Supplementary materials for

MALDI-TOF mass spectrometry applied for animal species identification based on bone samples

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S1. Scientific classification of the animal samples analyzed in the present study

Table S1. Scientific classification of the animal samples studied in the present work.

		classification			
sample	**species**	**class**	**order**	**family**	**genus**
1	*L. crocea* (LC)	Actinopteri	Perciformes	Sciaenidae	Larimichthys
2	*L. polyactis* (LP)	Actinopteri	Perciformes	Sciaenidae	Larimichthys
3	*C. alburnus* (CA)	Actinopteri	Cypriniformes	Cyprinidae	Culter
4	*P. leptocephalus* (PL)	Actinopteri	Cypriniformes	Cyprinidae	Pseudaspius
5	*N. virgatus* (NV)	Actinopteri	Perciformes	Nemipteridae	Nemipterus
6	*D. eleginoides* (DE)	Actinopteri	Perciformes	Nototheniidae	Dissostichus
7	*S. s. domestica* (SSD)	Mammalia	Artiodactyla	Suidae	Sus
8	*G. g. domesticus* (GGD)	Aves	Galliformes	Phasianidae	Gallus

S2. Detailed information of the samples studied in the present study

Table S2. Size and weight of the animal samples analyzed in the present study. *D. eleginoides* (DE) sample was obtained as slices, and *S. s. domestica* (SSD) and *G. g. domesticus* (GGD) samples were obtained as pieces, the size and weight of the whole samples are not available (N/A). The probable origins of SSD & GGD are also N/A.

sample	species	probable origin	trader	biological replicates (samples)	size / cm	weight / g
1	*L. crocea* (LC)	Ningbo, Zhejiang	Xianwaixian flagship store	6	23.4 ±1.5	149.2 ±26.7
2	*L. polyactis* (LP)	Qingdao, Shandong	Yulang flagship store	6	17.6 ±0.9	70.0 ±6.3
3	*C. alburnus* (CA)	Hangzhou, Zhejiang	Qiandao Yuxiang	6	26.6 ±1.7	108.3 ±19.4
4	*P. leptocephalus* (PL)	Hangzhou, Zhejiang	Qiandao Yuxiang	6	34.1 ±2.0	260.0 ±44.2
5	*N. virgatus* (NV)	Zhuhai, Guangdong	The Emperor of the Ship Seafood (Chuan Zhi Huang Seafood)	6	23.9 ±1.3	146.7 ±27.1
6	*D. eleginoides* (DE)	Qingdao, Shandong	Fishy aroma (Yu Wei Xiang)	3	N/A	N/A
7	*S. s. domestica* (SSD)	N/A	Guzong Road, Pudong New Area, Shanghai	3	N/A	N/A
8	*G. g. domesticus* (GGD)	N/A	Guzong Road, Pudong New Area, Shanghai	3	N/A	N/A

S3. Impact of matrices on mass spectrometric analysis

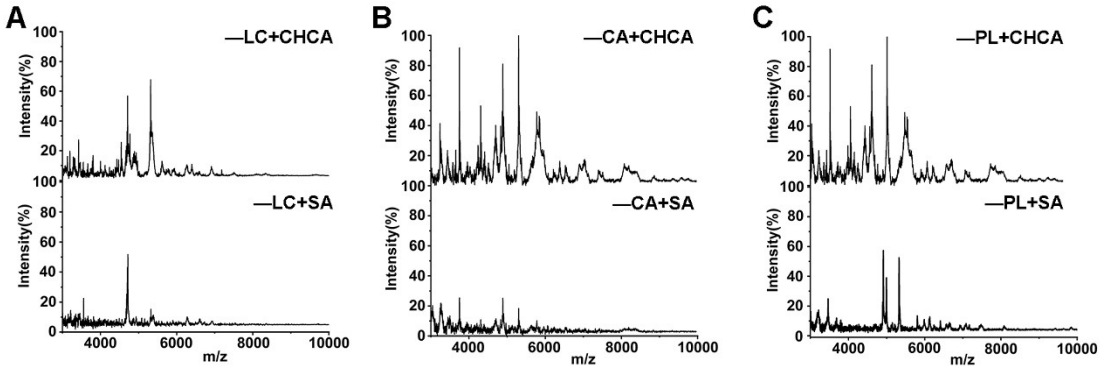

Fig. S1. Representative MALDI-TOF mass spectra for processed samples of bones of fish species *Larimichthys crocea* (LC), *Culter alburnus* (CA) and *Pseudaspius leptocephalus* (PL) when using matrix α-cyano-4-hydroxycynnamic acid (CHCA) and sinapinic acid (SA), respectively. Experimentally, 0.5 g of fish bone sample and 5 mL of 10% (v/v) formic acid (FA) solution in a beaker was boiled on a hot plate for 5.2 min. The obtained extracts were analyzed under the matrix of CHCA and SA, respectively.

S4. Cross validation (CV) for the mass spectra of fishes belonging to a same order, family and genus, respectively

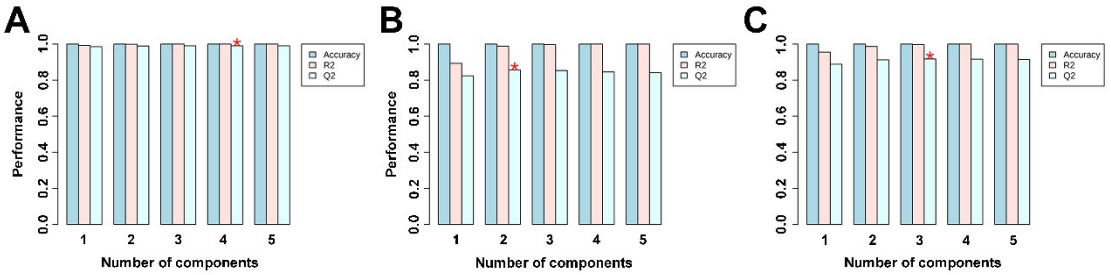

Fig. S2. Cross validation (CV) results of the datasets collected from fish species that belong to the same order, family and genus, respectively. Q2 is an estimate of the predictive ability of the model. In each CV plot, the predicted data are compared with the original data, and the sum of squared errors is calculated. The prediction error is then summed over all samples, which is predicted residual sum of squares (short as PRESS). For convenience, the PRESS is divided by the initial sum of squares and subtracted from 1 to resemble the scale of the R2 which provides a measure of model fit to the original data. The analyzed fish samples include: (A) at the order level, *D. eleginoides* (DE) and *N. virgatus* (NV); (B) at the family level, *C. alburnus* (CA) and *P. leptocephalus* (PL); (C) at the genus level, *L. crocea* (LC) and *L. polyactis* (LP).

Table S3. The measured R2 and Q2 in Figure S2C.

measure	**1comps**	**2comps**	**3comps**	**4comps**	**5comps**	**average (avg)**	**RSD**
R2	0.9549	0.9867	0.9975	0.9996	0.9999	0.988±0.019	1.9%
Q2	0.8883	0.9128	0.9177	0.9164	0.9147	0.910±0.012	1.3%

S5. Mass spectral data for each animal sample

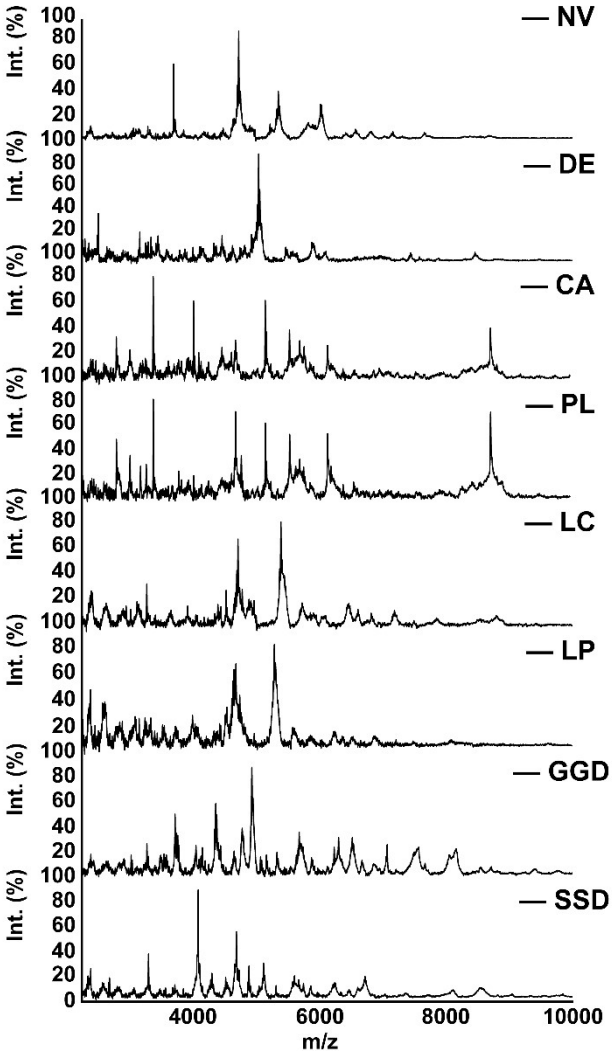

Fig. S3. Representative MALDI-TOF mass spectra of analyzed species. Bone sample of *Larimichthys crocea* (LC), *Larimichthys polyactis* (LP), *Culter alburnus* (CA), *Pseudaspius leptocephalus* (PL), *Nemipterus virgatus* (NV), *Dissostichus Eleginoides* (DE), *Sus scrofa domestica* (SSD) and *Gallus gallus domesticus* (GGD) were treated and analyzed. The bone extracts of the samples were analyzed by MALDI-TOF MS. Nine mass spectra were usually obtained for each species.