Comparative functional anatomy of hindlimb muscles and bones with reference to aquatic adaptation of the sea otter

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ABSTRACT. Although the sea otter (*Enhydra lutris*) is a complete aquatic species, spending its entire life in the ocean, it has been considered morphologically to be a semi-aquatic animal. This study aimed to clarify the unique hindlimb morphology and functional adaptations of *E. lutris* in comparison to other Mustelidae species. We compared muscle mass and bone measurements of five Mustelidae species: the sea otter, Eurasian river otter (*Lutra lutra*), American mink (*Neovison vison*), Japanese weasel (*Mustela itatsi*) and Siberian weasel (*M. sibirica*). In comparison with the other 4 species, *E. lutris* possessed significantly larger gluteus, popliteus and peroneus muscles, but smaller adductor and ischiopubic muscles. The popliteus muscle may act as a medial rotator of the crus, and the peroneus muscle may act as an abductor of the fifth toe and/or the pronator of the foot. The bundles of the gluteus superficialis muscle of *E. lutris* were fused with those of the tensor fasciae latae muscle and gluteofemoralis muscles, and they may play a role in femur abduction. These results suggest that *E. lutris* uses the abducted femur, medially rotated crus, eversion of the ankle and abducted fifth digit or extended interdigital web as a powerful propulsion generator. Therefore, we conclude that *E. lutris* is a complete aquatic animal, possessing differences in the proportions of the hindlimb muscles compared with those in other semi-aquatic and terrestrial mustelids.
KEY WORDS: hindlimb, muscle, mustelidae, sea otter, swimming locomotion

The purpose of this study is to clarify the sea otter (*Enhydra lutris*) as a complete aquatic species, based on its unique hindlimb morphology and functional adaptations. Previous investigators have morphologically described the muscles and skeleton of *E. lutris* appendages [2, 15–17], but there have been no comparative morphological studies between *E. lutris* and other closely related species in the Mustelidae family, except for one study on bone density [13]. *Enhydra lutris* has often been morphologically compared with pinnipeds and described as intermediate between terrestrial species and pinnipeds [17, 35, 36, 38]. However, to clarify the evolutionary adaptation of *E. lutris* from a terrestrial habitat to a complete aquatic lifestyle, its appendages should be functionally and morphologically compared with less aquatic species to which it is phylogenetically closely-related [34].

Such closely related species in the Mustelidae family include the Japanese weasel (*Mustela itatsi*), the Siberian weasel (*M. sibirica*), the American mink (*Neovison vison*) and the Eurasian river otter (*Lutra lutra*). These species and *E. lutris* show different levels of dependence on terrestrial and semi-aquatic ecology. *M. itatsi* and *M. sibirica* are closely

related species, which diverged each other about 1.6 million years ago [30]. *M. itatsi* preys on fish in rivers [18], whereas *M. sibirica* does not [32, 37]. Further, *N. vison* and *L. lutra* depend on fish for 5–70% [1, 6, 7] and for 80% over [4] of their diets, respectively. *Enhydra lutris* feeds on sea urchins, octopus, clams and fish [20, 24]. These findings demonstrate that although *M. itatsi*, *N. vison*, *L. lutra* and *E. lutris* all hunt underwater for aquatic prey, they show differences in the degree of aquatic resources use.

The hindlimb structures act as the main propulsion generator during submerged swimming in highly aquatic Mustelidae species [8, 11, 12, 39, 40]. Therefore, by comparing the hindlimb bones and muscles of *E. lutris* with the four other Mustelidae species that show different levels of dependence on terrestrial and semi-aquatic ecology, we can investigate the functional and morphological gradations from a terrestrial habitat to an aquatic lifestyle [13]. Also, examining such closely related species might minimize any phylogenetic influences on hindlimb morphology.

MATERIALS AND METHODS

Specimens: Hindlimb bones were measured in 83 individuals among the five species, and also, 26 individuals were dissected in order to weigh the hindlimb muscle mass. The specimens used in this study are listed in Supplementary Table 1. We used only male specimens of *M. itatsi* and *M. sibirica* and omitted female specimens, because of their strong sexual dimorphism. In particular, it was pointed out

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that the feeding ecology of the female of *M. itatsi* differed from that of the males [19]. There is a possibility that the female of *M. itatsi* does not use the aquatic resources. Both male and female specimens of the other three species were used, since their both sexes also depend on the aquatic habitat [3, 20, 26].

Muscle mass and skeletal length measurements: Muscle mass was recorded since it is considered to be proportional to the maximum power generated by the muscle [5]. The hindlimb muscle nomenclature used in the present study and equivalent names used in previous studies are listed in Supplementary Table 2 [9, 14, 16, 21]. All carcasses had been frozen at −20°C until dissection. During dissection, the left-side hindlimb muscles were exposed. Some of the muscles were fused and could not be divided; so 28 muscle groups were used for the measurements (Table 1). The presemimembranosus muscle was not used in the analysis, because it was absent in some specimens of *N. vison*. The hindlimb muscles were removed from the carcasses. Then, adipose and connective tissues were removed before the muscles were weighed to the nearest 0.001 g using an electronic balance (UX420H, Shimadzu Corp., Kyoto, Japan). Eleven osteological characteristics were measured to the nearest 0.1 mm using calipers (Fig. 1 and Table 2). The measurements were using definitions from a previous study [29], except for FGT, IL and PL. The FGT measurement was defined as the breadth of the femoral head to the greater trochanter of the femur, in order to obtain clear examination of lever arm length of the gluteus superficialis muscle in the lateral direction. The IL and PL pelvis measurements were taken in addition to the existing measurements described by Samuels *et al.* [29].

Definition of aquatic tendency: Aquatic tendency was defined using the dietary data of each species based on the weight ratios of fish remnants in total feces weight in the four species, except for *E. lutris* [4, 6, 18, 22, 37]. The percentages of consumed fish and aquatic tendencies were as follows; *M. sibirica*, 0.0%; *M. itatsi*, 17.7%; *N. vison*, 23.9%; and *L. lutra*, 81.1%. Since *E. lutris* lives their entire lives on the sea surface [23], we considered their aquatic tendency to be 100%.

We defined the terms "aquatic", "semi-aquatic" and "terrestrial" as follows. Aquatic animal: species that possess the ability to live on and/or in the water, e.g. *E. lutris*. Semi-aquatic animal: species that feed on fish and show terrestrial adaptation, e.g. *L. lutra*, *N. vison* and *M. itatsi*. Terrestrial animal: species that are completely adapted to terrestrial habitats and never feed on fish, i.e. they do not possess the ability to hunt fish, e.g. *M. sibirica*.

Statistical analysis: Statistical tests were performed to examine the relationships between hindlimb morphological characteristics and ecological aquatic tendency in Mustelidae. The tests included interspecific pair-wise comparisons, principal components analysis (PCA) and partial Mantel tests. The muscle masses were divided by the geometric means (GM) calculated from obtained 27 masses of muscle groups, except for SOL, since *E. lutris* lacks SOL. The bone measurements were divided by the GM of femoral length (FL), tibial length (TL) and pelvic length (PL). Analysis

Table 1. Muscles and muscle groups examined in this study and their abbreviations

Abbreviations	Contained muscles
ILLPS	Iliopsoas
GSFCTFL	Gluteus superficialis, Gluteofemoralis (Caudofemoralis), Tensor fasciae latae
GMPI	Gluteus medius, Piriformis
GPAC	Gluteus profundus, Iliocapsularis
OE	Obturator externus
OIGE	Gemelli, Obturator internus
QF	Quadratus femoris
RF	Rectus femoris
V3	Vastus lateralis, Vastus medialis, Vastus intermedius
BFSTTE	Biceps femoris, Tenuissimus, Semitendinosus
SMCR	Semimembranosus cranialis
SMCA	Semimembranosus caudalis
SAR	Sartorius
GLA	Gracilis
PEC	Pectineus
ADD	Adductor
TCREHL	Tibialis cranialis, Extensor hallucis longus
TCA	Tibialis caudalis
FI3	Peroneus longus, Peroneus digiti quinti, Peroneus brevis
EDL	Extensor digitorum longus
GLAT	Gastrocnemius caput latelaris
GMED	Gastrocnemius caput medialis
PLA	Plantaris
FHL	Flexor hallucis longus
FDL	Flexor digitorum longus
POP	Popliteus
PESB	Extensor digitorum brevis, Flexor digitorum brevis, Quadratus plantae, Lumbricales, Interosseus, Calcaneometatarsalis
SOL	Soleus

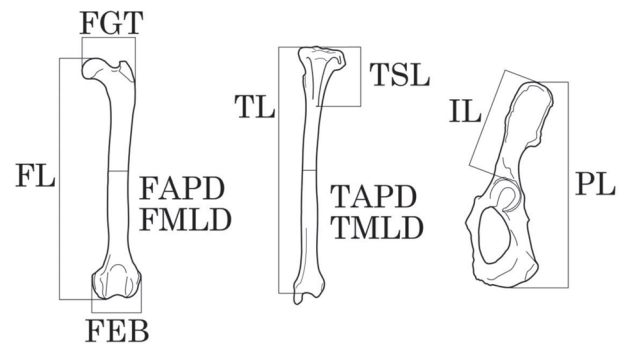Muscular names were reffered to the previous studies [9, 14, 16, 21].

Fig. 1. Bone measurements used in this study. The abbreviations are listed in Table 2.

of variance (ANOVA) was conducted to detect significant differences in the muscular and skeletal measurements between the five species. Homogeneity of variance was tested using the Bartlett's test for post-hoc comparisons. Pair-wise

Table 2.   Bone measurements used in the analysis

Abbreviation	Measurement definitions
FL[*]	Maximum length of the femur from the upper rim of the femoral head to the medial condyle parallel to the shaft.
FGT*	Mediolateral breadth from the femoral head to the greater trochanter.
FAPD	Anteroposterior diameter in the mid-shaft of the femur.
FMLD	Mediolateral diameter in the mid-shaft of the femur.
FEB*	Biepicondylar breadth of the femur.
TL*	Length of the tibia from the anterior rim of the medial condyle to the anterior rim of the talar trochlear articulation parallel to the shaft.
TSL*	Length from the anterior rim of the medial condyle to the distal extension of the tibial tuberosity (spine) parallel to the shaft.
TAPD	Anteroposterior diameter the mid-shaft of the tibia.
TMLD	Mediolateral diameter the mid-shaft of the tibia.
PL*	Maximum length of the pelvis from the posterior end of the ischium to the anterior end of the iliac wing.
IL*	Length from anterior rim of acetabulum to distal end of iliac wing.

The bone measurements are illustrated in Fig. 1. We referred to Samuels *et al.* (2013) [29] for these measurements, except for FGT, PL and IL. Asterisks indicate the measurements used in the principal component analysis.

comparisons were made using the Games–Howell method to reveal the interspecific statistical significance between the muscle and bone measurements. The relative positions of the species were visualized in morphological space using PCA and by drawing biplots with the first (PC1) and second (PC2) principal components. The variables used in the PCA were the scaled measurement values calculated from the respective GM values. Seven skeletal measurement variables were used in the PCAs to avoid deficient values; these were FL, FGT, FEB, TL, TSL, PL and IL (Table 2). Correlation function matrices were used for both PCAs.

The partial Mantel tests were performed to estimate the functional and morphological correlations between hindlimb morphology and the degree of the aquatic tendency in Mustelidae, after phylogenetic effects had been removed. The partial Mantel test clarifies the relationships between two matrices controlling another matrix [23]. We prepared Euclidean distance matrices of the five mustelids for morphology, ecology and phylogeny. The morphological distance matrices were prepared with PC1, PC2 and PC3 from the PCA results of the muscle and bone data. The ecological distance matrix was compiled from sine-transformations of the aquatic tendency ratios described earlier. The phylogenetic distance matrix was constructed of molecular data obtained from the sequence homology of the cytochrome *b* gene [25] (Table 3). All statistical analyses were performed using R version 3.0.1 [27].

RESULTS

Muscle mass measurements: The mean values, standard errors and interspecific significant differences of the 28 muscle weight measurements that were scaled by geometric

Table 3.   Extracted data of cytochrome *b* gene patristic distances for 1,140 bp in the 5 species that were applied to the partial Mantel test; from Marmi *et al.* [25]

	E. lutris	*L. lutra*	*N. vison*	*M. itatsi*
L. lutra	18.4			
N. vison	24.4	24.4		
M. itatsi	22.0	22.1	18.6	
M. sibirica	22.1	22.1	18.7	6.4

The accession numbers of National Center for Biotechnology Information for samples used in making the phylogenetical distance matrix from cytochrome *b* analyses by Marmi *et al.* [25] are as follows: *M. sibirica*, AB026108, AB051242, AB051243; *M. itatsi*, AB026104; *N. vison*, AB026109, AF057129; *L. lutra*, AF057124, X94923; *E. lutris*, AB051244, AF057120, X94924.

mean are shown in Table 4. In ANOVA, there were significant differences between species for the muscle groups, except for SMCR, SAR, GLA, TCREHL, PLA, FDL and PESB. Interspecific pair-wise comparison analyses also revealed significant differences in the relative mass of the muscle groups between species (Table 5). There were significant differences between *E. lutris* and at least one other species for 18 muscle groups (GSFCTFL, GMPI, OE, OIGE, QF, RF, V3, BFSTTE, SMCR, SMCA, GLA, ADD, FI3, GLAT, GMED, FHL, POP and SOL) (Table 4). Some muscle groups showed significant differences between *E. lutris* and all four other species. For example, the masses of GSFCTFL, GMPI, OE, FI3 and POP muscle groups were significantly larger in *E. lutris* than in the other four species, but those of RF, SMCA, ADD and SOL were significantly smaller. In the case of SOL, this is because it does not exist in *E. lutris*. Notably, in four muscle groups (GSFCTFL, RF, ADD and FI3), the only significant difference was between *E. lutris* and the other four species.

Not all muscle groups in *E. lutris* were significantly different to all four other species. For example, the GMED muscle group was significantly larger in *E. lutris* than in *L. lutra*, *M. itatsi* and *M. sibirica,* but not significantly different to that of *N. vison*. Some other muscle groups were smaller in *E. lutris* than in some of the other species; QF, SMCR, GLA and FHL muscle groups were significantly smaller than those of *L. lutra*; OIGE, V3, GLA, GLAT and FHL were significantly smaller than those of *N. vison*; OIGE, QF, V3, SMCR and FHL were significantly smaller than those of *M. itatsi*; and OIGE, QF, V3, BFSTTE, GLA and GLAT were significantly smaller than those of *M. sibirica*.

There were also significant differences between the muscle masses of the four other species. For example, the masses of 19 muscle groups in *L. lutra* were statistically significantly different from at least one other species (ILLPS, GSFCTFL, GMPI, GPAC, OE, OIGE, QF, RF, V3, BFSTTE, SMCR, SMCA, GLA, ADD, FI3, GMED, FHL, POP and SOL). The mass of GMPI in *L. lutra* was larger than in *N. vison,* but that of OIGE was smaller. Also, *L. lutra* had significantly larger OE and GMED muscle mass than *M. itatsi*, but smaller ILLPS, OIGE and SMCA muscle groups. Furthermore, in comparison to *M. sibirica, L. lutra* had

Table 4. Mean values, standard deviations and interspecific significant differences of measurements for geometric mean scaled muscle masses

Groups	*Enhydra lutris*			*Lutra lutra*			*Neovison vison*			*Mustela itatsi*			*Mustela sibirica*		
	mean	SD[a]	vs.[b]	mean	SD	vs.	mean	SD	vs.	mean	SD	vs.	mean	SD	vs.
ILLPS	1.75	0.32		1.23	0.14	I S	1.59	0.25		2.03	0.20	L	1.82	0.31	L
GSFCTFL	3.16	0.32	L V I S	1.75	0.09	E	1.64	0.20	E	1.73	0.11	E	1.82	0.19	E
GMPI	4.07	0.38	L V I S	2.23	0.14	E V	1.79	0.14	E L	1.93	0.17	E	1.98	0.28	E
GPAC	0.42	0.09		0.34	0.05	S	0.32	0.04	S	0.27	0.03	S	0.22	0.02	L V I
OE	0.99	0.15	L V I S	0.56	0.05	E I S	0.50	0.04	E	0.45	0.06	E L	0.41	0.05	E L
OIGE	0.23	0.06	V I S	0.23	0.01	V I S	0.41	0.08	E L	0.41	0.04	E L	0.38	0.06	E L
QF	0.08	0.03	L I S	0.18	0.01	E	0.14	0.04		0.18	0.03	E	0.24	0.05	E
RF	1.30	0.20	L V I S	1.90	0.14	E	1.99	0.20	E	2.09	0.07	E	2.20	0.27	E
V3	2.13	0.41	V I S	2.97	0.35	S	3.35	0.08	E I S	3.61	0.09	E V	3.93	0.26	E L V
BFSTTE	5.02	0.46	S	5.62	0.34	S	5.53	0.47	S	5.98	0.57		6.66	0.40	E L V
SMCR*	1.95	0.14	L I	2.62	0.25	E	2.31	0.31		2.30	0.19	E	2.55	0.34	
SMCA	1.16	0.11	L V I S	2.48	0.18	E I	2.26	0.16	E I	3.13	0.22	E L V	3.14	0.52	E
SAR*	2.10	0.39		1.70	0.15		1.84	0.25		2.12	0.32		2.11	0.31	
GLA*	1.03	0.19	L V S	1.54	0.22	E	1.81	0.29	E	1.41	0.08		1.56	0.18	E
PEC	0.44	0.06		0.35	0.06		0.33	0.04		0.27	0.12		0.35	0.05	
ADD	1.98	0.16	L V I S	2.67	0.22	E	2.67	0.23	E	2.62	0.29	E	2.61	0.19	E
TCREHL*	1.14	0.48		1.43	0.14		1.27	0.06		1.22	0.09		1.23	0.11	
TCA	0.61	0.32		0.33	0.05		0.30	0.04		0.25	0.06		0.26	0.08	
FI3	1.29	0.09	L V I S	0.77	0.11	E	0.82	0.06	E	0.77	0.04	E	0.70	0.05	E
EDL	0.76	0.12		0.64	0.10		0.56	0.03		0.59	0.04		0.50	0.05	
GLAT	1.13	0.11	V S	1.23	0.08		1.41	0.10	E	1.39	0.15		1.59	0.24	E
GMED	2.39	0.13	L I S	2.05	0.10	E I S	1.68	0.49		1.71	0.09	E L	1.76	0.15	E L
PLA*	1.13	0.14		1.21	0.05		1.10	0.12		1.13	0.08		1.17	0.05	
FHL	0.54	0.05	L V I	0.91	0.06	E	1.03	0.12	E	0.91	0.07	E	0.91	0.31	
FDL*	0.26	0.05		0.28	0.05		0.30	0.05		0.34	0.05		0.26	0.05	
POP	0.51	0.02	L V I S	0.30	0.04	E	0.34	0.02	E S	0.29	0.04	E	0.24	0.03	E V
PESB*	1.01	0.16		1.10	0.20		1.04	0.11		0.89	0.21		0.88	0.24	
SOL	0.00	0.00	L V I S	0.17	0.03	E	0.25	0.05	E S	0.16	0.01	E	0.14	0.02	E V

Muscle groups are defined in Table 1. Asterisks indicate no significant differences between the 5 species for that muscle group ($P>0.05$). a) SD, standard deviations. b) vs., species that showed significant differences in univariate ANOVA tests at the $P<0.05$ level using Games-Howell's tests post hoc procedure (E, *Enhydra lutris*. L, *Lutra lutra*. V, *Neovison vison*. I, *Mustela itatsi*. S, *Mustela sibirica*.)

larger GPAC, OE and GMED muscle groups, but smaller ILLPS, OIGE, V3 and BFSTTE muscle groups.

For *N. vison,* 16 muscle groups showed statistically significant differences from at least one other species (GSFCTFL, GMPI, GPAC, OE, OIGE, RF, V3, BFSTTE, SMCA, GLA, ADD, FI3, GMED, FHL, POP and SOL). V3 and SMCA were significantly smaller than those of *M. itatsi*; GPAC, POP and SOL were significantly larger than in *M. sibirica*; V3 and BFSTTE were significantly lower than those of *M. sibirica*.

M. itatsi and *M. sibirica* indicated statistically significant differences from at least one species in 17 muscle groups (ILLPS, GSFCTFL, GMPI, GPAC, OE, OIGE, QF, RF, V3, SMCR, SMCA, ADD, FI3, GMED, FHL, POP and SOL) and 18 muscle groups (ILLPS, GSFCTFL, GMPI, GPAC, OE, OIGE, QF, RF, V3, BFSTTE, SMCA, GLA, ADD, FI3, GLAT, GMED, POP and SOL), respectively. However, the only significant difference between *M. itatsi* and *M. sibirica* was for GPAC, which was significantly larger in *M.itatsi*.

No exclusive significant differences were observed between *L. lutra* and the three Mustelidae species, except for *E. lutris* in a comparison between *E. lutris* and the others.

Although no significant differences were detected, *L. lutra* tended to have relatively larger distal hindlimb muscles (TCREHL, PLA and PESB) than those of the other species.

PC1 accounted for 47.4% of the variance and primarily separated *E. lutris* from the other species (Fig. 2 and Table 5). PC1 was negatively correlated with OE, GMPI, FI3, POP and GSFCTFL, but it was positively correlated with V3, RF and SMCA. PC2 accounted for 13.2% of variance in the dataset and primarily separated *L. lutra* and *N. vison* from the other species. PC2 was negatively correlated with PESB and TCREHL, but it was positively correlated with ILLPS and SAR.

Table 6 shows the results of the partial Mantel test using the ecological, phylogenetic and morphological matrices prepared from PCA scores of the muscular weights. The coefficient of correlation between morphology (PC1) and ecology was 0.70 ($P<0.05$).

Bone length measurements: There were significant differences in FL and PL bone length measurements between the five species (Table 7). FL was shorter in species that possessed the higher aquatic tendency (*E. lutris* and *L. lutra*),

Table 5. Factor loadings of muscle masses in PCA

Abbreviations	PC1	PC2	PC3
ILLPS	0.13	0.65	−0.47
GSFCTFL	−0.88	0.36	0.04
GMPI	−0.92	0.22	0.16
GPAC	−0.78	−0.42	−0.09
OE	−0.96	0.01	0.02
OIGE	0.59	0.09	−0.63
QF	0.75	0.13	0.27
RF	0.88	0.14	0.05
V3	0.90	0.24	−0.11
BFSTTE	0.68	0.46	0.39
SMCR	0.57	−0.17	0.53
SMCA	0.87	0.21	0.04
SAR	0.08	0.72	0.15
GLA	0.64	−0.22	0.08
PEC	−0.52	−0.03	0.04
ADD	0.73	−0.07	0.18
TCREHL	0.11	−0.76	0.25
TCA	−0.70	0.34	0.05
FI3	−0.92	0.11	−0.12
EDL	−0.77	−0.13	0.11
GLAT	0.68	0.22	0.10
GMED	−0.71	0.03	0.39
PLA	−0.01	−0.45	0.35
FHL	0.64	−0.41	−0.21
FDL	0.22	−0.24	−0.54
POP	−0.92	0.03	−0.20
PESB	−0.28	−0.76	−0.15
SOL	0.75	−0.44	−0.18
EV[a]	13.28	3.70	2.04
PVE[b]	47.44	13.22	7.30
CPVE[c]	47.44	60.66	67.96

The abbreviations are defined in Table 1. a) EV, Eigen values. b) PVE, The percentage of the variation explained. c) CPVE, The cumulative percentage of the variation explained.
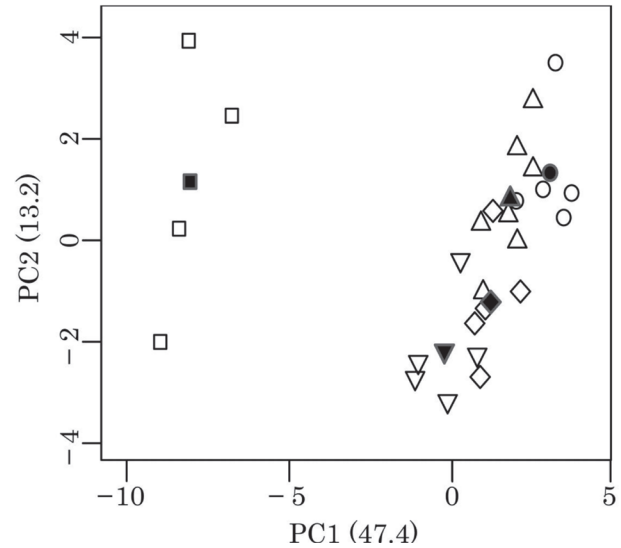

Fig. 2. Plot of PC1 and PC2 scores of muscle masses for the five mustelid species. PC1; the first principal component. PC2; the second principal component. Numbers in parentheses represent the percentage of the variation explained by the component. □, *Enhydra lutris*. ▽, *Lutra lutra*. ◇, *Neovison vison*. △, *Mustela itatsi*. ○, *Mustela sibirica*. Filled symbols indicate mean values of each species.

Table 6. The *P* values of partial Mantel test of muscle masses

	Mo[a] vs. Ec[b]	Mo vs. Ph[c]	Ec vs. Ph	Mo vs Ec con.[d] Ph	Mo vs Ph con. Ec
PC1	0.74	0.37	0.45	0.70	n.s.[e]
PC2	n.s.	n.s.	0.45	n.s.	n.s.
PC3	n.s.	n.s.	0.45	n.s.	n.s.

PC1 score indicates significance level. a) Mo, Morphological distance matrix. b) Ec, Ecological distance matrix. c) Ph, Phylogenetic distance matrix. d) con., conditioned on. e) n.s., not significant.

being the shortest in *E. lutris*. In contrast, PL was longer in these species with more aquatic tendency and was longest in *E. lutris*. IL was significantly larger in *E. lutris* than in the other species.

The percentage of variation explained by PC1 was 84.4%, which separated the samples into the following four plots on the basis of descending scores; *E. lutris*, *L. lutra*, *N.vison* and lastly both *Mustela*(Fig. 3 and Table 8). Both FL and TL correlated negatively with PC1, whereas FGT, FEB, TSL, PL and IL correlated positively. The percentage of variation explained by PC2 was 8.9%, and a separation of plots was found between *E. lutris* and both *N. vison* and *L. lutra*. *M. itatsi* and *M. sibirica* had intermediate scores and were not separated from other species. The factor loading of TSL correlated negatively with PC2.

Table 9 shows the results of the partial Mantel tests of the ecological, phylogenetic and morphological matrices. The coefficient of correlation conditioned on the phylogeny between the morphology (PC1) and the ecology was 0.89 ($P<0.05$).

DISCUSSION

Several studies have reported on the swimming motion of *E. lutris* [17, 36, 40], but no studies have described sequence data of the swimming motion and the movements of each hindlimb joint. Howell [17] studied the hindlimb skeleton of *E. lutris* and predicted that its submerged swimming motion differed from that of pinnipeds. He suggested that the swimming motion of *E. lutris* includes placing the feet horizontally to the rear with the soles up, one either side of the tail and oscillating them in the sagittal plane, in a motion similar to that of whale flukes. He suggested that the swimming motion of *E. lutris* is characterized by dorsoventral undulation of the trunk and by movements of both hindpaws in the dorsoventral direction, with the soles facing dorsally and/ or caudally. However, Howell [17] did not comment on the movements of the hindlimb joints. Our results are consistent with the action predicted by Howell [17]. The GSFCTFL, GMPI, OE, FI3 and POP muscle groups are proportionately

Table 7. Mean values, standard deviations and interspecific significant differences of geometric mean scaled bones measurements

Items	*Enhydra lutris* n[a]	mean	SD[b]	vs.[c]	*Lutra lutra* n	mean	SD	vs.	*Neovison vison* n	mean	SD	vs.	*Mustela itatsi* n	mean	SD	vs.	*Mustela sibirica* n	mean	SD	vs.
FL	13	0.81	0.01	L V I S	10	0.92	0.01	E V I S	10	1.01	0.01	E L I S	26	1.05	0.01	E L V S	24	1.09	0.01	E L V I
FGT	13	0.31	0.01	L V I S	10	0.28	0.01	E V I S	10	0.26	0.00	E L I S	23	0.23	0.01	E L V	24	0.23	0.01	E L V
FAPD	13	0.10	0.01	V I S	5	0.09	0.00	V I	10	0.07	0.00	E L I S	24	0.08	0.01	E L V	24	0.08	0.00	E V
FMLD	13	0.14	0.01	L V I S	5	0.10	0.01	E V I S	10	0.08	0.01	E L	24	0.08	0.00	E L	24	0.08	0.00	E L
FEB	13	0.24	0.01	V I S	10	0.23	0.01	V I S	10	0.20	0.01	E L I S	23	0.19	0.01	E L V S	24	0.18	0.01	E L V I
TL	13	0.93	0.01	L V I S	10	0.99	0.02	E V I S	10	1.06	0.01	E L S	26	1.06	0.01	E L S	24	1.04	0.01	E L V I
TSL	13	0.44	0.02	I S	10	0.44	0.02	V I S	10	0.42	0.01	L I S	26	0.39	0.02	E L V S	24	0.38	0.02	E L V I
TAPD	13	0.10	0.01	V I S	5	0.10	0.01	V I S	9	0.08	0.01	E L	24	0.08	0.01	E L	24	0.08	0.01	E L
TMLD	13	0.07	0.01	V I S	5	0.07	0.00	V I S	9	0.06	0.00	E L	24	0.06	0.00	E L	24	0.06	0.00	E L
PL	13	1.32	0.02	L V I S	10	1.10	0.03	E V I S	10	0.94	0.01	E L I S	26	0.90	0.01	E L V S	24	0.89	0.01	E L V I
IL	13	0.59	0.03	L V I S	10	0.49	0.02	E	10	0.49	0.01	E	26	0.48	0.01	E	23	0.48	0.01	E

The abbreviations of measurements are defined in Table 2. a) n, sample size. b) SD, standard deviations. c) vs., species that showed significant differences in univariate ANOVA tests at the $P<0.05$ level using Games-Howell's tests post hoc procedure (E, *Enhydra lutris*. L, *Lutra lutra*. V, *Neovison vison*. I, *Mustela itatsi*. S, *Mustela sibirica*)
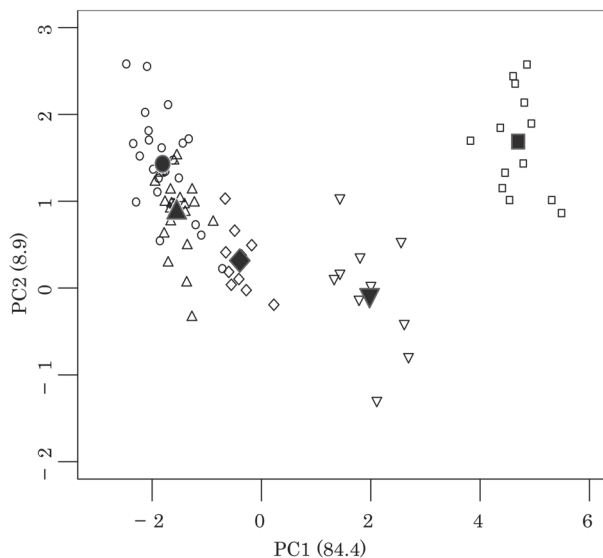

Fig. 3. Plot of PC1 and PC2 scores of bone measurements for the five mustelid species. PC1; the first principal component. PC2; the second principal component. Numbers in parentheses represent the percentage of the variation explained by the component. □, *Enhydra lutris*. ▽, *Lutra lutra*. ◇, *Neovison vison*. △, *Mustela itatsi*. ○, *Mustela sibirica*. Filled symbols indicate mean values of each species.

approximately two times larger in *E. lutris* than in the other species, but the RF, SMCA and ADD are significantly smaller in *E. lutris* than in the other species (Table 4). These results indicate that *E. lutris* utilizes GSFCTFL, GMPI, OE, FI3 and POP more powerfully than the other four species since muscle mass is proportional to work capacity [5]. Therefore, we suggest that during submerged swimming, the power-stroke phase of *E. lutris* comprises abduction of the femur by the GSFCTFL and GMPI muscles, medial rotation of the crus by the POP group, eversion movements of the ankle and also abduction of the fifth toe or extension of interdigital

web by the FI3 group, in the FI3 group the peroneus brevis and the peroneus longus enable abduction of the fifth toe, and the peroneus digiti quinti extends the interdigital digit. The gluteus superficialis, gluteofemoralis and tensor fasciae latae muscles in the GSFCTFL group are fused in *E. lutris* [16, 17]. This muscle group covers the lateral side of the hip joint; it attaches the lateral femoral ridge of the inferior border of the greater trochanter to the upper border of the lateral femoral condyle [16]. Therefore, GSFCTFL may act as an abductor of the hip joint. In addition, it is pointed out that the piriformis muscle of *E. lutris* acts as an abductor on femur [16]. Movement of these three muscles at the hip, knee and ankle joints is not required for running on land. Therefore, the greater mass and power of these muscles in *E. lutris* suggests that these movements of the hindlimb are used for the swimming motion of this species.

The other mustelids are equipped with large muscles in the distal part of the hindlimbs (TCA, EDL, GMED and PESB), according to their aquatic tendency (Table 4). However, the evolutionary specializations observed in *E. lutris* were not observed in *M. sibirica*, *M. itatsi*, *N. vison* or *L. lutra*. The hindlimb morphology of terrestrial and semi-aquatic mustelids is regarded as a continuum. The North American river otter (*Lontra canadensis*) swims using dorsoventral movement of the trunk, whereas seals swim using lateral movement of the trunk [10]. It is possible that none of the five species examined in this study swim using lateral movement of the trunk. The swimming motion of *M. itatsi* and *M. sibirica* has not yet been studied, but Dunstone described the swimming motion of *N. vison*, which used all four limbs with either diagonally opposite legs simultaneously under the body [8]. We suspect that the swimming motion of both of these mustelids may be similar to that of *N. vison*, as the hindlimb muscle distribution morphologically resembles in these species. Terrestrial animals run on the ground with an anterior–posterior motion of the appendages under the trunk. Therefore, the results of our study suggest that the semi-aquatic animals investigated in this study swim with a motion similar to terrestrial running.

Table 8. Factor loadings of bone measurements in PCA

Abbreviations	PC1	PC2	PC3
FL	−0.98	−0.02	0.00
FGT	0.96	−0.06	−0.13
FEB	0.94	−0.17	−0.23
TL	−0.93	−0.22	0.10
TSL	0.75	−0.62	0.22
PL	0.99	0.13	−0.01
IL	0.87	0.38	0.30
EV[a]	5.91	0.62	0.22
PVE[b]	84.39	8.88	3.10
CPVE[c]	84.39	93.27	96.37

The abbreviations of bone measurements are defined in Table 2. a) EV, Eigen values. b) PVE, The percentage of the variation explained. c) CPVE, The cumulative percentage of the variation explained.

Table 9. The *P* values of partial Mantel test of bone measurements

	Mo[a] vs. Ec[b]	Mo vs. Ph[c]	Ec vs. Ph	Mo vs. Ec con.[d] Ph	Mo vs. Ph con. Ec
PC1	0.89	0.64	0.45	0.88	0.60
PC2	n.s.[e]	n.s.	0.45	n.s.	n.s.
PC3	n.s.	n.s.	0.45	n.s.	n.s.

PC1 score indicates significance level in whole cases. a) Mo, Morphological distance matrix. b) Ec, Ecological distance matrix. c) Ph, Phylogenetic distance matrix. d) con., conditioned on. e) n.s., not significant.

Taylor [38] compared the roughness of iliac crest of *E. lutris* with that of harbor seal (*Phoca vitulina*) and *L. canadensis* quaritatively, and noticed more aquatic species held rougher crest. He suggested that the sartorius muscles (SAR), which originate from the crest, for adducting the femur were augmented in size with the increase in importance of the hindlimbs as a paddle from his observations. This aquatic adaptation tendency referred to by Taylor was not observed in any of the five species examined in our result, which showed no significant differences. Although, the mean SAR value in *E. lutris* (mean value 2.10) was proportionately larger than that of *L. lutra* (mean value 1.70), the mean values in other three mustelids: *N. vison*, 1.84; *M. itats*, 2.12 and *M. sibirica*, 2.11, were relatively larger than that in *L. lutra*. These data show that the aquatic tendency is not associated with the size increasing of SAR that was proposed by Taylor [38], at least not in Mustelidae.

Various studies have shown femoral shortening in aquatic or semi-aquatic animals [28, 29, 33, 35, 38]. Samuels *et al.* [29] reported that femoral shortening brought the paddling limb closer to the body and thus reduced induced drag during the recovery stroke when swimming. In our study, we also found femoral shortening (lower FL values) in *E. lutris* and *L. lutra* species which had higher aquatic tendency (Table 7). Additionally, it is interesting to note the significant difference in FL between *M. itatsi* (mean value 1.05) and *M. sibirica* (mean value 1.09), which hunts fishes but it does not well adapt to swimming, although both species diverged each other about 1.6 million years ago [30]. The shortening of femur enables the foot to close to the body axis [35]. It seems that the closing contributes to efficient swimming.

Smith and Savage [31] suggested that more aquatically adapted animals possess smaller gluteus and larger ischiopubic muscles, which include the semimembranosus, biceps femoris, semitendinosus and tenuissimus after comparing the iliac length in proportions to the pelvic bones among a marten (*Martes* sp.), a river otter (*Lutra* sp.) and a seal (*Phoca* sp.). However, our results do not support this suggestion since *E. lutris* had the largest relative mass of gluteus muscles, and it and *L. lutra* also had smaller relative mass of the semimembranosus, biceps femoris, semitendinosus

and tenuissimus muscles (Table 4). In addition, the IL length value of *E. lutris* (mean value 0.59) was significantly much larger than that of the other four species (mean value; *L. lutra* and *N. vison*, 0.49; *M. itatsi* and *M. sibirica*, 0.48) (Table 7). There are morphological differences, since the body of seal moves bilaterally, but that of both *E. lutris* and *L. lutra* moves dorsoventrally when they swim [35]. The difference between the results of Smith and Savage and ours was caused by following reason: *E. lutris* developed their gluteal muscles and became to aquatic; however, seals adopt another development for aquatic.

In conclusion, our muscle and bone measurement analyses revealed that the hindlimb morphology of the mustelids has obvious functional and morphological relationships with their ecology rather than with their phylogeny (Tables 6 and 9). We could confirm that *E. lutris* had unique hindlimb morphological characteristics compared to the other Mustelidae. In our study, we defined the aquatic tendency of each species from ecological information. Based on this information, we defined *E. lutris* as having full aquatic tendency, whereas the other four species had semi-aquatic or fully terrestrial tendencies. Furthermore, the muscle and bone analyses indicated morphological differences associated with hip joint abductors between *E. lutris* and the four other species that were categorized as terrestrial or semi-aquatic species. Therefore, we suggest the hindlimb of *E. lutris* clearly reflects their complete aquatic lifestyle. This hindlimb structure has resulted in *E. lutris* acquiring a more abductable femur, as it evolved away from the other semi-aquatic mustelidae and their need to partly survive on land. The *E. lutris* abductable femur may enable the species to develop maneuverability when they swim by raising the center of mass near to the body axis, the same as the shortening of femur. We conclude that *E. lutris* is a complete aquatic animal, possessing differences in the proportions of the hindlimb muscles compared with those in other semi-aquatic and terrestrial mustelids. The semi-aquatic species share similar muscle proportions with terrestrial rather than with aquatic species.

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