 BC to 3200 BC	Koot et al. (2008)	Alveolar	oxy	Excluded
NL	Rijswijk, Ypenburg (RYP4)	v13-2-332	146 cm	3900–3700 BC to 3200 BC	Koot et al. (2008)	Alveolar	oxy	oxy[a]
NL	Rijswijk, Ypenburg (RYP4)	v13-3-480	205 cm	3900–3700 BC to 3200 BC	Koot et al. (2008)	Alveolar	oxy	oxy[a]
NL	Rijswijk, Ypenburg (RYP4)	v8-2-684	>1 m	3900–3700 BC to 3200 BC	Koot et al. (2008)	Alveolar	oxy	oxy

Table 5
(Continued)

Country	Locality	Sample identifier	Reconstructed TL	Date	Reference(s)	Surface pattern	Taxonomic identification based on morphology	Taxonomic identification based on genetics
NL	Vlaardingen	VLA (17-XII-1959)	>1 m	2380–2240 BC	Clason (1967); van Beek (1990)	Alveolar	oxy	oxy[a]
NL	Vlaardingen	VLA19d	>1 m	2380–2240 BC	Clason (1967); van Beek (1990)	Alveolar	oxy	oxy[a]
NL	Vlaardingen	VLA371/4	>1 m	2380–2240 BC	Clason (1967); van Beek (1990)	Alveolar	oxy	oxy[a]
UK	London, Billingsgate Lorry Park (BIG82)	2913	221 cm	Mid–late 14th c. AD	Roskams (1999)	Alveolar	oxy	oxy
UK	London, Old Custom House (CUS73)	A626	178 cm	Late 13th–mid 14th c. AD	Tatton-Brown (1974)	Alveolar	oxy	oxy[a]
UK	London, Royal Mint (MIN86)	1891	/	AD 1560–1758	Grainger and Phillpotts (2010)	Alveolar	oxy	oxy[a]
UK	London, The Seal House Site (SH74)	280	min. 97 cm	15th c. AD	Schofield (1974)	Alveolar	oxy	oxy[a]
NL	Nijmegen, Canisiuscollege	5594	>1 m	AD 70–120	Haalebos (1993)	Alveolar	oxy	oxy
NL	Vlaardingen	VLA441b	min. 115 cm	2380–2240 BC	Clason (1967); van Beek (1990)	Tubercular, with some alveoli	stur	oxy[a]
NL	Vlaardingen	VLA548b	min. 99.13 cm	2380–2240 BC	Clason (1967); van Beek (1990)	Tubercular, with some alveoli (edge of bone)	stur	oxy[a]
NL	Barendrecht-Zuidpolder 20-58 Trace 1	336	/	Early Bronze Age–Middle Bronze Age	Moree et al. (2002)	Tubercular, with some alveoli	stur	Excluded
NL	Brakel, Molenkampseweg	100119	min. 125 cm	Second half 1st–first quarter 3rd c. AD	Schrijer et al. (2005)	Tubercular	stur	stur[a]
NL	Vlaardingen	VLA613a/2	>1 m	2380–2240 BC	Clason (1967); van Beek (1990)	Alveolar	oxy	stur[a]
UK	London, Billingsgate Lorry Park (BIG82)	2937	120 cm	Mid–late 14th c. AD	Roskams (1999)	Alveolar	oxy	stur[a]
NL	Rijswijk, Ypenburg (RYP4)	V18-5-543	min. 146 cm	3900–3700 BC to 3200 BC	Koot et al. (2008)	Tubercular	stur	stur[a]
NL	Vlaardingen	VLA18G	~1 m	2380–2240 BC	Clason (1967); van Beek (1990)	Tubercular, with some alveoli	stur	stur[a]
UK	London, Fleet Valley Project (VAL88)	7437	min. 173 cm	AD 1666–1720	Mccann and Orton (1989)	Tubercular, with some alveoli	stur	stur

BE: Belgium; NL: The Netherlands; UK: United Kingdom;
[a]mtDNA only.

Table 6
Morphological and molecular species identification of *A. sturio* (stur) and *A. oxyrinchus* (oxy) archaeological remains. Data from Ludwig et al. (2008), Chassaing et al. (2013) and this study

Archaeological remains			Molecular identification				
			stur	oxy	Hybrid	Total	% Miscl.
Morphological identification	Chassaing et al. (2013)	stur	1			1	**0%**
		oxy	**1**[a]	3[b]		4	**25%**
		Total	2	3		5	
		% Miscl.	**50%**	**0%**			**20%**
	Ludwig et al. (2008)[a]	stur	11	**23**		34	**68%**
		oxy	**4**	172		176	**2.3%**
		Total	15	195		210	
		% Miscl.	**27%**	**11.8%**			**12.8%**
	This study	stur	4	**2**[a]		6	**33%**
		oxy	**2**[a]	24[c]	1	27	**8%**
		Indet.			1	1	
		Total	6	26	2	34	
		% Miscl.	**33%**	**8%**			**12%**

Hybrids and unidentified specimens were not included for the calculation of the percentage of misclassifications. The number and proportion of misclassified specimens are indicated in boldface. Indet.: indeterminate; Miscl.: misclassified.
[a]Molecular species identification based on mtDNA only.
[b]Molecular species identification of 1 specimen based on mtDNA only.
[c]Molecular species identification of 14 specimens based on mtDNA only.

Conclusion

In this study we evaluated the surface pattern of dermal bones as a characteristic to distinguish *A. oxyrinchus* from *A. sturio*. Our results indicate that dermal bones with an alveolar pattern are most likely to be *A. oxyrinchus*. However, it appears that the identification of dermal bones with a tubercular pattern is not so straightforward. This surface pattern type is said to be particular to *A. sturio* (Magnin, 1964; Kottelat and Freyhof, 2007; Desse-Berset, 2009), but our results indicate that small *A. oxyrinchus* (less than ~1 m TL) can also display this pattern type. Our analysis of museum specimens and archaeological remains also showed that an alveolar pattern on a dermal bone could become more tubercular towards the edges of the bone. Moreover, in some *A. oxyrinchus,* the lateral scutes towards the posterior end of the row display a more tubercular pattern compared with those in the anterior end of the row, which display an alveolar surface pattern.

When identifying complete fish to a species, the number of scutes and the presence of a fontanel between the parietals and frontals are relevant, but the surface pattern on the scutes or other dermal bones can also help. In archaeological material, which is no longer in articulation, the surface pattern is the only morphological criterion available. When the pattern is alveolar, the specimen is most likely to be *A. oxyrinchus*, regardless of the size of the fish. When the pattern is tubercular, the pattern should not be used as a criterion for the identification of bones derived from individuals smaller than ~1 m reconstructed/estimated TL. However, when a bone from an individual larger than 1 m TL displays the tubercular surface pattern, it is most likely *A. sturio*. Scutes from the end of the lateral row or bone fragments consisting of the edges of bony plates with a tubercular surface pattern cannot with certainty be assigned to *A. sturio*.

Our analysis showed that hybrid specimens could not be distinguished from purebred animals based on the surface pattern.

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Appendix

Table A1
Details of the museum specimens included in the study. For abbreviations of institutions, see Table 2

Museum collection and inventory number	Type of preservation	Date of capture	Locality	Remark	Museum identification
AML 8797	Mounted	1896	North Sea		*A. sturio*
BAI 1884	Osteological	1896	Port of IJmuiden (NL)		*Acipenser*
DCB 721	Osteological + frozen (head)		NL		*Acipenser*
KUL MD N/A	Mounted				*A. sturio*
MHNN Z19398	Mounted	1886	Loire river (FR)		*Acipenser*
MHNN Z19558	Mounted	1880	Nantes, Loire river (FR)		*Acipenser*
MHNN Z58274	Mounted	1896	Nantes (FR)		*Acipenser*
MHNN Z58275	Mounted				*Acipenser*
MNHN-IC-B-2598	Mounted	1869	Nova Scotia (CA)		*A. oxyrinchus*
MNHN-IC-0000-3108	Mounted	1851	Seine river (FR)	Holotype *A. yarrellii* Duméril 1867	*A. sturio*
MNHN-IC-0000-3110	Mounted	1815	USA	Holotype *A. (antaceus) lecontei* Duméril 1867 & *A. sturio*	*A. sturio*
MNHN-IC-0000-3113	Mounted	1822	USA	Syntype *A. (huso) milberti* Duméril 1870 & *A. sturio*	*A. sturio*
MNHN-IC-0000-3115	Mounted	1823	Seine estuary (FR)	Syntype *A. valenciennii* Duméril 1870	*A. sturio*
MNHN-IC-0000-3119	Mounted		Côtes-d'Armor, Brittany (FR)		*A. sturio*
MNHN-IC-0000-3493	Mounted	1830	Oyapok (GUF)	Holotype *A. cayennensis* Duméril 1867 & *A. sturio* & *A. oxyrinchus* (Vasil'eva, 1997)	*A. oxyrinchus*
MNHN-IC-0000-3494	Mounted	1822	New York (USA)	Syntype *A. (huso) milberti* Duméril 1870 & *A. sturio* & *A. oxyrinchus* (Vasil'eva, 1997)	*A. oxyrinchus*
MNHN-IC-0000-3573	Mounted; only head and first scutes observed			Syntype *A. valenciennii* Duméril 1870	*A. sturio*
MNHN-IC-0000-3574	Mounted; only head and first scutes observed		West Coast (USA)	Holotype *A. (antaceus) hallowellii* Duméril, 1867 & *A. sturio* & *A. oxyrinchus* (Vasil'eva, 1997)	*A. oxyrinchus*
MNHN-IC-0000-4843	Liquid		Nova Scotia (CA)	Holotype *A. (huso) honneymani* Duméril 1870	*A. oxyrinchus*
MNHN-IC-0000-9114	Mounted	1822	New York (USA)	Holotype *Acipenser (huso) macrorhinus* Duméril, 1870	*A. oxyrinchus*
MNHN-IC-1869-0127	Mounted	1869	Nova Scotia (CA)		*A. oxyrinchus*
MRSN N/A	Mounted; heavily overpainted	1873	Haine river (BE)		*Acipenser*
NHM 1859.3.15.1	Mounted	1859	Trigmouth (UK)		*A. sturio*
NHM 1865.5.23.3	Mounted	1865	London Market (UK)		*A. sturio*
NHM 1886.8.24.1	Mounted	1886	British coast (UK)		*A. sturio*
NHM 1931.12.7.1	Liquid	1931	North Sea		*A. sturio*
NHM 1986.5.21.1	Liquid	1986	Grimsby (UK)		*A. sturio*
NHM 2005.6.22.6	Mounted	2005	North America		*A. sturio*
NHM N/A	Frozen		Scotland (UK)		*A. sturio*
NMB N/A	Mounted; poor condition	1860	Niawier, Nijewier-ster opfeart (NL)		*Acipenser*
NRM 94	Liquid	1932	Töro Island (SE)	Paralectotype *A. sturio*	*A. oxyrinchus*
NRM 1709	Liquid	1890	Dalarö (SE)		*A. sturio*
NRM 8948	Liquid		Genoa (IT)		*A. sturio*
NRM 8950	Liquid		Potomac River (USA)		*A. oxyrinchus*
NRM 13336	Liquid	1991	Böckers bank (SE)		*A. sturio*
NRM 18265	Mounted	pre-1839			*A. sturio*
NRM 21705	Liquid	1838	Bohuslän (SE)		*A. sturio*
NRM 21707	Liquid	1932	Torö island (SE)		*A. sturio*
NRM 21708	Liquid	1910	Skagerrak (SE)		*A. oxyrinchus*
NRM 21710	Liquid	1895	Mörkhult (SE)		*A. sturio*
NRM 21711	Liquid	pre-1736		Paralectotype *A. sturio* (Freyhof & Kottelat 2009)	*A. oxyrinchus*
NRM 21712 (large)	Liquid				*A. sturio*

Table A1
(Continued)

Museum collection and inventory number	Type of preservation	Date of capture	Locality	Remark	Museum identification
NRM 21712 (medium)	Liquid				*A. sturio*
NRM 21712 (small)	Liquid				*A. sturio*
NRM 35435	Mounted	1856	Venice (IT)		*A. sturio*
NRM 35438	Mounted				*A. sturio*
NRM 35439	Mounted		Norrland (SE)		*A. sturio*
NRM 35442	Liquid				*A. sturio*
NRM 36001	Liquid; head only	1985	Hanstholm Kommune (DK)		*A. sturio*
NRM 36002	Mounted	1881	Kristianstad (SE)	Tip of tail broken	*A. sturio*
NRM 36074	Liquid	1837	Bohuslän (SE)		*A. sturio*
NRM 49317	Liquid				*Acipenser*
NRM 55538	Liquid				*A. sturio*
NRM 60292	Liquid	2009	Falsterborev (SE)	Reintroduced	*A. oxyrinchus*
NRM 60821	Liquid	2010	Sölvesborgs Kommun (SE)	Reintroduced	*A. oxyrinchus*
NRM 61784	Liquid	2011	Enangersfjärden bay (SE)	Reintroduced	*A. oxyrinchus*
RBINS 1528	Liquid	1883			*A. sturio*
RBINS 4449	Liquid	1935	Helgoland (DE)		*A. sturio*
RBINS 24792	Osteological	2011		Aquaculture (Canadian stock)	*A. oxyrinchus*
RBINS N/A (1)	Mounted				*Acipenser*
RBINS N/A (2)	Mounted				*Acipenser*
RBINS N/A (5)	Dried				*Acipenser*
SML N/A	Skin; head missing	1875	Lokeren, Durme river (BE)		*Acipenser*
UUZM UPSZTY 170	Mounted				*Acipenser*
UUZM UPSZTY N/A	Mounted	1869	SE		*Acipenser*

BE: Belgium; CA: Canada; DE: Germany; DK: Denmark; FR: France; GUF: French Guiana; IT: Italy; NL: The Netherlands; SE: Sweden; UK: United Kingdom; USA: United States.

Table A2
Details of the genetic analysis of the archaeological samples and museum specimens of *A. oxyrinchus* (oxy) and *A. sturio* (stur), with indication of sample number, type of sample and the number of sequenced PCR products. For abbreviations of institutions, see Table 2

			mtDNA			nDNA			
Museum collection and inventory number/Locality	Sample identifier	Tissue type	90 bp from cyt *b*	124 bp from cyt *b*	Sequence	*LS68*	Sequence	*D161*	Sequence
MNHN-IC-B-2598	ICTI5250	Fin clip	4	4	oxy	5	oxy/oxy	6	oxy/oxy
MNHN-IC-0000-3108	ICTI5241	Fin clip	2	2	oxy	N/A		N/A	
MNHN-IC-0000-3110	ICTI5242	Fin clip	4	4	oxy	N/A		4	oxy
MNHN-IC-0000-3113	ICTI5244	Fin clip	3	2	oxy	N/A		N/A	
MNHN-IC-0000-3115	ICTI5245	Fin clip	1	1	oxy	N/A		N/A	
MNHN-IC-0000-3493	ICTI5246	Fin clip	2	2	stur	N/A		2	stur
MNHN-IC-0000-3494	ICTI5247	Fin clip	2	2	oxy	N/A		N/A	
MNHN-IC-0000-3573	ICTI5248	Fin clip	N/A	N/A		N/A		N/A	
MNHN-IC-0000-4578	ICTI5249	Tissue	2	2	oxy	N/A		N/A	
MNHN-IC-1869-0127	ICTI5240	Fin clip	3	2	oxy	N/A		2	oxy
NMB N/A		Dry tissue	N/A	N/A		N/A		N/A	
NRM 21712 (large)		Skin + underlying tissue	4	4	oxy	9	oxy/stur	7	oxy/stur
NRM 21712 (small)		Skin + underlying tissue	4	4	oxy	5	oxy/stur	6	oxy/stur
NRM 27172 (medium)		Skin + underlying tissue	4	4	oxy	6	oxy	5	oxy/stur
NRM 35438		Tissue	2	2	oxy	N/A		N/A	
NRM 35442		Skin + underlying tissue	N/A	N/A		N/A		N/A	
NRM 49317		Skin + underlying tissue	3	2	oxy	N/A		1	oxy
NRM 55538		Skin + underlying tissue	3	N/A	oxy	N/A		N/A	
RBINS 1528		Skin + underlying tissue	1	N/A	oxy	N/A		N/A	
RBINS 4449		Skin + underlying tissue	N/A	N/A		N/A		N/A	
RBINS 24792		Bone	1	1	oxy	1	oxy	1	oxy
RBINS N/A (1)	RBINS 1	Fin clip	2	2	stur	N/A		N/A	
RBINS N/A (2)	RBINS 2	Fin clip	3	3	stur	3	oxy/oxy	4	oxy/stur
SML N/A		Fin clip	4	1	oxy				
Antwerpen, Zwartzusterstraat Put 1		Bone (indet.)	4	4	oxy				
Antwerpen, Zwartzusterstraat Put 17		Bone (indet.)	4	4	oxy			4	oxy/stur
Barendrecht-Zuidpolder 20-58 Trace 1 328		Bone (lateral scute)	4	0	oxy				
Barendrecht-Zuidpolder 20-58 Trace 1 336		Bone (indet.)	1	0	stur				
Brakel, Molenkampseweg 100054		Bone (accessory bone of skull)	4	1	oxy				
Brakel, Molenkampseweg 100119		Bone (cleithrum)	4	4	stur				
Den Haag, Oldenbarneveltlaan (JOB84) 32		Bone (lateral scute)	4	4	oxy				
Den Haag, Oldenbarneveltlaan (JOB84) 531		Bone (postorbitale)	4	1	oxy			5	oxy/stur
Ename, Castrum N/A		Bone (scute)	4	4	oxy				
Gent, E. Braunplein (EB'96) 1A II		Bone (subopercle)	4	4	oxy	1	oxy		
Hekelingen III 23d 456 III		Bone (frontal/parietal)	0	1	oxy				
Kapel-Avezaath, Stenen Kamer 98STKV002075		Bone (frontal/parietal)	4	4	oxy	7	oxy/oxy	7	oxy/oxy
Kerk-Avezaath, Huis Malburg 97MALOBOT034A		Bone (cleithrum)	4	4	oxy				
Londerzeel, Burcht (LM1/092) N/A		Bone (frontal/parietal)	4	4	oxy	1	oxy		
Londerzeel, Burcht (LM1/092) N/A		Bone (indet.)	4	4	oxy				
London, Billingsgate Lorry Park (BIG82) 2913		Bone (subopercle)	4	4	oxy	2	oxy/oxy		
London, Billingsgate Lorry Park (BIG82) 2937		Bone (supracleithrum)	4	4	stur				
London, Fleet Valley Project (VAL88) 7437		Bone (subopercle)	4	4	stur			5	stur/stur
London, Old Custom House (CUS73) A626		Bone (subopercle)	2	0	oxy				

Table A2
(Continued)

Museum collection and inventory number/Locality	Sample identifier	Tissue type	mtDNA			nDNA			
			90 bp from cyt *b*	124 bp from cyt *b*	Sequence	*LS68*	Sequence	*D161*	Sequence
London, Royal Mint (MIN86) 1891		Bone (subopercle)	4	4	oxy			5	oxy/oxy
London, The Seal House Site (SH74) 280		Bone (subopercle)	4	4	oxy			4	oxy
Nijmegen, Canisiuscollege 5594		Bone (lateral scute)	4	4	oxy	1	oxy	4	oxy/oxy
Rijswijk, Rijksweg A4 (RZO93) 173		Bone (indet.)	4	2	oxy				
Rijswijk, Rijksweg A4 (RZO93) 321		Bone (frontal)	4	4	oxy	6	oxy	5	oxy/oxy
Rijswijk, Ypenburg (RYP4) v18-5-543		Bone (dermopterotic)	4	2	stur				
Rijswijk, Ypenburg (RYP4) V1-1-79		Bone (branchiostegal)	1	0	oxy				
Rijswijk, Ypenburg (RYP4) v13-2-332		Bone (dermopterotic)	4	2	oxy				
Rijswijk, Ypenburg (RYP4) v13-3-480		Bone (dermosphenotic)	4	3	oxy				
Rijswijk, Ypenburg (RYP4) v21-5-256		Bone (lateral scute)	4	4	oxy				
Rijswijk, Ypenburg (RYP4) v8-2-684		Bone (indet.)	4	4	oxy	3	oxy/oxy	2	oxy
Vlaardingen VLA (17-XII-1959)		Bone (ventral scute)	4	4	oxy			1	oxy
Vlaardingen VLA18G		Bone (lateral scute)	4	4	stur			1	stur
Vlaardingen VLA19d		Bone (dorsal scute)	4	4	oxy				
Vlaardingen VLA371/4		Bone (ventral scute)	3	4	oxy				
Vlaardingen VLA441b		Bone (subopercle)	4	4	oxy				
Vlaardingen VLA548b		Bone (frontal/parietal)	4	4	oxy				
Vlaardingen VLA613a/2		Bone (dorsal scute)	4	3	stur				