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Differentiation and phylogeny of the olivaceous warbler *Hippolais pallida* species complex

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Abstract The pattern of phenotypic and molecular variation within the polytypic olivaceous warbler *H. pallida* was examined. This species is distributed in the southern parts of the western Palaearctic, central Asia and in the arid parts of northern Africa, and also in parts of the sub-Saharan Sahel zone. Based on morphology, five subspecies, at times assigned to three groups, have been identified. By comparing morphological, behavioural, vocal and molecular variation, we investigated the phenotypic and phylogenetic relationships within the *Hippolais pallida* group. The morphological and genetic data of the present study support the view of splitting the olivaceous warbler into a western (former subspecies *opaca*) and an eastern form (former subspecies *elaeica*, *pallida*, *reiseri* and *laeneni*). *Opaca* is consistently and significantly larger than the other taxa in all size measurements, even if it does share morphological properties with *elaeica* in characters associated with migration. The song of the polytypic *pallida* differs clearly from that of *opaca*, and is remarkably consistent within its wide range which comprises four subspecies. The molecular analysis, and the resulting phylogenetic pattern, clearly separated *opaca* on a single branch distant from the other subspecies. The eastern form consists of two non-overlapping haplotype groups: *elaeica* with relatively diverse mtDNA variation, and the three African subspecies *pallida*, *reiseri* and *laeneni*, which all share the same or closely related mtDNA haplotypes. It remains open whether the African taxa should be regarded as three valid subspecies of the Eastern olivaceous warbler, or if they are better treated as a single African subspecies.

Keywords *Hippolais pallida* · Morphology · Phylogeny · Sylviidae · Taxonomy

Introduction

Traditionally, avian taxonomy has been based on morphological and behavioural traits, incorporating differences in size and plumage, vocalisation and reproductive behaviour. However, in taxa where variation of such traits is limited and hard to measure objectively, e.g. in many genera within the family Sylviidae, phylogenetic relationships are hard to disentangle. In such groups, DNA sequences can contribute a large number of phylogenetically informative characters that will aid the traditional taxonomy (Shirihai et al. 2001). Although genetic divergence as such does not delineate species (Ferguson 2002), the distance and pattern of sequence divergence will indicate for how long and effective the compared taxa have been isolated (Nichols 2001). Because speciation correlates with time, genetic divergence will indicate how likely the two taxa are to behave as different species according to a defined species concept. On the other hand, the scientific community has not reached a consensus on the species concept. Hence, the debate of whether closely related taxa are species or not will continue. Meanwhile, an improved understanding of differences between closely related taxa will shed light on the speciation process, regardless of the species concept applied.

The reed warbler group is a species-rich clade traditionally subdivided into three genera (*Acrocephalus*, *Hippolais* and *Chloropeta*) comprising species with similar morphology and behaviour (Sibley and Monroe

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1990, 1993; Helbig and Seibold 1999). Phylogenetic relationships based on mitochondrial cytochrome *b* sequences have challenged both inter- and intrageneric relationships, which have traditionally been based on morphology (Leisler et al. 1997; Helbig and Seibold 1999). For example, *H. pallida*, *H. caligata* and *H. rama* seem to be more closely related to the *Acrocephalus* warblers than to the other *Hippolais* species.

Here we examine the pattern of phenotypic and molecular variation within the polytypic olivaceous warbler *H. pallida*. This species (group) is distributed in the southern parts of the western Palaearctic, Central Asia and arid parts of northern Africa, and also in parts of the sub-Saharan Sahel zone. Based on morphology, five subspecies, at times assigned to three groups, have been identified (e.g. Sibley and Monroe 1990, 1993; Cramp 1992; Baker 1997; Urban et al. 1997 and references therein): (1) *opaca* which breeds in Iberia and NW Africa and winters across the Sahel and the Sudan savannahs from the Atlantic coast to Cameroon, and (2) *elaeica*, which breeds in SE Europe and SW Asia and winters mainly in eastern Africa. These two taxa can be said to represent two separate groups. The third group, the 'pallida group', is divided into three subspecies: (3) *pallida*, which breeds in Egypt (and possibly N Sudan), of which the northernmost subpopulation migrates south into Sudan, (4) *reiseri*, which breeds in oases in the Sahara from W Algeria (and apparently S and SE Morocco) to SW Tunisia (Nefta, Douz) and W Libya (Fezzan), at least northern populations apparently migrate to south of the desert in winter, and (5) *laeneni*, which is resident in the arid Sahel-belt in sub-Saharan Africa, in Niger, Nigeria, the Chad region and W Sudan.

Interestingly, *opaca* and *reiseri* seem to breed parapatrically (or possibly locally sympatrically) in Morocco and Algeria (Roselaar in Cramp 1992; Urban et al. 1997; Svensson 2001). Based on morphological differences, Roselaar (in Cramp 1992) suggested a split of the *pallida*-group into three 'incipient species', *opaca*, *pallida* (with suggested subspecies *reiseri* and *laeneni*), and *elaeica*. Helbig and Seibold (1999) did not treat the African taxa, but suggested a split into two species based on molecular data, the monotypic western olivaceous warbler *H. opaca* and the polytypic eastern olivaceous warbler *H. elaeica*. Finally, Svensson (2001) treated all five taxa and reached the same conclusion as Helbig and Seibold (1999) based on behavioural and morphological data.

Variation in plumage and body size is very limited between taxa in this group; they are basically grey-brown above and off-white below with a short pale supercillium. In fresh plumage, the eastern form *elaeica* has an olive tinge on the upperparts and a faint but obvious pale wing panel, both of which are lacking in *opaca*, the western form. The three African taxa, which are largely found in the vicinity of arid habitats including deserts and semi-desert, are smaller with more rounded wings, browner above and, in the case of *laeneni*, slightly paler. For further plumage characters, see Svensson (2001) and below.

The dull and more or less featureless appearance makes field identification difficult. This probably contributed to the confusion regarding the distribution of these taxa, and also as to the distribution of their close relatives, booted warbler *H. caligata* and Sykes's warbler *H. rama* (cf. Cramp 1992; Baker 1997; Urban et al. 1997; Svensson 2001). It is, for instance, unlikely that *laeneni* breeds anywhere in the Nile Valley or that *elaeica* winters in India, to give just two examples of published but questionable ranges. The level of difficulty is further demonstrated by the fact that a *Hippolais* population breeding on the north Somalian coast (Ash and Pearson 2002) has still not been conclusively identified. A study of song and DNA seems desirable before it can be established whether these birds belong to *H. p. pallida* or to Sykes's warbler, or represent a hitherto undescribed taxon.

Here we compare morphological, behavioural, vocal and molecular variation to resolve the phylogeny of the *Hippolais pallida* group.

Methods

Olivaceous warblers were studied both in the field and in museum collections (Table 1). Fieldwork was undertaken in Spain and Morocco (*opaca*), The Gambia

Table 1 Origin of collected samples of olivaceous warblers *Hippolais pallida* and places of study

Taxa	Site	Morphology	Behaviour	Blood	Feather	Tissue	Sequence	Recordings
laeneni	Nigeria	X	X	X				X
opaca	Nigeria	X		X				
	The Gambia	X						
	Spain		X					
	Morooco	X	X		X			X
reiseri	The Gambia	X						
	Mus. Paris					X		
	Tunisia	X	X		X			X
elaeica	Genebank						X	
	Bulgaria	X	X		X			
	Turkey	X	X					X
	Kazakhstan	X	X		X			X
pallida	Egypt	X	X		X			X

(*opaca*, *reiseri*, unpublished data provided by J.M.B. King), Tunisia (*reiseri*), Nigeria (*opaca*, *laeneni*), Egypt (*pallida*), and in Greece, Bulgaria, Turkey and Kazakhstan (*elaeica*). The number of examined specimens is shown in Table 2.

Vocalisation

Notes on vocalisation are largely based on field notes and recordings made by L.S. Recordings of *laeneni* (Chappuis 2000, J. Wilson, unpublished data) and *opaca* (Chappuis 2000) were also used.

Morphology

Morphological measurements were taken from trapped birds and specimens examined in museum collections. Wing-length was measured to the closest 0.5 mm (maximum chord for live birds, method 3, Svensson 1992); bill-length, both to skull and to feather-base, to the closest 0.1 mm; bill-width partly (in Nigeria) according to the picture on p.178 in Svensson (1992), partly (Svensson 2001, and later field work) at the inner edge of the nostrils, and to the closest 0.1 mm; tail-length in mm; and tail graduation, i.e. the difference between the longest and shortest tail-feathers measured on the folded tail to the closest millimetre. Wing formula was recorded as is customary, primaries measured from tip of wing to tip of each primary on the closed wing, and with primaries numbered ascendently (from outside towards body). Additional measurements taken were the distance between the tip of the outermost, diminutive, primary P1 and the tip of P2, the distance between P1 and the longest primary-covert, and, for a part of the material, the distance between the central tertial and the other two tertials, and from wingtip to tip of outermost secondary. Tarsus length on live birds (at least in Nigeria) was measured according to Fig. 18B (with toes bent approximately 90° to the tarsus) and museum specimens according to Fig. 18A in Svensson (1992).

We employed principle component analysis using the program SYSTAT on 15 morphological traits on 196 specimens in museum collections (*opaca* $n = 41$, *elaeica* $n = 110$, *laeneni* $n = 9$, *pallida* $n = 12$ and *reiseri* $n = 24$), all measurements taken by one of the authors (L.S.). Principle components with eigenvalues >1 were extracted from the correlation matrices between the variables using varimax rotation (Wilkinson 1991).

Molecular analysis

Samples consisting of blood (~20 μl), toe pads or feathers (Table 1) were put in 500 μl of SET buffer and incubated over night at 55°C together with 0.15 mg of proteinase K. DNA was isolated using a standard phenol-chlorophorm extraction protocol. To amplify the 5′ end of the cyto-

Table 2 Wing length, tail length, tarsus length and body mass for the five taxa of the *H. pallida* complex

Taxa	Wing length (mm)			Tail length (mm)			Tarsus (mm)			Body mass (g)			Source		
	Mean	Range	*n*	Mean	Range	*n*	Mean	Range	*n*	Mean	Range	*n*	Type	Origin	Reference
pallida	63.2	59–66	22	51.0	47–54	22	21.1	20.3–22.3	13				Specimens	Egypt	Svensson (2001)
elaeica	66.8	62–72	199	51.6	46–56	200	20.8	19.0–21.8	138				Live + specimens		Svensson (2001)
laeneni	62.7	56–68	266	52.2	46–57	168	20.2	16.0–22.7	173	9.2	7.6–11	266	Live birds	Nigeria	
laeneni	62.3	59.5–65	14	50.4	46–53	14	19.8	19.0–21.4	14				Specimens		Svensson (2001)
reiseri	64.0	59–68	52	52.6	49–56	10	21.9	20–24	11	9.7	7.6–11	52	Live birds	The Gambia	M. King, unpublished data
reiseri	65.3	60.5–70	29	54.0	50–60	28	21.2	19.8–22.4	17				Live + specimens	Various	Svensson (2001), L. Svensson, unpublished data
opaca	69.5	64–73	13	56.6	52–61	11	23.0	21.5–25.7	12	12.3	9.3–16.8	13	Live birds	Nigeria	
opaca	67.7	63–72	71	58.6	56–62	12	22.1	21.1–23.2	20	10.2	8.5–12.5	71	Live birds	The Gambia	M. King, unpublished data
opaca	69.2	63.5–74	84	56.4	51–62	86	22.5	21.0–24.2	81				Specimens	Various	Svensson (2001)

chrome *b* gene we used the universal vertebrate primers H15149 and L14841 (Kocher et al. 1989). PCR reactions were performed in 25 μl volumes, containing 25 ng of template DNA, 0.4 μM of each primers, 0.125 mM of each nucleotide and 1.5 mM $MgCl_2$. Amplifications were initiated by heating the samples to 94°C for 3 min followed by 35 cycles consisting of 30 s at 94°C, 30 s at 52°C and 30 s at 72°C. Reactions were terminated with a 10-min step at 72°C. We used 2.5 μl of the final reaction run on a 2% agarose gel in 0.5×TBE buffer to check the success of the PCR reaction. Positive samples were sequenced with the H15149 primer using the Big Dye terminator cyclic sequencing kit loaded on an ABI PRISM 310 (Perkin Elmer). Sequences were aligned using BioEdit (Hall 1999) and for some relevant taxa we added published sequences from the GenBank International Nucleotide Sequence Database. Newly obtained sequences were deposited in the GenBank data base (AY736360-71). Phylogenetic analyses and calculations of nucleotide diversity were performed with the program MEGA 2.0 (Kumar et al. 2001).

Results

Vocalisation

Whereas *opaca* has a rather distinctive song, the song of polytypic *pallida* differs clearly from *opaca*, and is remarkably consistent throughout its wide range comprising four subspecies.

The song of *opaca* often opens with a few repeated call notes, *chek chek chek*..., then runs into a varied, fluent but well-articulated phrase of varying length, often 15–35 s (but can be extended much longer) of unmistakable *Hippolais* character. It can recall both melodious warbler *H. polyglotta* (though pace is slower and more even) and reed warbler *Acrocephalus scirpaceus* (but includes fewer repeated notes in twos and threes and is more varied). The song sounds ‘talkative’, well-articulated, and often contains some nasal notes, suggesting affinity with melodious and icterine warblers *H. icterina*.

The song of *pallida* is less pleasing to the ear, being more monotonous and hoarse-voiced, a cyclically repeated pattern of scratchy, rather blurred or stuttering notes which run up and down the scale. Regardless of subspecies, the song is remarkably similar throughout the vast range (*contra* D.H. Vincent in Cramp 1992) from Nigeria and Algeria through SE Europe and Middle East to Kazakhstan. There is actually very little resemblance between this and the song of reed warbler, but to the inexperienced ear it could perhaps be confused with the song of masked shrike *Lanius nubicus*, which also has a scratchy and monotonous phrase.

Both *opaca* and *pallida* have a ‘fat’ or ‘thick’ clicking *chek* call with a slightly nasal undertone, *opaca* usually slightly stronger and harder, but the two are probably not separable. Variations of this call, like a short series of *chet-et-et-et-et* or faster, almost like dry trills *cher-r-r-r*, occur in both groups and are not thought to be distinctive.

Behaviour

There is a behavioural difference in that *opaca* keeps its tail still when feeding and when moving from perch to perch in canopy, whereas all four subspecies of *pallida* frequently flick or ‘dip’ their tail downward from horizontal and back, rather like a chiffchaff *Phylloscopus collybita*. Hence, it is well known that *elaeica* often gives a call and tail-dip for each movement when feeding. We also established this behaviour in Nigeria for *laeneni*, in Egypt for *pallida* and in Tunisia for *reiseri*.

The only tail-movement known in *opaca*, incidentally shared by *pallida*, is a fine shivering of the tail while singing.

Plumage

All taxa treated here have similar plumages, being brown or greyish-brown above and off-white below with a varying degree of cream or buff tinge to sides of breast and flanks. The appearance of all these taxa varies a lot with status of plumage, changing from warmer grey-brown (in some with an olive cast) in fresh to a duller and paler brownish-grey in worn plumage.

Opaca is quite uniform brownish above without a noticeably paler wing panel. Flight feathers are brown, only slightly darker than rest of upperparts. The supercilium is very indistinct and generally not reaching behind the eye.

There is a fair amount of geographical variation within the widely distributed *H. pallida*. Nominate *pallida* and its close relatives *reiseri* and *laeneni* are all warm brown above, with especially *laeneni* being paler and sandier, while *pallida* is slightly darker and more rufous-tinged above with a little darker flight-feathers. However, differences are slight, and many birds are difficult to separate on coloration alone. Of the three African subspecies, only *reiseri* normally has a faintly paler wing panel formed by buff edges to tertials and secondaries. *Elaeica* is similar to *pallida*, but slightly more grey-tinged above, with an olive cast in fresh plumage, and the folded wing in fresh plumage has a slight pale panel formed by paler greyish edges to long tertials and secondaries.

Average differences in pattern of the outer tail feathers exist between all taxa involved, *opaca* having on average least prominent pale tips and edges to the outer tail feathers, *reiseri* quite well-marked white edges, in particular to the inner webs, and *pallida* and *laeneni* being somewhat intermediate in these respects (for details see Svensson 2001).

Juveniles of *laeneni*, and apparently also of the other subspecies, have two diffuse wing-bars and are on average slightly warmer brown than adults.

Biometry and structure

Separation of the taxa based on biometry is often possible, but requires accurate measurements and the comparison of several criteria. General size (expressed as length of wing and tail), bill size and bill shape, tarsus length, and wing formula are the most important ones to assess (cf. Tables 2, 3, 4, 5). The taxon *opaca* was found to be significantly larger than both *laeneni* and *reiseri*, except for tarsus length in comparison with *reiseri* (Tables 4, 5)

Bill size and shape is an important discriminating character, *opaca* having a long and strong bill with convex sides in lateral view (rarely nearly straight sides), and lower mandible being rounded rather than keeled, giving the bill an almost ‘swollen’ look. This is significantly different from both *laeneni* and *reiseri* (Table 4), which, like all other taxa, have a thinner and more pointed bill with straight or even concave sides in lateral view (Table 4). However, it should be noted that the bill of *reiseri* can have a slightly convex outline in its outer part (not unlike that of many Sykes’s warblers).

Bill size as such is not sufficient for identification of every individual, since there is overlap between smaller *opaca* and those *elaeica* and *reiseri* with the largest bill. However, a combination of bill size and bill shape, together with plumage characters and tarsus length should identify virtually every bird. A summary of the biometry of the five taxa is presented in Table 2. *H. opaca* from The Gambia and Nigeria had similar wing-length, but slightly different tail and tarsus length. The difference might be due to slightly different measurement techniques.

Wing formula

The most migratory subspecies, *opaca* and *elaeica*, have the most pointed wings, whereas the other three taxa have a rather similar and more rounded wing-shape (Table 3). It is sometimes useful to examine a few key criteria, like the length of the outermost short primary in relation to the tips of the primary-coverts, the position of the second outermost primary in relation to the length of the other primaries, the primary projection expressed as the distance between the tip of the outermost secondary and the wing tip, and the distance between tips of first and second outermost primaries. The presence or prominence of an emargination of the outer web of the sixth primary is also useful to note. For details see Table 3 and Svensson (2001).

Principle component analysis of morphology

Three principle components showed eigenvalues >1 and together they explained 68.1% of the variation in the data (Table 6). From the coefficients in Table 6 we interpret PC1 to mainly describe wing length, PC2

Table 3 Wing shape

	P1 > Pc	P1	P1–P2	P2	P3	P5	P6	P7	P8	P9	P10	S1	*n*	Origin	Reference
elaeica	4.7 (1.5–7.5)		30.4 (26.5–33)	4.6 (2.5–7)	0.0 (0–1)	1.0 (0–2.5)	3.8 (1–6.5)	6.8 (3–9.5)	9.1 (5.5–14)		12.9 (10–15.5)	14.9 (11.5–18)	195	Various	Svensson (2001)
pallida	4.9 (2–8.5)		27.5 (24–30.5)	4.9 (3.5–6.5)	0.1 (0–1)	0.5 (0–1.5)	2.8 (1–4)	5.3 (3.5–6.5)	7.7 (5–9)		11.2 (9–14)	13.3 (11.5–15)	22	Various	Svensson (2001)
laeneni	5.6 (2–9)	32.1 (28–38)		5.7 (2–8)	0.4 (0–2)	0.6 (0–3.5)	2.6 (0.5–5.5)	4.9 (3–9)	6.8 (4–11)	8.6 (6–12)	10.3 (7–14)	12.6 (8–17)	130	Nigeria	
laeneni	5.3 (3.5–8.5)		26.3 (23–29.5)	5.7 (4–7.5)	0.4 (0–1.5)	0.3 (0–1)	1.6 (0.5–3)	3.8 (2–5.5)	6.2 (5–8)		10.5 (9.5–12)	12.3 (10.5–13.5)	14	Chad	Svensson (2001)
reiseri	6.2 (5–8)										10.7 (9–13)		8	The Gambia	M. King, unpublished data
reiseri	6.3 (3–9)		28.0 (26–32)	4.8 (3–6)	0.1 (0–0.5)	0.4 (0–1)	2.5 (1.5–4)	5.1 (4–7)	7.2 (6–9)		11.1 (9–13)	13.1 (11.5–15)	29	Various	Svensson (2001)
opaca	7.0 (5–9)	35.0 (30–38)		7.3 (6–11)	0.6 (0–3)	0.5 (0–1.5)	3.2 (1.5–5)	5.8 (4–8)	8.3 (7–10)	10.2 (8–12)	12.2 (11–14)	15.0 (13–16)	10	Nigeria	
opaca	5.5 (3–8)										11.8 (10–13)		12	The Gambia	M. King, unpublished data
opaca	6.4 (4–9.5)		28.6 (25–31.5)	6.6 (4–8)	0.1 (0–1)	0.6 (0–2)	3.4 (1.5–4.5)	6.3 (5–7.5)	8.7 (7.5–10.5)		13.0 (11–16)	15.1 (13–18)	84	Various	Svensson (2001)

Table 4 Statistical comparisons, independent sample *t*-test (two-way)

	Wing lenght	Tail length	Tarsus	Bill to skull	Bill to feather	Bill width
opaca/*laeneni* Nigeria	$t_{227} = 12.17$, $P < 0.001$	$t_{177} = 6.80$, $P < 0.001$	$t_{183} = 9.51$, $P < 0.001$	$t_{165} = 8.32$, $P < 0.001$	$t_{182} = 7.58$, $P < 0.001$	$t_{184} = 10.08$, $P < 0.001$
opaca/*reiseri* The Gambia	$t_{121} = 10.86$, $P < 0.001$	$t_{20} = 7.84$, $P < 0.001$	$t_{29} = 0.65$, $P = 0.52$	$t_{45} = 7.72$, $P < 0.001$	$t_{21} = 7.43$, $P < 0.001$	$t_{46} = 14.30$, $P < 0.001$
opaca Nigeria / *opaca* The Gambia	$t_{82} = 2.93$, $P = 0.004$	$t_{21} = 1.75$, $P = 0.09$	$t_{30} = 2.72$, $P = 0.011$	$t_{35} = 0.27$, NS	$t_{24} = 0.22$, NS	$t_{38} = 1.34$, NS
laeneni Nigeria / *reiseri* The Gambia	$t_{319} = 0.50$, NS	$t_{176} = 0.55$, NS	$t_{182} = 5.64$, $P < 0.001$	$t_{179} = 0.25$, NS	$t_{179} = 1.10$, NS	$t_{192} = 0.30$, NS

Table 5 Bill measurements (mean and range in mm) according to the methods of Svensson (2001, and later additions)

	Bill to skull	Bill to feathering	Bill width outside nostrils	Bill width inside nostrils	*n*	Origin	Reference
elaeica	15.9 (14.1–17.5)			4.2 (3.6–4.9)	198/0/185	Various	Svensson (2001)
pallida	15.2 (13.9–17.0)			4.3 (3.9–4.6)	22/0/22		Svensson (2001)
laeneni	15.3 (11.4–17.4)	11.0 (9.0–13.0)	3.5 (2.4–4.2)		161/172/174	Nigeria	This study
laeneni	15.0 (14.0–15.8)			4.2 (3.7–4.6)	13/0/14	Chad	Svensson (2001)
reiseri	15.4 (13.5–18.5)	11.3 (10.3–12.1)	3.5 (2.9–4.0)		20/9/20	The Gambia	M. King, unpublished data
reiseri	15.3 (14.4–16.1)			3.1 (2.7–3.4)	27/0/28	Various	Svensson (2001); this study
opaca	17.9 (15.8–20.1)	13.2 (10.6–15.7)	4.3 (3.9–4.6)		8/10/10	Nigeria	This study
opaca	18.0 (15.4–19.5)	13.9 (11.8–17.5)	4.4 (4.1–4.6)		27/14/28	The Gambia	M. King, unpublished data
opaca	17.6 (16.4–18.9)			5.0 (4.6–5.5)	83/0/81	Various	Svensson (2001)

overall size and PC3 bill size inversely related to overall size. All three principle components showed significant differences between the five taxa using ANOVA (PC1, $F_{4,191} = 40.6$, $P < 0.001$; PC2 $F_{4,191} = 138.1$, $P < 0.001$; PC3, $F_{4,191} = 5.7$, $P < 0.001$). Relative wing length, as interpreted from PC1, was longest in *elaeica* followed by *opaca* and the African taxa (Fig. 1). PC2, suggestively describing overall size, showed the largest values in *opaca* and small and similar values in the other four taxa. PC3 showed smallest values in *laeneni*, indicating that this taxon has a relatively small bill for its size.

Molecular results

Analysis of partial cytochrome *b* sequences revealed that *opaca* is very different and clearly separated from the other four olivaceous warbler taxa (Fig. 2). The average sequence divergence, controlling for within taxon diversity, was 0.101 ± 0.020 between *opaca* and the four other taxa (Kimura 2-parameter distance). There was no significant resolution within the clade containing *elaeica* and the three African subspecies *pallida*, *laeneni* and *reiseri*. However, detailed inspection of the data revealed that *elaeica* does not share any haplotype with the African taxa (Table 7). In fact, *elaeica* can be discriminated from all the African taxa by having a C rather than a T on position 162. However, mapping of this single character on the phylogenetic tree (Fig. 2) suggests that it has mutated repeatedly and thus cannot be used to resolve the deeper relationships within this species group. In the neighbour-joining tree, the four *elaeica* haplotypes found are placed basal to all the haplotypes found among the African taxa. Within the African taxa, there was one common haplotype (B) that dominated in all three taxa. The nucleotide diversity was 5 times higher within *elaeica* than within the African taxa analysed together (Table 8).

Table 6 Standardised coefficients for the three extracted principle component scores

Variable	PC1	PC2	PC3
Wing length	0.112	0.194	−0.194
Tail length	−0.004	0.246	−0.154
Tarsus	−0.026	0.183	0.185
Bill length	0.01	0.174	0.122
Bill depth	−0.071	0.006	0.457
Bill width	−0.037	0.16	0.201
Primary 1 (P1)	−0.105	0.11	0.203
P2	−0.019	0.21	−0.204
P3	0.042	0.064	−0.507
P5	0.123	−0.128	0.152
P6	0.173	−0.057	0.06
P7	0.187	−0.025	0.018
P8	0.193	−0.005	−0.017
P10	0.19	0.045	−0.121
Secondary 1	0.207	0.053	−0.181
% of variation explained	32.7	25.4	10.0

Discussion

The breeding distribution of the olivaceous warbler species complex covers a large geographical area, and unusually for migratory songbirds, includes parts of western Eurasia, northern Africa and parts of sub-Saharan Africa. The morphology differs, although slightly, between some of the populations within the distribution range, which has been the basis for the delineation of five subspecies. Recent studies (Helbig and Seibold 1999; Svensson 2001), based on molecular sequences as well as morphological and behavioural observations, have supported a split into two olivaceous warbler species: a monotypic western species *H. opaca* (western olivaceous warbler), and an eastern species *H. pallida* comprising the four subspecies *elaeica*, *pallida*, *reiseri* and *laeneni* (eastern olivaceous warbler). Hitherto, however, no full investigation of all recognised taxa has been performed, and the phylogenetic relationships have not been completely clarified.

In the present study, we found *opaca* to be consistently and significantly larger in all size measurements, i.e. it had longer wings, tarsus and tail, compared to the other four taxa. These differences, and the pointed wing formula, reflect the migratory behaviour of this species. However, it is important to note that *elaeica* also migrates long distances, from breeding areas in SE Europe and SW Asia to wintering quarters in sub-Saharan Africa. *Opaca* and *elaeica* thus share morphological properties associated with migration, but *opaca* is consistently larger in each measured morphological trait. If

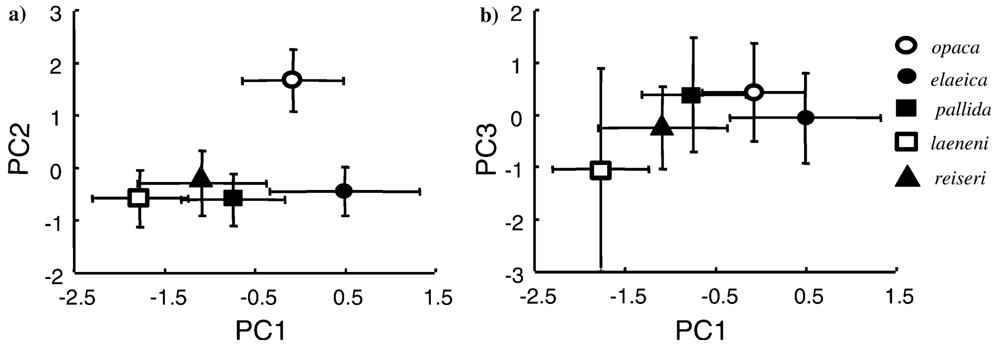

Fig. 1 Mean values (±SD) of PC2 (**a**) and PC3 (**b**) plotted against PC1 for each of the five taxa

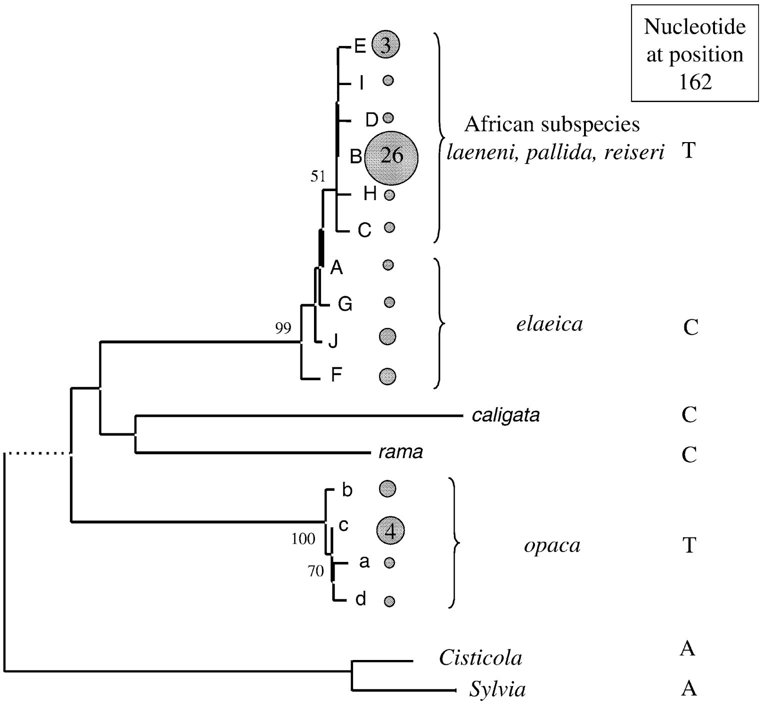

Fig. 2 Phylogeny of selected *Hippolais* warblers from partial cytochrome *b* sequences (303 nc). The tree was estimated using the neighbour-joining method with a Kimura-2 parameter genetic distance and was rooted with *Cisticola juncidis* and *Sylvia borin* sequences. The sizes of the *shaded circles* correspond to sample sizes and letters (*A–J*, **a–d**) to the haplotypes. Bootstrap values are indicated for branches with a support >50%. The *opaca* haplotype "a" correspond to GenBank AJ004790 and the *elaeica* haplotypes "A" to AJ004791 (Helbig and Seibold 1999). The haplotypes B-J and **b–d** have GenBank accession numbers AY736360-71

Table 7 Distribution of mitochondrial haplotypes inferred from partial cytochrome b sequences across different subspecies of eastern olivaceous warblers *Hippolais pallida*. Source of DNA is given in parentheses

Haplotype	Variable sites	Subspecies				Total
	112222 389661559 326246284 ATTCTACCT	*pallida* (feathers)	*laeneni* (blood)	*reiseri* (toe pad[a], feathers)	*elaeica* (feathers)	
A*	●●●●●●●●●				1	1
B	●●●T●●●●●	6	17	3		26
C	●C●T●●●●●	1				1
D	●●CT●●●●●	1				1
E	G●●T●●●●●		1	2		3
F	●●●●C●T●C				2	2
G	●●●●●●T●●				1	1
H	●●●T●G●●●		1			1
I	●●●T●●●T●		1			1
J	●●●●●●●●C				2	2
Total		8	20	5	6	39

[a] The two specimens from MNHN Paris (CG1951N475 & CG1966N2272) both had haplotype B

PC1 is describing wing morphology along an axis representing adaptations to residency versus migratory lifestyles, then *elaeica* is even more shaped for migration than is *opaca*. Another key feature separating *opaca* from the other taxa is the shape of the bill, which is likely to reflect adaptations to prey. The observed differences, therefore, suggest differences in feeding ecology.

Table 8 Nucleotide diversity within taxa of olivaceous warblers *Hippolais pallida*

Taxa	Sequence diversity $\pi(\times 10^{-3})$	SE ($\times 10^{-3}$)	*n*
elaeica	5.54	3.08	6
African taxa	1.35	0.68	33
opaca	3.09	1.74	8

The molecular analysis and the resulting phylogenetic pattern clearly separated *opaca* from the other taxa (Fig. 2). Unexpectedly, in our tree based on relatively short, partial cytochrome *b* gene sequences, *H. rama* and *H. caligata* nested within the olivaceous warbler cluster as the sister group to the eastern olivaceous warbler. There was, however, no statistical support for this branching pattern, but it emphasizes the relatively large genetic divergence between western and eastern olivaceous warblers.

We found no significant support for a division into three incipient species, contrary to the suggestion of Roselaar (in Cramp 1992). Instead, the lack of significant resolution within the clade containing *elaeica* and the three African subspecies supports the inclusion of *elaica*, *pallida*, *reiseri* and *laeneni* in a single species. The four different, and unique, *elaeica* mitochondrial haplotypes were found to be basal to all haplotypes found among the African subspecies. Furthermore, the *reiseri*, *pallida* and *laeneni* subspecies shared one common haplotype that also was the most frequently found haplotype among these subspecies. Finally, the nucleotide diversity was significantly higher within *elaeica* than within the three African subspecies combined (Table 6). Taken together, these results suggest that the African clade was derived relatively recently from an ancestral *elaeica* haplotype. Interestingly, this scenario would imply that the present pattern of residency or short distance migration among the African taxa is recently derived from the ancestral state of long-distance migration as it is presently seen in *elaeica*.

Because the sequences we have analysed are short, we cannot rule out that more resolution within the African group might be achieved with longer sequences. Also, the short sequences are not suitable to date the divergence and radiation of the African taxa. Geological and paleoclimatic records indicate that the savannahs which for long covered most of the present Sahara desert started to disintegrate about 4,000–6,000 years BP (e.g. Gasse 2000), and it is tempting to associate the isolation of the African taxa from *elaeica* with this major habitat transformation. Whether the pattern of change in migratory behaviour has proceeded from migratory in *elaeica* to residency in the African taxa, or vice versa, cannot be resolved with statistical confidence with the present data. The diversity of migratory strategies exhibited by the Eastern olivaceous warbler is nonetheless adding to an increasing number of studies showing that the migratory behaviour is a particular flexible trait that readily evolves in response to changes in the environment (e.g. Helbig 2003; Pérez-Tris et al. 2004). We leave the question open whether the African taxa should be regarded as three valid subspecies of the Eastern olivaceous warbler, or if they are better considered to form a single subspecies.

Zusammenfassung

Differenzierung und Phylogenie des Blassspötter Hippolais pallida—Komplexes

Die phänotypische und molekulare Differenzierung des Blassspötter-Komplexes wurde anhand von morphologischen Maßen, Lautäußerungen und mtDNA-Sequenzen untersucht. Der Komplex ist mit fünf Taxa in der südlichen Westpaläarktis, Nordafrika, der Sahelzone und in Zentralasien verbreitet. Die phänotypischen und molekularen Daten stimmen in einer deutlichen Differenzierung zwischen der westlichen Form *opaca* einerseits und den vier anderen Taxa *elaeica*, *pallida*, *reiseri* und *laeneni* überein. Letztere sind im Genstammbaum (Cyt b–Gen) gegenüber *opaca* gesichert monophyletisch. *Opaca* ist in allen Maßen größer als die anderen Taxa, teilt aber mit elaeica Anpassungen der Körperproportionen an ausgeprägtes Zugverhalten. Der Gesang von opaca unterscheidet sich eindeutig von den vier anderen Formen, die untereinander im Gesang bemerkenswert einheitlich sind. Unter den Taxa der *pallida*-Gruppe (ohne *opaca*) steht die östliche Form *elaeica* mit deutlich höherer mtDNA-Variation einer monophyletischen Gruppe aus drei afrikanischen Taxa (*pallida*, *reiseri*, *laeneni*) gegenüber. Ob die drei letzteren Formen valide Unterarte darstellen oder besser in einer Unterart vereinigt werden sollten, bleibt offen.

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