Are phenotypic traits useful for differentiating among *a priori Coregonus* taxa?

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A key for three putative species apparently found in three geographic areas, *i.e. Coregonus clupeoides* (in Scotland), *Coregonus stigmaticus* (in England), and *Coregonus pennantii* (in Wales) given in a recent review was tested quantitatively using 544 individuals from nine populations. The classification success of the key was very low (27%). It was concluded that there is currently no robust evidence for the recognition of the three putative species. Furthermore, the use of phenotypic characters alone to distinguish putative species in postglacial fish species such as those of the genus *Coregonus* that show homoplasy in many of these traits is questioned. In the absence of further evidence, it was concluded that a single highly variable species best describes the pattern of phenotypic variation in these U.K. populations. On this basis it is argued that taxonomic subdivision of U.K. European coregonids is inappropriate and that *Coregonus lavaretus* should prevail as the species name applicable to all populations. © 2012 The Authors

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INTRODUCTION

The definition of which nominal species and populations do, and do not, comprise a true species is an important biological concept with potentially significant consequences. Accurate species definitions allow diversity to be robustly catalogued and managed. Species definition is particularly important, both as a political and a conservation concept, as it is generally species that are protected by law, discussed

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in the media or recognized by the general public. It is also essential for information exchange. How to define a species, however, is not completely clear: there are >20 different species definitions in current use (Mayden, 1997; Adams & Maitland, 2007). This is a particularly significant issue for those taxa that can exhibit great variation between and within individual sites, including some freshwater fishes and in particular those living in postglacial lakes (Campbell, 1985; Kahilainen & Østbye, 2006; Adams & Maitland, 2007).

Coregonines (whitefishes) are holarctic species which are found throughout the cooler regions of the northern hemisphere, and are renowned for the level of phenotypic variation displayed both between and within populations. This has resulted in considerable debate regarding the allocation and definition of species (Berg, 1962; Wheeler, 1969; McPhail & Lindsey, 1970). Phenotypic plasticity (Lindsey, 1981), alternative evolutionary scenarios (*i.e.* allopatric *v.* sympatric speciation) (Douglas *et al.*, 1999, 2005), and introgression (Svärdson, 1957), have all been identified as being responsible for the existence of different coregonine forms. These can be recognized as different morphs, or even subspecies, and complicate the use of morphological and anatomical characters in their taxonomy. Traditionally, coregonids have been segregated into forms by counts of meristic characteristics, such as gill rakers, which have a high hereditary component (Svärdson, 1950, 1951). In fishes, however, some meristic characters can be affected by interactions between species (Lindsey, 1981), or contrasting environmental conditions (Svärdson, 1951; Barlow, 1961; Swain & Lindsey, 1986). The radiation of extant coregonine species has occurred comparatively recently [*i.e.* during the repeated glaciations of the Pleistocene 0·012−2·6 million years before present (B.P.)], thus relatively low levels of genetic variation contrasts with high levels of morphological differentiation observed among coregonines (Bernatchez *et al.*, 1996). It has therefore been suggested that genetic analyses have more discriminatory power to reveal phylogenetic patterns, while morphological patterns are better indicators of adaptive processes in these species (Bernatchez *et al.,* 1996, 1999; Østbye *et al.,* 2006).

According to Maitland & Campbell (1992) and Davies *et al.* (2004) three coregonid species occur naturally in the U.K. and Ireland: the Arctic cisco *Coregonus autumnalis* (Pallas 1776), the vendace *Coregonus albula* (L. 1758) and the European whitefish *Coregonus* spp. *lavaretus* (L. 1758). The British populations of *Coregonus* are thought to have originated from anadromous ancestors that migrated from refugia after the last glaciation, *c.* 10 000 B.P. Extant populations are now lacustrine (Maitland, 1970). The houting *Coregonus oxyrinchus* (L. 1758) (Maitland & Campbell (1992) and Davies *et al.* (2004) was the only anadromous coregonid in the U.K., but is now considered to be extinct in the U.K. (Maitland & Lyle, 1991; Freyhof & Schoeter, 2005). As with many coregonids, the species designations of these fishes have undergone many changes (Etheridge, 2009).

In Britain, coregonids are afforded legal protection due to their rarity. Seven native populations of *C. lavaretus* (Maitland & Campbell, 1992; Davies *et al.,* 2004) occur in Britain as four populations in England, two populations in Scotland and one population in Wales. As a conservation measure, two refuge populations of *C. lavaretus* have been successfully established in Loch Sloy and Carron Valley Reservoir Scotland using parental stock from Loch Lomond (Maitland & Lyle, 1990). Additional refuge populations of *C. lavaretus* sourced from other native populations are in the process of being established (Winfield *et al.*, 2002; Etheridge, 2009). The

conservation interest in these taxa makes the understanding of taxonomic diagnosis and geographic distribution particularly crucial.

The generally accepted view of European coregonid taxonomy has recently been challenged by Kottelat & Freyhof (2007), who claim to have clarified much of the uncertainty in the systematics of the Coregonidae. Kottelat & Freyhof (2007) recognize 59 separate species of *Coregonus* across Europe and suggest that many more are likely to be accepted in future. Within the U.K., the seven native populations of *C. lavaretus* have been identified as three different endemic species by Kottelat & Freyhof (2007). According to those authors, both Scottish populations of *C. lavaretus* are endemic *Coregonus clupeoides* Lacépède 1803, all four English populations of *C. lavaretus* are endemic *Coregonus stigmaticus* Regan 1908 and the single Welsh population of *C. lavaretus* is endemic *Coregonus pennantii* Valenciennes 1848. In addition, all U.K. populations of *C. albula* are considered to be endemic *Coregonus vandesius* Richardson 1836, while Irish populations of *C. autumnalis* are endemic *Coregonus pollan* Thompson 1835. Before changes of such a radical degree are adopted into widespread use, however, the supporting case for them needs to be rigorously assessed.

This paper focuses on all seven native populations and two refuge populations of *C. lavaretus* (Maitland & Campbell, 1992; Davies *et al.,* 2004) in the U.K. The key presented by Kottelat & Freyhof (2007) uses phenotypic traits to differentiate the native populations into the three geographically separated putative species noted above: *C. clupeoides* (Scotland), *C. stigmaticus* (England) and *C. pennantii* (Wales). The aims of the current paper are, first, to critically evaluate the accuracy of the proposed key to U.K. *Coregonus* populations and, second, to evaluate the reliability of the phenotypic traits for separating individuals from a known site into the 'expected' putative species. This was achieved by assuming that the link between site and putative species presented by Kottelat & Freyhof (2007) is correct, *i.e*. an individual sampled from within the defined range of *C. clupeoides* was a putative *C. clupeoides* and so forth. The results are discussed in relation to the usefulness of phenotypic traits in identification of species of *Coregonus*.

METHODS

Coregonids representing the three putative species discussed by Kottelat & Freyhof (2007) (*C. clupeoides, C. stigmaticus* and *C. pennantii)* were collected from nine study sites (Table I). Four study sites situated in Scotland contain putative *C. clupeoides*: Loch Lomond (LL) and Loch Eck (LE) (natural populations), and Loch Sloy (LS) and Carron Valley Reservoir (CR) (conservation refuge populations of Loch Lomond origin). Four study sites located in England contain putative *C. stigmaticus*: Brotherswater (BW), Haweswater Reservoir (HW), Red Tarn (RT) and Ullswater (UW). One study site, Llyn Tegid (LT) is located in Wales and contains putative *C. pennantii*. Only U.K. *C. lavaretus* were investigated; individuals of putative *C. vandesius* and putative *C. pollan* are accepted as distinct species (*C. albula* and *C. autumnalis*, respectively, (Maitland & Campbell, 1992; Davies *et al*., 2004) and thus individuals were not sampled in this study.

Multi-panel Norden benthic gillnets, which comprise 12 panels, ranging from 5 to 55 mm, knot-to-knot mesh, were set in each of the sites except Haweswater where fishes were obtained from entrapment in the water abstraction system. Nordic nets are not selective for coregonids over the modal size range 78−613 mm fork length (L_F) (Jensen, 1986). All fishes were examined fresh, or frozen within 4 h of removal from the gillnet or water abstraction system.

TABLE I. Study site and *Coregonus* capture information

| | | | Site data | | | | Netting data | |
Site	Region	Latitude and Longitude	SA (km^2)	MD (m)	ASL (m)	Status	Dates	n
LL	Scotland	56° 05′ N; 4° 36′ W	71·0	190	8	Native	09 November 2005–24 January 2006	99
							15 May 2008–23 July 2008	9
LE	Scotland	56° 06′ N; 4° 59′ W	4·6	42	9	Native	09 January 2006	103
							29 July 2008	32
LS	Scotland	56° 16′ N; 4° 47′ W	1·0	Ca.40	287	Introduced	21 December 2005–28 December 2005	34
							28 July 2008	35
CR	Scotland	56° 02′ N; 4° 06′ W	3·0	Ca.10	223	Introduced	03 January 2006–05 January 2006	53
							31 July 2008	6
BW	England	54° 30′ N; 2° 55′ W	0·19	16	173	Native	04 July 2008	19
HW	England	54° 03′ N; 2° 48′ W	3·9	57	246	Native	Winter 2007–2008	11
RT	England	54° 31′ N; 3° 31′ W	0·098	25	718	Native	15 August 2008 & 05 September 2008	21
UW	England	54° 34′ N; 2° 54′ W	8·9	63	145	Native	04 July 2008–12 September 2008	29
LT	Wales	52° 54′ N; 3° 37′ W	4·14	42	170	Native	15 December 2008	43

SA, surface area; MD, maximum depth; ASL, height above sea level; *n*, number of *Coregonus* sampled. LL, Loch Lomond; LE, Loch Eck; LS, Loch Sloy; CR, Carron Valley Reservoir; BW, Brotherswater; HW, Haweswater Reservoir; RT, Red Tarn; UW, Ullswater; LT, Llyn Tegid.

In the laboratory, fishes were thawed if necessary. The L_F ($\pm$ 1 mm) and mass (M) ($\pm$ 0·01 g) were measured. Measurements ($\pm$ 0·1 mm) and meristic counts that are featured in the Kottelat & Freyhof's (2007) key to coregonids in the British Isles were taken from the fishes. The first branchial gill arch on the left side was removed and stored in 70% ethanol; the gill rakers were subsequently counted.

Specimens held at the Natural History Museum, London were also examined (Appendix), to provide additional preserved specimens for examination, as only limited netting for fresh specimens was possible. These specimens had been preserved in formalin and stored in ethanol. In total 50 specimens were examined that could be used to test the key: putative *C. clupeoides* from Loch Lomond ($n = 11$) and Loch Eck ($n = 2$); putative *C. stigmaticus* from Haweswater ($n = 14$) and Ullswater ($n = 4$), and putative *C. pennantii* from Llyn Tegid ($n = 19$). Measurements and counts were recorded as described above.

Individuals of British coregonids from sites identified by Kottelat & Freyhof (2007) as containing putative *C. clupeoides*, putative *C. stigmaticus* and putative *C. pennantii* were identified using the published key as follows. Key dichotomy 1: *C. pollan* have a terminal mouth, remaining British coregonids have a sub-terminal mouth. Key dichotomy 2: *C. oxyrinchus* have a snout length that is two eye diameter lengths, *i.e.* eye diameter lengths in snout length (N_{EDxSL}) = 2, the number of gill rakers (N_{GR}) = 36−44, and are anadromous. The remaining British coregonids have $N_{EDxSL} > 2$, N_{GR} = 29−41, and are lacustrine. Key dichotomy 3: *C. stigmaticus* have spots on the back and flank, *i.e.* presence of spots (P_S) = yes, and a head length that is 3−3·3 interorbital distance lengths, *i.e.* interorbital distance lengths in head length (N_{IDxHL}) = 3·0−3·3. The remaining British coregonids do not have spots, *i.e.* P_S = no, and N_{IDxHL} = 3·3−3·7. Key dichotomy 4: *C. clupeoides* have 7·5−8·5 scale rows between the lateral line and pelvic-fin base, *i.e.* scale rows between the lateral line and pelvic fin (N_{ScLP}) = 7·5−8·5, the number of circumpeduncular scale rows (N_{ScC}) = 20−21, and the number of anal-fin rays (N_{AFR}) = 9·0−11·5. *Coregonus pennantii* have N_{ScLP} = 8·0−10·0, N_{ScC} = 22−24 and N_{AFR} = 11·0−13·5.

The numbers of fishes identified to putative species using the published key and the method of specimen storage (fresh, frozen and preserved) were tested with χ^2. It was expected that, *e.g.* fishes from a *C. clupeoides* site (in Scotland), would be assigned as *C. clupeoides* in the key. If this was not the case, the result was classed as 'unexpected' (cf. 'expected'). Mann−Whitney U-test and Fisher's exact test were used to explore for site-level differences in the phenotypic traits used in the key to differentiate between putative species. Principal components analysis (PCA) was used to combine all the key phenotypic traits and provide new variables in which most of the variation was explained. The differences in principle component (PC) scores for individuals were explored using a general linear model (GLM), using region (putative species), and site nested within region as grouping factors. To further examine PC scores, discriminant function analysis (DFA) was used to assign fish using phenotypic traits at both region (putative species) and site level. Statistical analyses were conducted using SPSS v. 13 (SPSS Inc.; www.ibm.com).

RESULTS

TESTING OF THE KEY

The phenotypic key provided by Kottelat & Freyhof (2007) was highly unreliable for allocating fishes to putative species. Most individuals could not be identified to any species (73%). Dichotomy 1 and 2 did not result in many unidentifiable fishes (only 0·4%), but dichotomies 3 and 4, resulted in large numbers of unidentifiable individuals (Table II). There was a significant difference in number of specimens allocated to each result (*i.e.* identified or unidentified) depending on the storage method (fresh, frozen or preserved in alcohol) (χ^2, d.f. = 2, $P < 0·001$), with frozen fishes being identified more often (32%) using the key than fresh fishes (15%) or museum specimens preserved in alcohol (14%).

TABLE II. Key allocation numbers for fish from *Coregonus stigmaticus* (England), *Coregonus clupeoides* (Scotland) and *Coregonus pennantii* (Wales) sites

Key level	Dichotomy[a]	Trait[b]	*C. stigmaticus* site	*C. clupeoides* site	*C. pennantii* site	Total	Result
1	A	Terminal mouth	0	0	0	0	*C. pollan*
	B	Sub-terminal mouth	98	384	62	544[c]	
	C	Other than above	0	0	0	0[d]	
2	a	$N_{EDxSL} = 2$	0	0	0	0	
	b	$N_{EDxSL} < 2$	98	383	62	543	
	a	$N_{GR} = 36\text{–}44$	54	51	53	158	
	b	$N_{GR} = 29\text{–}41$	85	255	54	394	
	A	Only *a* traits	0	0	0	0	*C. oxyrinchus*
	B	Only *b* traits	98	383	61	542[b]	
	C	Other than above	0	1	1	2[c]	
3	a	$P_S = yes$	29	1	0	30	
	b	$P_S = no$	69	382	62	513	
	a	$N_{IDxHL} = 3.0\text{–}3.3$	22	154	39	215	
	b	$N_{IDxHL} = 3.3\text{–}3.7$	2	231	26	259	
	A	Only *a* traits	2	0	0	2	*C. stigmaticus*
	B	Only *b* traits	2	231	26	259[b]	
	C	Other than above	94	152	35	281[c]	
4	a	$N_{ScLP} = 7.5\text{–}8.5$	0	55	7	62	
	b	$N_{ScLP} = 8\text{–}10$	2	190	23	215	
	a	$N_{ScC} = 20\text{–}21$	0	74	20	74	
	b	$N_{ScC} = 22\text{–}24$	1	140	12	141	
	a	$N_{AFR} = 9\text{–}11.5$	0	94	2	96	
	b	$N_{AFR} = 11\text{–}13.5$	2	212	24	238	
	A	Only *a* traits	0	10	0	10	*C. clupeoides*
	B	Only *b* traits	2	122	11	135	*C. pennantii*
	C	Other than above	0	99	15	114[c]	

[a]Lower case letters, individual character dichotomy outcomes within a key level; upper case letters, key dichotomy outcomes for a key level.

[b]L_F, fork length; N_{EDxSL}, eye diameter lengths in snout length; N_{GR}, number of gill rakers; P_S, presence of spots; N_{IDxHL}, interorbital distance lengths in head length; N_{ScLP}, number of scale rows between the lateral line and pelvic fin; N_{ScC}, number of circumpeduncular scale rows; N_{AFR}, number of anal-fin rays.

[c]British coregonid, moved to next key dichotomy.

[d]Unidentifiable (removed from analysis).

The key was also unsuccessful in allocating fishes from a known site in a geographical region to the putative species present in that region (Table III). Ninety-eight fishes were sampled from sites considered by Kottelat & Freyhof (2007) to contain *C. stigmaticus*. Using the key, however, only two putative *C. stigmaticus* were identified; both individuals were from putative *C. stigmaticus* sites. Three hundred and eighty-four fish were sampled from sites considered by Kottelat & Freyhof (2007) to contain *C. clupeoides*. Using the key, however, only 10 putative *C. clupeoides* were identified; these 10 individuals were from putative *C. clupeoides* sites. Sixty-two specimens were sampled from sites considered by Kottelat & Freyhof (2007) to contain *C. pennantii*. Using the key, however, 135 putative *C. pennantii* were identified; 11 of these individuals were from the putative *C. pennantii* site. Thus the key resulted in just 23 of 544 individuals ($< 5\%$) being identified as expected (or

correctly). Those from Scottish sites (putative *C. clupeoides*) were often identified as *C. pennantii*, from the Welsh site.

ASSESSMENT OF PHENOTYPIC TRAITS

When comparing L_F and traits used in Kottelat & Freyhof's (2007) key, more significant differences were found between regions (Table IV; Bonferroni corrected $P < 0.0002$, 57%) than within regions (Table V; Bonferroni corrected $P < 0.0005$, 27%). When only traits that distinguish between putative species in the different regions in Kottelat & Freyhof's (2007) key were compared, *i.e.* P_S and N_{IDxHL} (separating putative *C. stigmaticus* from other British coregonids) or N_{ScLP}, N_{ScC} and N_{AFR} (separating putative *C. clupeoides* from putative *C. pennantii*), more significant differences were found between sites in different regions (see shaded areas in Table IV, 69%) than between sites in the same region (see shaded areas in Table V, 23%). Measurements taken from specimens, however, were often outside the trait range described in the key (Fig. 1). It was found that the putative *C. clupeoides* and *C. stigmaticus* sites contained populations of smaller sized or larger sized individuals, but putative *C. pennantii* was found in only one site which contains smaller individuals (Fig. 1). In those fishes from sites not in the same region (*i.e.* from different putative species), the most similar (the fewest significant differences in traits) were CR and LT, while the most different (the greatest number of significant differences in traits) were LE and UW. In those fishes from sites within the same region (*i.e.* same putative species), the most similar were BW and HW, while the most different were LL and LE.

All key traits were found to be significantly correlated with L_F (all comparisons, Pearson correlation, $P < 0.01$) and many traits (52%) were multico-linear (Pearson correlation, $P < 0.05$). Therefore PC scores were extracted from a PCA of all key traits and L_F. The PC1 explained 24% of the total variation in the examined characters and was most positively correlated with L_F (0.90) and then N_{EDxSL} (0.78). The PC2 explained 22% of the total variation in the examined characters and was most positively correlated with N_{IDxHL} (0.76) and negatively correlated with P_S (−0.70). The PC3 explained 14% of the total variation in the examined characters and was most positively correlated with N_{ScLP} (0.67) and then N_{ScC} (0.55). The PC4 explained 13% of the total variation in the examined characters and was most positively correlated with N_{AFR} (0.80). There was a large overlap in all traits between different putative species and sites (Fig. 1).

The GLM analysis of these PC scores using the factors of region (equivalent to putative species) and site nested within region, indicated that while most of the variation observed [partial Eta squared (gives the contribution of each factor or interaction, taken as if it were the only variable) = 0.59] was explained by region ($F_{2,331} = 115.6$, $P < 0.001$), a significant though lesser amount (partial Eta squared = 0.29) was explained by site nested within region ($F_{6,331} = 22.4$, $P < 0.001$).

Discriminant analysis assigning individuals to region (or putative species) revealed two discriminant functions (DF). The first explained 69% of the variance (canonical $R^2 = 0.62$) and the second explained 31% of the variance (canonical $R^2 = 0.43$). Together these two DFs significantly differentiate the putative species ($\chi^2 = 509.4$, d.f. = 8, $P < 0.001$) (Fig. 2). The correlations between characters and DFs indicated that PC2 (N_{IDxHL} and P_S) ($r = 0.98$) had the most important contribution

TABLE III. Percentage of individuals from each site allocated to *Coregonus stigmaticus*, *Coregonus clupeoides*, *Coregonus pennantii* or unidentified using provided key

Key assignment[a]	Sites[b]	Putative C. clupeoides				Putative C. stigmaticus				Putative C. pennantii	Total
		LL ($n = 119$)	LE ($n = 137$)	LS ($n = 69$)	CR ($n = 59$)	BW ($n = 19$)	HW ($n = 25$)	RT ($n = 21$)	UW ($n = 33$)	LT ($n = 62$)	($n = 544$)
C. stigmaticus		0·0	0·0	0·0	0·0	0·0	8·0	0·0	0·0	0·0	0·4
C. clupeoides		3·4	1·5	5·8	0·0	0·0	0·0	0·0	0·0	0·0	1·8
C. pennantii		40·3	25·5	24·6	37·3	5·3	4·0	0·0	0·0	17·7	24·8
Unidentified		56·3	73·0	69·6	62·7	94·7	88·0	100·0	100·0	82·3	73·0

[a]Expected or 'correct' allocations shaded.
[b]LL, Loch Lomond; LE, Loch Eck; LS, Loch Sloy; CR, Carron Valley Reservoir; BW, Brotherswater; HW, Haweswater Reservoir; RT, Red Tarn; UW, Ullswater; LT, Llyn Tegid, *n*, sample size.

TABLE IV. Significant differences in a comparison of median values of traits between sites in different regions (containing different putative *Coregonus* species)

Population comparison[a]		L_F	N_{EDxSL}	N_{GR}	P_S	N_{IDxHL}	N_{ScLP}	N_{ScC}	N_{AFR}
A[d]	LL	BW			SD[c]		SD	SD		
	LL	HW			SD	SD	SD			
	LL	RT	SD	SD		SD	SD			
	LL	UW	SD			SD	SD		SD	
	LE	BW	SD			SD	SD	SD		
	LE	HW	SD			SD	SD	SD		
	LE	RT				SD	SD			SD
	LE	UW	SD			SD	SD	SD	SD	
	LS	BW	SD			SD	SD	SD	SD	
	LS	HW	SD			SD	SD	SD	SD	
	LS	RT	SD	SD		SD	SD			
	LS	UW	SD			SD	SD	SD	SD	
	CR	BW	SD	SD			SD	SD		
	CR	HW	SD	SD	SD	SD	SD	SD		
	CR	RT	SD	SD		SD	SD			
	CR	UW		SD	SD	SD	SD	SD	SD	
B[e]	LL	LT	SD	SD	SD					
	LE	LT		SD	SD		SD			SD
	LS	LT	SD	SD	SD		SD			
	CR	LT	SD	SD	SD					
C[f]	BW	LT	SD	SD	SD		SD	SD	SD	
	HW	LT	SD	SD		SD	SD		SD	
	RT	LT	SD	SD	SD	SD	SD			
	UW	LT	SD	SD		SD	SD		SD	

[a]LL, Loch Lomond; LE, Loch Eck; LS, Loch Sloy; CR, Carron Valley Reservoir; BW, Brotherswater; HW, Haweswater Reservoir; RT, Red Tarn; UW, Ullswater; LT, Llyn Tegid.

[b]L_F, fork length; N_{EDxSL}, eye diameter lengths in snout length; N_{GR}, number of gill rakers; P_S, presence of spots; N_{IDxHL}, interorbital distance lengths in head length; N_{ScLP}, number of scale rows between the lateral line and pelvic fin; N_{ScC}, number of circumpeduncular scale rows; N_{AFR}, number of anal-fin rays.

[c]SD, Mann–Whitney U-test significant at Bonferroni corrected significance $\alpha < 0.0002$.

[d]Putative *Coregonus clupeoides* (Scotland) compared with putative *Coregonus stigmaticus* (England); relevant trait comparisons in key shaded.

[e]Putative *C. clupeoides* (Scotland) compared with putative *Coregonus pennantii* (Wales); relevant trait comparisons in key shaded.

[f]Putative *C. stigmaticus* (England) compared with putative *C. pennantii* (Wales); relevant trait comparisons in key shaded.

to DFI which separated putative *C. stigmaticus* and putative *C. pennantii* from putative *C. clupeoides*. The most important contribution to DFII was from PC1 (L_F and N_{EDxSL}) ($r = 0.78$) which separated putative *C. pennantii* from putative *C. stigmaticus* and putative *C. clupeoides*. Classification success (*i.e.* allocation of fishes to region of origin on the basis of phenotypic traits) was generally high with 96 and 93% of putative *C. clupeoides* and putative *C. pennantii* allocated to the correct region respectively, however, only 67% of putative *C. stigmaticus* were allocated to

TABLE V. Significant differences in a comparison of median values of traits between sites in a region (containing the same putative *Coregonus* species)

	Population comparison[a]		L_F	N_{EDxSL}	N_{GR}	P_S	N_{IDxHL}	N_{ScLP}	N_{ScC}	N_{AFR}
A[d]	LL	LE	SD[c]	SD			SD	SD		SD
	LL	LS	SD							
	LL	CR	SD	SD						
	LE	LS	SD	SD						SD
	LE	CR	SD	SD			SD			SD
	LS	CR	SD	SD						
B[e]	BW	HW								
	BW	RT	SD	SD						
	BW	UW					SD			
	HW	RT	SD							
	HW	UW					SD			
	RT	UW	SD	SD			SD		SD	

[a]LL, Loch Lomond; LE, Loch Eck; LS, Loch Sloy; CR, Carron Valley Reservoir; BW, Brotherswater; HW, Haweswater Reservoir; RT, Red Tarn; UW, Ullswater; LT, Llyn Tegid.

[b]L_F, fork length; N_{EDxSL}, eye diameter lengths in snout length; N_{GR}, number of gill rakers; P_S, presence of spots; N_{IDxHL}, interorbital distance lengths in head length; N_{ScLP}, number of scale rows between the lateral line and pelvic fin; N_{ScC}, number of circumpeduncular scale rows; N_{AFR}, number of anal-fin rays.

[c]SD, Mann–Whitney U-test significant at Bonferroni corrected significance $\alpha < 0.0005$.

[d]Comparison of putative *Coregonus clupeoides* (Scotland) sites; relevant trait comparisons in key shaded.

[e]Comparison of putative *Coregonus stigmaticus* (England) sites; relevant trait comparisons in key shaded.

the correct region (Fig. 2). Only Scottish fish (putative *C. clupeoides*) were allocated with $< 5\%$ error.

A discriminant analysis assigning individuals to U.K. European coregonid sites revealed four DFs, although only three explained $> 5\%$ of explainable variance. The first explained 59% of the variance (canonical $R^2 = 0.77$), the second explained 36% of the variance (canonical $R^2 = 0.70$), and the third explained 6% of the variance (canonical $R^2 = 0.24$). Together these DFs significantly differentiated the populations ($\chi^2 = 905.9$, d.f. $= 32$, $P < 0.001$) (Fig. 3). The correlations between characters and DFs indicated that PC1 (L_F and N_{EDxSL}) ($r = 0.98$) had the most important contribution to DFI which separated LT, LE, RT and LS from LL, HW, BW, UW and CR. The PC2 (N_{IDxHL} and P_S) ($r = 0.99$) had the most important contribution to DFII which separated UW, RT, BW, LT and HW, from LL, LE, CR and LS. The PC3 (N_{ScLP} and N_{ScC}) ($r = 0.80$) had the most important contribution to DFIII which separated LT, CR, LL, HW and LS, from RT, BW, UW and LE. The PC4 (N_{AFR}) ($r = 0.86$) had the most important contribution to DFIV which separated BW, CR, LL, LE, LT and RT, from UW, LS and HW. Classification success (*i.e.* allocation of fishes to site of origin on basis of phenotypic traits) varied considerably between site LT (95%), LE (83%), CR (75%), LL (71%), UW (65%), RT (60%), BW (53%), LS (41%) and HW (0%) (Fig. 3). Only LT fish were allocated with $< 5\%$ error.
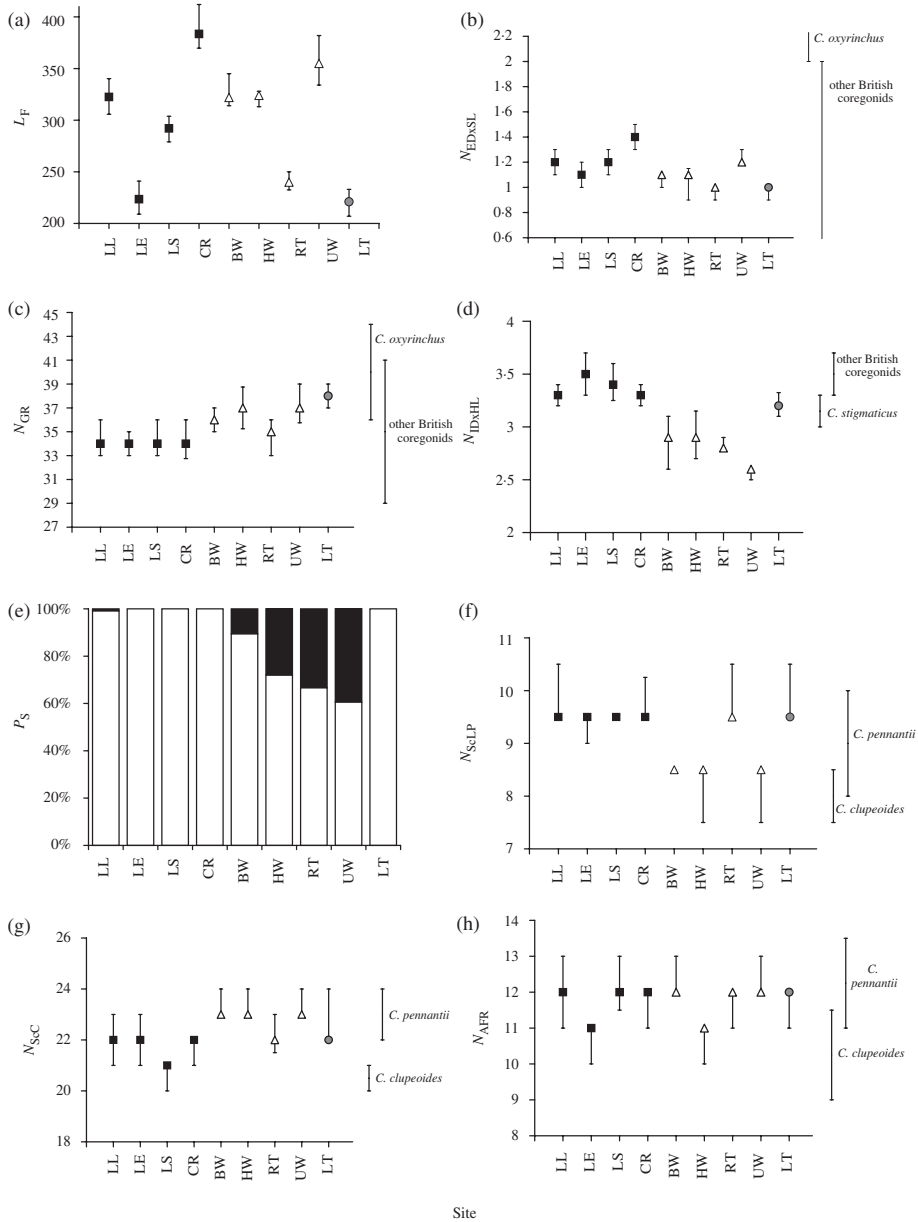
FIG. 1. (a)–(d), (f)–(h) Median of traits or (e) percentage frequency of fishes in populations of British *Coregonus* spp.; LL, Loch Lomond; LE, Loch Eck; LS, Loch Sloy; CR, Carron Valley Reservoir; BW, Brotherswater; HW, Haweswater Reservoir; RT, Red Tarn; UW, Ullswater; LT, Llyn Tegid. (a), fork length, (L_F) (b), eye diameter lengths in snout length (N_{EdxSL}), (c), number of gill rakers, (N_{GR}) (d), interorbital distance lengths in head length, (N_{IDxHL}) (e), presence of spots, (P_S) (f), number of scale rows between the lateral line and pelvic fin, (N_{ScLP}) (g) number of circumpeduncular scale rows, (N_{ScC}) and (h), number of anal-fin rays (N_{AFR}), (■), putative *C. clupeoides*; (●), putative *C. stigmaticus*;, (△), putative *C. pennantii*. Lower bars indicate 25% percentile, upper bars indicate 75% percentile, where no bar exists median and percentile are the same. Range of trait value for species in key indicated on each graph.
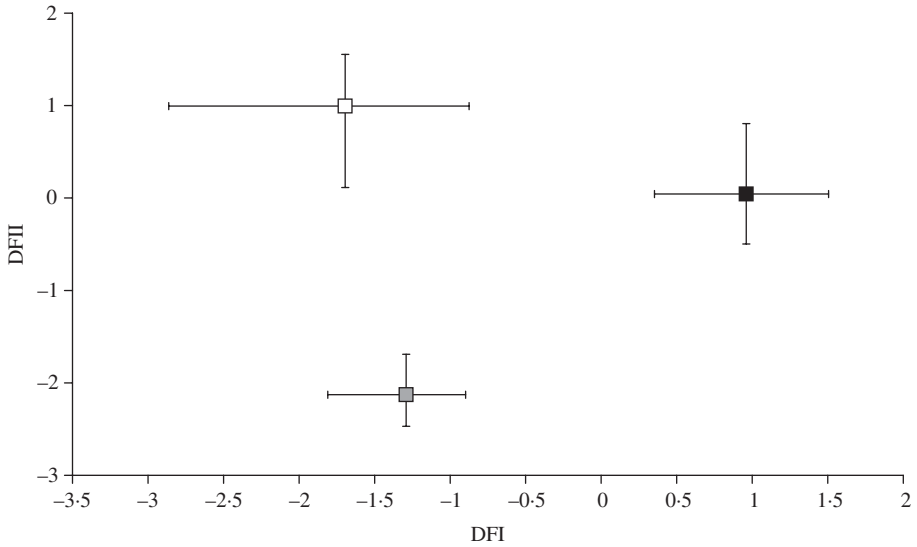
FIG. 2. Median discriminant function scores of individuals from putative *Coregonus clupeoides,* (■), *Coregonus stigmaticus*, (□) and *Coregonus pennantii* (■), regions. Lower bars indicate 25% percentile, upper bars indicate 75% percentile.
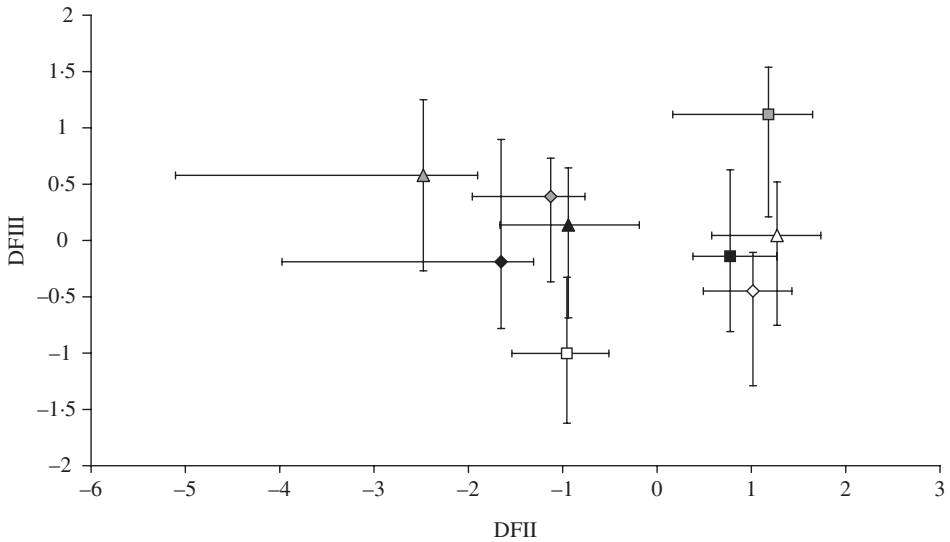

FIG. 3. Median discriminant function scores of individuals from Loch Lomond (■), Loch Eck (■), Loch Sloy, (△); Carron Valley Reservoir (◇), Brotherswater (◆), Haweswater Reservoir (▲), Red Tarn (◆) Ullswater (▲) and Llyn Tegid (□). Lower bars indicate 25% percentile, upper bars indicate 75% percentile.

DISCUSSION

TESTING OF THE KEY

Identification of putative British coregonid species using the key proposed by Kottelat & Freyhof (2007) is very poor and so offers little support for the revised

nomenclature. While dichotomies 1 and 2 resulted in few fishes being discarded as unidentifiable, no putative *C. pollan* or putative *C. oxyrinchus* were used in this analysis which focused only on populations of *C. lavaretus* (Maitland & Campbell, 1992; Davies *et al.,* 2004). Dichotomies 1 and 2 separate fish that are currently recognized as different species in Britain, respectively *C. autumnalis* and *C. oxyrinchus*, from those sampled in the present study. Recent genetic work by Hansen *et al*. (2008), however, has suggested that European populations of *C. oxyrinchus* (Maitland & Campbell, 1992; Davies *et al.*, 2004) may, in fact be conspecific with *C. lavaretus* (European whitefish). Though Freyhof & Schoeter (2005) consider *C. oxyrinchus* to be an endemic U.K. species and this species therefore to be extinct and European populations to be *Coregonus maraena* Bloch 1779.

There are several possible reasons for the inability of the published key to identify correctly many of the sampled individuals to putative species. The species descriptions may be inadequate. The taxonomic characters and the trait ranges utilized by Kottelat & Freyhof (2007) appear to be derived from historical species descriptions, and in particular the species descriptions and keys provided in Regan (1911). Historical descriptions, however, have sometimes been derived from the examination of only samples sizes that are too small be to representative of the variation exhibited within a species. For example, Lacépède (1803) described *C. clupeoides* from a second-hand account of 'several' Loch Lomond coregonids, and none apparently from Loch Eck. Regan (1908, 1911) described *C. stigmaticus* from 13 specimens from Haweswater and Ullswater, but none from Brotherswater or Red Tarn. It has been suggested that many more specimens (> 50) are required to be confident that the sample is adequately representing the full extent of intraspecific variation, *i.e.* that all possible morphotypes are accounted for (Walsh, 2000). Though used for much of the Kottelat & Freyhof (2007) key, Regan (1911) was, however, not apparently considered adequate to be used for the description of putative *C. pollan* and putative *C. vandesius* which were each split into species separated geographically, similarly to putative *C. clupeoides*, *C. stigmaticus* and *C. pennantii*. Putative *C. pollan* was split into *C. pollan* found in eastern Northern Ireland (Lough Neagh), *C. altior* found in western Northern Ireland (Lough Erne) and *C. elegans* found in the Republic of Ireland (Shannon lough system), while putative *C. vandesius* was split into *C. vandesius* found in Scotland in Lochmaben (extinct) and *C. gracilior* found in England in Derwent Water and Bassenthwaite (extinct).

Another possible reason for the lack of success in the use of the key is that the phenotypic traits used may be inadequate. Relatively few traits were used to identify these fishes; in addition, trait measurements were often not exclusive to a particular dichotomy (see Table II, this overlap can result in a single fish simultaneously occupying both dichotomies of some characters), which is unhelpful for clearly separating individuals to different species. A more important issue, however, is whether the phenotypic traits used in the key may be influenced by the environment, and thus whether they are useful for separating this group of fishes. Incorrect use of the key was also a possible cause of the lack of success of the key; but in this study counts and measurements were taken as described by Kottelat & Freyhof (2007) therefore it seems unlikely that user error could have resulted in such a low identification success. Ultimately, to be of practical value, a species key should be usable by general fish biologists and not just by taxonomic specialists.

These issues suggest that collecting and examining at least 50 individuals from each site to extend the range of these phenotypic traits, and provide additional traits or fewer trait overlaps, might produce a key that is able to identify correctly more individuals. This conclusion, however, stems from two potentially erroneous assumptions: (1) three robust species exist across these sites and (2) these phenotypic traits are useful in identifying these species.

ASSESSMENT OF THE PRESENCE OF *C. CLUPEOIDES*, *C. STIGMATICUS* AND *C. PENNANTII*

The percentage of fishes allocated to the correct (expected) species using Kottelat & Freyhof's (2007) key was extremely small (4%). The DFA was completed to determine whether three phenotypically separated species could be detected when strict trait ranges were not utilized for separation. There clearly is phenotypic variation in fishes between sites. Kottelat & Freyhof (2007) suggest that this might reflect the existence of multiple species in different geographical regions that show some overlap in phenotypic traits. Regardless of the statistical analysis employed here, however, British coregonid populations could not be consistently separated into the three geographically separated putative species using the phenotypic characters. While a large amount of variation was explained by putative species (59%), the variation between sites excluding the variation explained by the explanatory variable putative species (or region), was also highly significant (29%). There was also large overlap in DFA between individuals from different sites, and between individuals belonging to different putative species. In the first DFA, the most reliable separation was for putative *C. clupeoides*, while in the second DFA, LT individuals (putative *C. pennantii*) were the most reliably separated. In both DFAs, individuals of putative *C. clupeoides* were best separated by PC2 which utilized N_{IDxHL} and P_S (DFI in comparisons of region, DFII in comparisons of site). The PC1 was not useful in separating groups; this is unsurprising as most of the variation was explained by L_F, a highly variable trait in coregonids. Thus in the DFA comparing fishes from certain putative species (or regions), DFII separated putative *C. pennantii* effectively, since this putative species consists of only one population of small fishes; all other putative species contain several sites that contain both large and small fishes. Despite L_F not being a trait in the key, putative *C. pennantii* individuals were most often identified correctly using Kottelat & Freyhof's (2007) key. Individuals from HW were the only putative *C. stigmaticus* correctly identified, yet these fish could never be correctly allocated to a site using DFA, and *C. stigmaticus* was the most poorly differentiated group in the putative species level DFA.

Differences were also found between fishes from sites containing the same putative species (*i.e.* within the same region). Putative *C. clupeoides* from LL and LE are believed to have been separated for thousands of years (Maitland, 1970); these were the most different native populations of the same putative species when individual characters were considered. They are also very different when the site level DFA is considered, as are HW and UW individuals. This latter finding was somewhat unexpected as one-way genetic exchange between these populations is theoretically possible because large volumes of water are periodically pumped from UW to HW for storage purposes (I. J. Winfield, pers. obs.). While the differences between HW

and UW individuals have not been well studied (Bagenal, 1970), a number of studies have made comparisons between LL and LE fish. Despite being in the same geographical region, and LL and LE being only 22 km apart, the fishes in these sites show differences in head morphology and ecology, the main difference being that LL fishes are primarily planktivorous while LE fishes primarily feed on benthic macroinvertebrates (Pomeroy, 1991; Brown & Scott, 1994).

Considerable levels of phenotypic variation were found between individual fishes at each site. If the dichotomous key was considered to be correct and the described geographic separation of species is incorrect, four sites contain more than one putative species, while all contain an 'unidentified' coregonid. This does not follow the described locations of the putative species (Kottelat & Freyhof, 2007), although more extensive ranges of the putative species might be possible. Sympatric coregonid populations demonstrating a range from limited to complete reproductive isolation are known to exist within sites in Europe (Bernatchez *et al*., 1996; Douglas *et al*., 2005). Alternatively, introgression after secondary contact has been suggested to be responsible for the large amount of phenotypic variation in some fish taxa (Svärdson, 1970; Dowling & Secor, 1997). While previous workers have suggested the presence of more than one morph in both LL and LT populations, further investigations have found no evidence of this (Etheridge *et al*., 2010*a*), thus these populations appear to be phenotypically variable but monomorphic. Certainly the case of the refuge populations (LS and CR) in particular suggests that there are not multiple coregonid species present in LL. Fishes from LL were identified in the key allocations as *C. clupeoides*, *C. pennantii* and 'unidentified' coregonids. LS and CR fishes are conservation refuge populations sourced from the same genetic material from LL, which was collected from one spawning site over a short period of time. Yet a mix of putative species is also identified at these sites. It seems extremely unlikely that individuals from the refuge populations would represent more than one whitefish species, which when inadvertently crossed and introduced into new sites would still then be diagnosable as separate species. Generally, the presence of more than one species is also considered unlikely in the other sites examined here. The physical and biotic features usually found in a site with multiple coregonid populations or sub-populations present (*i.e*. large size, physical heterogeneity and fish species paucity) are not found combined in U.K. coregonid sites. It is far more likely that the apparent multiple species found in some sites reflects natural phenotypic variation in these populations. It is concluded that the classification of British coregonid populations into three species *C. clupeoides*, *C. stigmaticus* and *C. pennantii* is not supported by evidence available to date. In contrast it is likely that most of the differences between putative species result from regional level and site-level environmental differences.

ASSESSMENT OF PHENOTYPIC TRAITS IN COREGONID TAXONOMY

Phenotypic traits may reflect similarities due to a shared ancestor (homology), or due to responses to abiotic and biotic pressures (homoplasy). Phenotypic traits in postglacial fishes are also known to respond plastically to environmental pressures (Smith & Skúlason, 1996; Garduño-Paz *et al.,* 2010). Many postglacial fishes are known to exhibit homoplasy for morphological traits (Douglas *et al*., 2005; Østbye

et al., 2006; Hansen *et al.*, 2008), thus certain traits may not be useful for distinguishing between species. Crucially, most of the traits used in the key of Kottelat & Freyhof (2007) are known to be affected by environmental pressures. Morphological traits such as head measurements can be modified by physical habitat and foraging behaviour (Adams *et al.,* 2003; Garduño-Paz & Adams, 2010) and such patterns seen between fishes that specialize in utilizing divergent resources are repeatable across fish taxa (Robinson & Wilson, 1994; Smith & Skúlason, 1996). In addition, there is often a growth effect on morphology (allometry) in fishes, which suggests that body proportions are not good diagnostic traits as they change over the life of an individual (Svärdson, 1950). Meristic traits such as number of scales or fin rays can be modified during development by temperature, salinity, oxygen level, extent of light exposure and egg density (Lindsey, 1958; Barlow, 1961; Swain & Lindsey, 1986). Other meristic characters such as gill rakers do have a high genetic component (Svärdson, 1950, 1957), but they are also strongly influenced by diet with high gill raker numbered fishes usually specializing in zooplankton and low gill raker numbered fishes usually specializing in macroinvertebrates (Kahilainen & Østbye, 2006). Thus, gill raker number has been shown to respond to the introduction of, or release from competition with a sympatric specialist foraging fishes (Svärdson, 1951; Lindsey, 1981; Robinson & Wilson, 1994). Descriptive characters can be useful when they are always or usually present, but they can also be subjective. Spots observed on sampled coregonids were usually a darkish shadow that may or not have been a true spot and they were never as clear as suggested by Regan (1911). In addition, almost all fishes had smaller or less defined speckles on the scales which could have been described as spots. Thus a fish that shares some of these key traits with another in a different site is not necessarily closely related as the expressed traits may be due to common environmental influences.

Some traits appeared to be useful key traits in that no difference was found when comparing fishes from populations of the same putative species (i.e., the same geographic region). The N_{GR} (though not actually used in the relevant key dichotomies), and P_S, showed no significant differences between comparisons, while N_{ScLP}, showed no significant differences between comparisons in the relevant key dichotomy. These traits, however, were not good key traits when comparing fishes from populations of different putative species (*i.e*. different geographic region). No trait consistently showed significant differences between all site comparisons between different regions, although, N_{IDxHL} showed consistently significant differences between comparisons in the relevant key dichotomy. Thus there was an overall poor performance by those traits used in Kottelat & Freyhof's (2007) key: a reliable defining character should discriminate between populations of different species but not between populations of the same species.

Interestingly, taxonomic traits between LL (source) and LS and CR (refuge populations) were consistent. Only L_F and N_{EDxSL} were significantly different between these sites. A comparison of LL coregonids in comparison to the translocated populations in LS and CR found significant differences in growth rates and in head morphology between populations presumably due to phenotypic plasticity (Etheridge *et al.,* 2010b). It has been suggested that allometric traits may change the diagnosis of a species when moved to a different environment due to the often reported changes in growth in translocated coregonid populations (Svärdson, 1957). While

this has not been found in these translocated populations to any great extent, no CR fishes were ever identified as a putative *C. clupeoides* and the proportions of LS and CR fishes classed as unidentifiable were greater than for LL fishes. Etheridge *et al*. (2010*b*) found that after only four generations LS and CR fishes were found to be diverging phenotypically in different directions, presumably due to physical and biotic differences between sites. It is possible that, if this diversion continues, LS and CR fishes might ultimately be classed as different species using phenotypic characters. The value of phenotypic traits that have the potential to classify individuals from populations unequivocally known to be the same species, as different, must be questioned.

In conclusion, phenotypic traits are not always useful; this may particularly be the case for differentiation between phenotypically variable fish species such as coregonids because few traits can elucidate underlying genetic histories of their populations. Moreover, the high degree of phenotypic variation between and within the examined populations was not clearly apportioned into three clear species. If phenotypic differences between populations are mainly due to environmental influences, sites that are more similar and geographically closer are more likely to contain fishes with similar phenotypes. It is more likely that trait variation is the result of a single highly variable species where variability is the result of plasticity, founder effects and adaptation.

Taxonomic changes have potentially important implications for conservation and management (Mina *et al*., 2006; Adams & Maitland, 2007). Acceptance of a new taxonomy is likely to result in significant shifts in how the affected fishes are recognized, especially by non-experts (*e.g.* conservation managers, legislators, funding bodies and conservation pressure groups). At present *C. lavaretus* is protected in the U.K. under Schedule 5 of the Wildlife and Countryside Act (1981) and is listed as a priority species in the U.K. Biodiversity Action Plan (2007); *C. clupeoides*, *C. stigmaticus* and *C. pennantii* are not. With ambiguity surrounding their species designations, if the revised taxonomy of Kottelat & Freyhof (2007) is adopted in the U.K. then in theory these populations will have no legal protection. An increase in the conservation status of U.K. coregonids (*i.e.* recognizing endemic species, rather than rare British populations of a pan-European species) would be welcomed in principle, provided such a step could be justified. Formally recognizing a species that is difficult to define in practical terms cannot, in the long-term, be helpful and may undermine the trust of wider society in the pronouncements of biologists. The view of Kottelat & Freyhof (2007) that conservation should be focussed on the native populations (as they are clearly different) is fully endorsed here, but there is very little statistical support for the morphological separation of British European coregonids into the putative species they propose. Current management in the U.K. effectively treats each native coregonid population as a separate 'evolutionarily significant unit' (ESU). The initial, purely genetic concept of an ESU as proposed by Ryder (1986) has been modified elsewhere to include discrete phenotypic characters and restricted gene flow (DeGuia & Saitoh, 2007). As such this allows populations to be conserved and resources allocated depending on phenotypic, ecological and genetic distinctiveness. It is suggested that this approach will allow conservation of U.K. coregonids to reflect site specific variability (including that within regions), without relying on putative species that are distinguished through a flawed process. The view that these sites contain populations of a single but variable species is

strongly supported. Until and unless more detailed taxonomic and genetic evidence supports a different view, these European coregonid populations should be considered to be *C. lavaretus*.

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APPENDIX

Natural History Museum, London, (BMNH) specimens examined with repository, catalogue numbers, localities from which specimens were sampled and number examined (*n*).

Coregonus lavaretus

BMNH 1861.12.9.2-3, HW, *n* = 2; BMNH 1864.10.3.1, UW, *n* = 1; BMNH 1871.8.28.2, LT, *n* = 1; BMNH 1874.4.16.2, LT, *n* = 1; BMNH 1904.5.11.9, LT, *n* = 1; BMNH 1904.7.28.15-16, HW, *n* = 2; BMNH 1904.7.28.15-17, HW, *n* = 3;

BMNH 1905.8.1.1, LL, $n = 1$; BMNH 1905.8.14.1, LT, $n = 1$; BMNH 1905.9.22.1, LT, $n = 1$; BMNH 1909.11.30.1-3, LE, $n = 2$; BMNH 1910.10.17.1, LL, $n = 1$; BMNH 1929.8.29.1, UW, $n = 1$; BMNH 1934.1.6.1, LT, $n = 1$; BMNH 1937.1.28.1, LT, $n = 1$; BMNH 1952.10.1.1-12, LL, $n = 3$; BMNH 1967.4.26.1-15, LL, $n = 3$; BMNH 1967.4.26.16-30, LL, $n = 3$; BMNH 1977.10.5.6-50, LT, $n = 12$; BMNH 1978.6.27.3-7, UW, $n = 2$; BMNH 1986.11.14.1-3, HW, $n = 3$; BMNH 1986.2.16.4-7, HW, $n = 4$.