



A complete multilocus species phylogeny of the tits and chickadees (Aves: Paridae)

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ABSTRACT

The avian family Paridae (tits and chickadees) contains c. 55 species distributed in the Palearctic, Nearctic, Afrotropics and Indomalaya. The group includes some of the most well-known and extensively studied avian species, and the evolutionary history, in particular the post-glacial colonization of the northern latitudes, has been comparably well-studied for several species. Yet a comprehensive phylogeny of the whole clade is lacking. Here, we present the first complete species phylogeny for the group based on sequence data from two nuclear introns and one mitochondrial gene for 67 taxa of parids. Our results strongly support the inclusion of the Fire-capped Tit (*Cephalopyrus flammiceps*), currently placed in the Remizidae, as the most basal member of the Paridae. The Yellow-browed Tit (*Sylviparus modestus*) and the Sultan Tit (*Melanochlora sultanea*) constitute the next two sequential branches whereas the remaining tits fall into two large clades, one of which contains the seed hoarding and nest excavating species. The indicated clades within these two groups are largely congruent with recent classifications, but with several unforeseen relationships, such as non-monophyly of the Sombre Tit (*Poecile lugubris*) and the Marsh Tit (*Poecile palustris*), as well as non-monophyly of both the African gray and the African black tits. Further, our results support a close relationship between the White-fronted Tit (*Parus semilarvatus*) and the varied Tit (*Poecile varius*) as well as a close relationship between the White-naped Tit (*Parus nuchalis*) and the Yellow-cheeked and Black-lored tits (*Parus spilnotus* and *P. xanthogenys*). Finally, Hume's Ground-tit (*Pseudopodoces humilis*) is found to be closely related to the Green-backed Tit (*Parus monticolus*) and the Great Tit (*Parus major*). We propose a new classification that is in accordance with this phylogeny.

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1. Introduction

The tits and chickadees (Paridae) constitute a morphologically homogenous avian family (Gosler and Clement, 2007; Harrap and Quinn, 1996). Of the c. 55 species traditionally assigned to the group, only two, the Sultan Tit (*Melanochlora sultanea*) and the Yellow-browed Tit (*Sylviparus modestus*) deviate from the typical “tit-like” appearance of the other taxa. This morphological homogeneity is also reflected in many classifications, with all but the Sultan and Yellow-browed tits often being placed in a single genus, *Parus* (Dickinson, 2003; Harrap and Quinn, 1996; Sibley and Monroe, 1990; Snow, 1967). More recently a third species with strikingly deviate appearance, Hume's Ground-tit (*Pseudopodoces humilis*), previously considered the smallest of all corvids, was demonstrated to form part of the parid radiation (James et al., 2003). As

the name implies Hume's Ground-tit is primarily ground-living and distributed on the steppe landscape of the Tibetan Plateau. As with many other tit species it excavates its own nest hole, but in contrast to the other tits, the hole is dug in the ground. It has also been suggested that the Fire-capped Tit (*Cephalopyrus flammiceps*), which currently is placed in Remizidae (Penduline tits), may be a parid, “particularly in view of its habit of nesting in tree holes” (Gosler and Clement, 2007).

In more recent classifications of Paridae, three genera have typically been recognized (*Melanochlora*, *Sylviparus* and *Parus*). Yet it has also remained common practice to divide the largest of these genera, *Parus*, into several subgenera. For instance, Harrap and Quinn (1996) recognized 10 subgenera (*Poecile*, *Periparus*, *Pardaliparus*, *Lophophanes*, *Melaniparus*, *Parus*, *Machlolophus*, *Cyanistes*, *Sitiparus*, and *Baeolophus*), largely following Thielcke (1968). Some of these subgenera have in recent years been elevated to genera. For example, Gill et al. (2005) proposed the recognition of *Poecile*, *Baeolophus*, *Lophophanes*, *Periparus*, and *Parus* as distinct genera,

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in addition to *Melanochlora*, *Sylviparus* and *Pseudopodoces*. This arrangement was adopted by Gosler and Clement (2007) in their account of the Paridae for the *Handbook of the Birds of the World*. Although several aspects of this classification have been criticized (Päckert and Martens, 2008), we make use of this classification as a starting point for further discussion in this paper.

Although the number of taxa within the Paridae has remained relatively constant over time, the number of species recognized within the family has changed considerably over the years. Snow (1967) recognized 46 species of tits, but more recent checklists have recognized anywhere from 53 to 59 species (Clements, 2007; Dickinson, 2003; Gosler and Clement, 2007; Harrap and Quinn, 1996; Sibley and Monroe, 1990). These differences in species numbers are a reflection of the many uncertainties regarding the species status of several populations. In particular, the species status of some taxa presently lumped within the Great Tit, *Parus major*, which is widespread across the Palearctic, extending from Western Europe south to North Africa and east to China and Southeast Asia, continue to be debated. Within its range the Great Tit is divided into more than 30 subspecies (Gosler and Clement, 2007), which in turn are united in three groups (*major*, *minor* and *cinereus*) (Harrap and Quinn, 1996). Closely associated with these groups is the Turkestan Tit (*Parus bokharensis*), which in many recent classifications is treated as a distinct species (e.g. Dickinson, 2003; Gosler and Clement, 2007; Harrap and Quinn, 1996), but that earlier has been considered a subspecies of *Parus major* (Delacour and Vaurie, 1950; Vaurie, 1950); a view also supported by recent molecular data (Päckert et al., 2005). These studies suggest that *major* and *bokharensis* are conspecific whereas *minor* and *cinereus* constitute distinct species (Eck and Martens, 2006; Päckert et al., 2005).

Other taxa with uncertain species status include the Caspian Tit (*Poecile lugubris hyrcanus*) and the Songar Tit (*Poecile montanus songarus*), as well as other Central Asian forms of *Poecile* including the Black-bibbed Tit (*Poecile palustris hypermelaenus*) and the Spot-winged Tit (*Periparus melanolophus*). The first three taxa are sometimes considered distinct species (e.g. Harrap and Quinn, 1996) whereas the Spot-winged Tit appears to be nested within the Coal Tit (*Parus ater*) (Gill et al., 2005; Martens et al., 2006). The Canary Tit (*Cyanistes teneriffae*) is a recently accepted split from the Eurasian Blue Tit (*Cyanistes caeruleus*) (Salzburger et al., 2002b).

The tits and chickadees are widely distributed, with 18 species in Palearctic, 15 in the Indomalayan and Afrotropical regions and 12 in the Nearctic. Collectively tits and chickadees occur in a wide range of habitats and can be found in virtually all wooded biomes (Gosler and Clement, 2007). However, with the exception of few species such as the Great Tit and the Black-capped Tit (*Poecile atricapillus*), most species are specialized in their choice of habitat (Gosler and Clement, 2007). Several groups of passerines have historically been associated with the tits, but recent molecular studies have demonstrated that the sister group is the Penduline tits (Remizidae; e.g. Barker et al., 2004; Johansson et al., 2008). However, the closest relative of this larger group in turn remains unresolved (Johansson et al., 2008), and the group falls outside the three main clades of Passerida (Sylvioidea, Muscicapoidae and Passeroidea).

In this paper we present a phylogeny of all 56 species of parids recognized by Gosler and Clement (2007). We also include several of the subspecies that some authors consider to be distinct species, including the Caspian and Black-capped Tits. In all, the phylogeny includes 67 ingroup taxa (Table 1).

2. Materials and methods

2.1. Genes and laboratory procedures

Portions of two nuclear genes (Myoglobin, Ornithine Decarboxylase [ODC]) and one mitochondrial gene, NADH 2 (ND2), were

PCR-amplified and sequenced for this study. The analyzed portions of these genes include the complete second intron of Myoglobin and the complete introns 6 and 7, along with intervening exon 7 of ODC, and almost complete ND2 (1041 base pairs, bp).

Genomic DNA was isolated from muscle, blood or toe-pads from historical museum specimens using the QIAamp DNA Mini Kit (QIAGEN), and PCR-amplifications of the different markers were carried out with Ready-To-Go PCR Beads (Amersham Biosciences) following the manufacturer's recommendations. Primer sequences for PCR-amplification and sequencing are listed in Table 2. For blood and muscle samples, all PCR products were amplified in a single amplification except for Myoglobin, which was amplified as a nested PCR, with primers Myo2 and Myo3 used in the first amplification and Myo2 and Myo3F in the second (Johansson and Ericson, 2005). Toe pad samples from museum specimens were amplified in shorter (typically 175–250 bp), overlapping segments, using Paridae-specific primers. All amplifications were performed with an initial denaturation step at 95 °C, an annealing temperature typically between 53 °C and 59 °C and completed with a final extension at 72 °C for 5 min. Before sequencing the PCR products were cleaned either with ExoSAP-IT following the manufacturer's protocol or, in cases where multiple bands were obtained, the fragment of the expected size was cut from an agarose gel and cleaned using the QIAquick Gel Extraction Kit (QIAGEN). Purified PCR products were sequenced with BigDye Terminator Cycle Sequencing Kits (Applied Biosystems) and run on an ABI PRISM 3100 automated DNA sequencer. The same primers were used for sequencing as in the PCR, and the amplified DNA fragments were sequenced in both directions. Sequence fragments were assembled to complete sequences using SeqMan II (DNASTAR Inc., Madison, WI, USA).

All sequences generated for this study have been deposited in GenBank and the accession numbers are detailed in Table 1.

2.2. Alignment

The concatenated sequences were aligned by eye in BIOEDIT V7.0.1 (Hall, 1999). The alignment of the nuclear introns indicated a few instances of insertions and deletions in these sequences, but the sequences could be unambiguously aligned. The final alignment of the three gene segments included 2542 bp. All inserted gaps were treated as missing data in the phylogenetic analyses.

2.3. Phylogenetic analysis

We analyzed the data under Maximum Likelihood (ML) and Bayesian Inference (BI) criteria using RAXML (Stamatakis, 2006) and MrBayes 3.1.1 (Ronquist and Huelsenbeck, 2003), respectively. The model of sequence evolution for each partition was selected with the Akaike Information Criterion (Akaike, 1973) calculated in MrModeltest v2.2 (Nylander, 2004). Based on this analysis the GTR + Γ_4 model of nucleotide substitution was selected for myoglobin and ODC, whereas the GTR + Γ_4 + I substitution model was selected for ND2. Three sets of analyses were performed: (1) on each gene separately; (2) on the concatenated nuclear intron data set and (3) on the complete dataset. Several preliminary analyses were performed to explore the effect of chain temperature on the mixing behavior of the chains. We found that lowering the temperature to 0.05 resulted in better mixing of the chains than the default 0.2. For each BI analysis, two independent runs, each with four Metropolis-coupled MCMC chains, were run for 20 million generations and sampled every 1000 generations. Partitions were unlinked to allow independent parameter estimation for each one. At the end of each run the standard deviation of split frequencies were less than 0.01. Trees sampled before the chain reached apparent stationarity (burn-in) were discarded and the posterior

Table 1
List of samples and GenBank accession numbers.

Scientific name	subspecies	Specimen No.	Myo	Ref	ODC	Ref	ND2	Ref
<i>Poecile lugubris</i>	ssp.	NRM 20026298	KF183715	1	KF183803	1	KF183891	1
	<i>lugubris</i>	NRM 570735 ^a	KF183716	1	KF183804	1	KF183892	1
	<i>hyrcana</i>	BMNH 1907.12.21.341 ^a	KF183717	1	KF183805	1	KF183893	1
<i>Poecile palustris</i>	<i>palustris</i>	NRM 956361	KF183719	1	KF183807	1	KF183895	1
	<i>hypermelaenus</i>	IOZ1239	KF183720	1	KF183808	1	KF183896	1
<i>Poecile montanus</i>	<i>montanus</i>	NRM 956362	KF183721	1	KF183809	1	KF183897	1
	<i>affinis</i>	NRM 570741 ^a	KF183722	1	KF183810	1	KF183898	1
<i>Poecile carolinensis</i>	(1)	MVZ 178250	KF183727	1	KF183815	1	KF183903	1
	(2)	MVZ 178249	KF183728	1	KF183816	1	KF183904	1
<i>Poecile atricapillus</i>		NRM 20036275	KF183723	1	KF183811	1	KF183899	1
<i>Poecile gambeli</i>	(1)	MVZ 165817	KF183724	1	KF183812	1	KF183900	1
	(2)	MVZ 167837	KF183725	1	KF183813	1	KF183901	1
<i>Poecile sclateri</i>		NRM 570153 ^a	KF183726	1	KF183814	1	KF183902	1
<i>Poecile superciliosus</i>		NRM 570157 ^a	KF183714	1	KF183802	1	KF183890	1
<i>Poecile davidi</i>		BMNH 1901.5.4.151 ^a	KF183718	1	KF183806	1	KF183894	1
<i>Poecile cinctus</i>		NRM 946508	KF183730	1	KF183818	1	KF183906	1
<i>Poecile hudsonicus</i>		NRM 570156 ^a	KF183729	1	KF183817	1	KF183905	1
<i>Poecile rufescens</i>	(1)	MVZ 181977	KF183731	1	KF183819	1	KF183907	1
	(2)	MVZ 167891	KF183732	1	KF183820	1	KF183908	1
<i>Poecile varius</i>		NRM 570159 ^a	KF183713	1	KF183801	1	KF183889	1
<i>Periparus rufonuchalis</i>		FMNH 395843	KF183700	1	KF183788	1	KF183876	1
<i>Periparus rubidiventris</i>	<i>rubidiventris</i>	T. Price 01766	KF183701	1	KF183789	1	KF183877	1
	<i>beavani</i>	T. Price N019	KF183702	1	KF183790	1	KF183878	1
<i>Periparus melanolophus</i>		T. Price 01748	KF183699	1	KF183787	1	KF183875	1
<i>Periparus ater</i>		NRM 976516	KF183698	1	KF183786	1	KF183874	1
<i>Periparus venustus</i>		NRM 20096362	KF183697	1	KF183785	1	KF183873	1
<i>Periparus elegans</i>		ZMUC 117211	KF183695	1	KF183783	1	KF183871	1
<i>Periparus amabilis</i>		BMNH 1911.11.16.268 ^a	KF183696	1	KF183784	1	KF183872	1
<i>Lophophanes cristatus</i>		NRM 20036743	KF183703	1	KF183791	1	KF183879	1
<i>Lophophanes dichrous</i>		T. Price 9597	KF183704	1	KF183792	1	KF183880	1
<i>Baeolophus wollweberi</i>		NRM 570151 ^a	KF183705	1	KF183793	1	KF183881	1
<i>Baeolophus inornatus</i>	(1)	MVZ 172903	KF183709	1	KF183797	1	KF183885	1
	(2)	MVZ 173182	KF183710	1	KF183798	1	KF183886	1
<i>Baeolophus ridgwayi</i>		MVZ 171985	KF183711	1	KF183799	1	KF183887	1
<i>Baeolophus bicolor</i>	(1)	NRM 20086098	KF183707	1	KF183795	1	KF183883	1
	(2)	MVZ 178245	KF183708	1	KF183796	1	KF183884	1
<i>Baeolophus atricristatus</i>		NRM 570162 ^a	KF183706	1	KF183794	1	KF183882	1
<i>Parus guineensis</i>		S. Bensch	KF183674	1	KF183762	1	KF183850	1
<i>Parus leucomelas</i>	<i>leucomelas</i>	NRM 570396 ^a	KF183676	1	KF183764	1	KF183852	1
	<i>insignis</i>	NRM 570168 ^a	KF183675	1	KF183763	1	KF183851	1
<i>Parus niger</i>	(1)	MVZ uncat. JF711	KF183670	1	KF183758	1	KF183846	1
	(2)	MVZ RB3097	KF183671	1	KF183759	1	KF183847	1
	(3)	MVZ uncat. JF761	KF183672	1	KF183760	1	KF183848	1
<i>Parus carpi</i>		BMNH 1957.35.510 ^a	KF183673	1	KF183761	1	KF183849	1
<i>Parus albiventris</i>		ZMUC 117186	KF183669	1	KF183757	1	KF183845	1
<i>Parus leuconotus</i>	(1)	U. Ohlson	KF183681	1	KF183769	1	KF183857	1
	(2)	NRM 570163 ^a	KF183682	1	KF183770	1	KF183858	1
<i>Parus funereus</i>		NRM 570158 ^a	KF183680	1	KF183768	1	KF183856	1
<i>Parus rufiventris</i>		NRM 570164 ^a	KF183679	1	KF183767	1	KF183855	1
<i>Parus pallidiventris</i>	(1)	MVZ uncat. RCKB1104	KF183677	1	KF183765	1	KF183853	1
	(2)	MOM 2007.2.228	KF183678	1	KF183766	1	KF183854	1
<i>Parus fringillinus</i>	(1)	NRM 570165 ^a	KF183693	1	KF183781	1	KF183869	1
	(2)	BMNH 1946-34-37 ^a	KF183694	1	KF183782	1	KF183870	1
<i>Parus fasciiventer</i>	(1)	MVZ RB3096	KF183686	1	KF183775	1	KF183863	1
	(2)	MVZ RB3095	KF183687	1	KF183774	1	KF183862	1
	(3)	NRM 570737 ^a	KF183688	1	KF183776	1	KF183864	1
<i>Parus thruppi</i>		NRM 570155 ^a	KF183692	1	KF183780	1	KF183868	1
<i>Parus griseiventris</i>		MVZ uncat. RCKB1169	KF183685	1	KF183773	1	KF183861	1
<i>Parus cinerascens</i>	(1)	BH2401011122003	KF183689	1	KF183777	1	KF183865	1
	(2)	MVZ uncat. JF1149	KF183690	1	KF183778	1	KF183866	1
	(3)	MVZ uncat. JF2300	KF183691	1	KF183779	1	KF183867	1
<i>Parus afer</i>	(1)	MVZ uncat. HS15	KF183683	1	KF183771	1	KF183859	1
	(2)	MVZ uncat. HS27	KF183684	1	KF183772	1	KF183860	1
<i>Parus major</i>	<i>major</i>	NRM 956363	AY228310	2	KF183747	1	AY136587	3
	<i>cinereus</i>	IOZ11398	KF183659	1	KF183748	1	KF183836	1
	<i>minor</i>	IOZ1632	KF183660	1	KF183749	1	KF183837	1
<i>Parus bokharensis</i>		NRM 571033 ^a	KF183661	1	KF183750	1	KF183838	1
<i>Parus monticolus</i>		T. Price N053	KF183658	1	KF183746	1	KF183835	1
<i>Parus nuchalis</i>		BMNH 1939.3.20.1 ^a	KF183662	1	KF183745	1	KF183834	1
<i>Parus xanthogenys</i>	<i>xanthogenys</i>	BMNH 1949.23.3492 ^a	KF183668	1	KF183756	1	KF183844	1
	<i>aplnotus</i>	BMNH 1949. Whi. 1.4772 ^a	KF183667	1	KF183755	1	KF183843	1
<i>Parus spilonotus</i>	<i>rex</i>	NRM 20086493	KF183664	1	KF183752	1	KF183840	1
	<i>subviridis</i>	NRM 570754 ^a	KF183666	1	KF183754	1	KF183842	1
	<i>basileus</i>	NRM 570753 ^a	KF183665	1	KF183753	1	KF183841	1

Table 1 (continued)

Scientific name	subspecies	Specimen No.	Myo	Ref	ODC	Ref	ND2	Ref
<i>Parus holsti</i>		NRM 570160 ^a	KF183663	1	KF183751	1	KF183839	1
<i>Parus semilarvatus</i>		ZMUC 352	KF183712	1	KF183800	1	KF183888	1
<i>Pseudopodoces humilis</i>		IOZ N2004	KF183657	1	KF183744	1	KF183833	1
<i>Cyanistes caeruleus</i>	<i>caeruleus</i>	NRM 966240	KF183655	1	KF183742	1	KF183831	1
	<i>ultramarinus</i>	U. Olsson	KF183654	1	KF183741	1	KF183830	1
<i>Cyanistes teneriffae</i>	<i>teneriffae</i>	NRM 586295 ^a	KF183653	1	KF183740	1	KF183829	1
<i>Cyanistes cyanus</i>	<i>hyperrhiphaeus</i>	NRM 570152 ^a	KF183656	1	KF183743	1	KF183832	1
<i>Sylviparus modestus</i>		T. Price 19597	KF183651	1	KF183738	1	KF183827	1
<i>Melanochlora sultanea</i>		NRM 570161 ^a	KF183652	1	KF183739	1	KF183828	1
Remizidae								
<i>Auriparus flaviceps</i>		FMNH394359	KF183647	1	KF183733	1	KF183822	1
<i>Anthoscopus minutus</i>		W83645	KF183648	1	KF183734	1	KF183823	1
<i>Anthoscopus parvulus</i>		BMNH 1940.12.4.28 ^a	KF183649	1	KF183735	1	KF183824	1
<i>Remiz pendulinus</i>		ZMUC O1852	AY228319	2	KF183736	1	KF183825	1
<i>Cephalopyrus flammiceps</i>		NRM 570107 ^a	KF183650	1	KF183737	1	KF183826	1
Outgroups								
<i>Stenostira scita</i>			EU680607	4	EU680768	4	DQ125993	5
<i>Elminia nigromitrata</i>			GQ369640	6	GQ369667	6	GQ369687	6
<i>Hyliota flavigaster</i>			EU680608	4	EU680733	4	DQ125983	5
<i>Bombycilla garrulus</i>			AY228286	2	EU680709	4	DQ466855	7
<i>Regulus regulus</i>		NRM20016439	DQ466835	7	EU680761	4	KF183821	1
<i>Modulatrix stictigula</i>			EU680619	4	EU680743	4	GU816833	8
<i>Dicaeum australe</i>			AY228294	2	GU816903	8	GU816835	8
<i>Passer montanus</i>			AY228311	2	DQ785937	9	GU816845	8
<i>Certhia familiaris</i>			DQ011861	10	EU680713	4	DQ466857	7
<i>Troglodytes troglodytes</i>			AY228325	2	EU680775	4	GQ369683	6
<i>Muscicapa striata</i>			FJ357982	11	FJ358082	11	DQ861967	12
<i>Sturnus vulgaris</i>			AY228322	2	EU680769	4	EU551977	13
<i>Alauda arvensis</i>			AY228284	2	EF625336	14	DQ125975	5
<i>Pycnonotus barbatus</i>			GQ369651	6	FJ358086	1	GQ369695	6
<i>Picathartes gymnocephalus</i>			AY228314	2	GU816900	8	GU816831	8

Abbreviations: BMNH = Natural History Museum, Tring, United Kingdom, FMNH = Field Museum of Natural History, Chicago, USA, IOZ = Institute of Zoology, Chinese Academy of Sciences, Beijing, China; MVZ = Museum of Vertebrate Zoology, University of California, Berkeley, USA, NRM = Swedish Museum of Natural History, Stockholm, Sweden, ZMUC = Zoological Museum, University of Copenhagen, Denmark.

References: 1, This study; 2, Ericson and Johansson (2003); 3, Sefc et al. (2003); 4, Johansson et al. (2008); 5, Fuchs et al. (2006); 6, Fuchs et al. (2009); 7, Zuccon et al. (2006); 8, Fjeldsa et al. (2010); 9, Irestedt et al. (2006); 10, Alstrom et al. (2006); 11, Gelang et al. (2009); 12, Pasquet et al. (2006); 13, Zuccon et al. (2008); 14, Johansson et al. (2007).

^a Samples taken from museum study skins.

probabilities for the topology were estimated from the remaining generations.

The ML analyses were conducted in RAXML v7.2.6 (Stamatakis, 2006), as implemented in RAXMLGUI v0.93 (Silvestro and Michalak, 2012). We performed three ML analyses on the concatenated dataset with rapid bootstrap (1000 replicates) and a thorough ML search for 1 run and 1000 replicates under a GTR + G model. The dataset was partitioned by gene and branch-lengths were calculated independently for each partition.

The sister group to Paridae and Remizidae is currently unknown (Johansson et al., 2008). Therefore, sequence data from all major deep lineages in Passerida were downloaded from GenBank and included as outgroups (Table 1). The tree was rooted with *Picathartes gymnocephalus* (Johansson et al., 2008).

3. Results

The resulting trees from the Maximum Likelihood and Bayesian Inference analyses of the combined dataset were largely congruent and recovered two main clades (Fig. 1). The first of these includes all the African tits, the Great Tit complex, Hume's Ground-tit (*Pseudopodoces*) and the *Cyanistes* species. The second clade includes the chickadees (*Poecile*), the Varied Tit (*Poecile varius*), the White-fronted Tit (*Parus semilarvatus*), the Eurasian crested tits (*Lophophanes*), Nearctic crested tits (*Baeolophus*) and the Coal Tit and allies (*Periparus*). Basal relative to these two clades is the Sultan Tit (*Melanochlora sultanea*) and basal again relative to these clades is the Yellow-browed Tit (*Sylviparus modestus*). The Fire-capped Tit (*Cephalopyrus flammiceps*), currently placed in the Remizidae, is placed as the most basal member of the Paridae,

rather than with the Remizidae. Most of the indicated relationships are highly supported and only a few nodes have a posterior probability lower than 0.95.

The individual gene trees are also largely congruent but differ in resolution and overall support. There are, however, some conflicts between the trees, but in most cases the support for the alternative placement is weak (posterior probability [pp] < 0.95). One of the more notable conflicts relates to the basal splits of *Parus/Pseudopodoces*. Both myoglobin and ODC support a close relationship of *Pseudopodoces* with *Parus major* and *P. monticolus* (in ND2 the placement is unresolved relative to several other taxa), but in ODC this species is placed as sister to *P. monticolus* (pp = 0.96) rather than basal to the *P. major* complex and *P. monticolus* as indicated in the combined tree. The second conflict concerns the placement of *P. nuchalis*, which in the myoglobin tree is placed basal relative to the *Pseudopodoces/P. major/P. monticolus* clade (pp = 0.94), whereas its position in both the ODC and ND2 trees is identical to the combined tree (Fig. 1). A third conflict concerns the monophyly of the North American *Poecile* species. In the combined tree, as well as in the myoglobin and ND2 trees, this group is monophyletic. In the ODC gene tree, *P. palustris* and *P. davidi* are placed inside this clade, with strong support (pp = 0.99). However, short branch-lengths indicate that a few characters are responsible for this association. A similar situation occurs within the *silonotus-xanthogenys* complex, where *P. silonotus subviridis* is placed basal relative to the other members of that clade in the ODC tree. The final conflict concerns the placement of the two *Lophophanes* species relative to *P. semilarvatus/P. varius*. In the ODC gene tree these two groups have changed their relative position compared to their placement in the combined tree. The support for this is low (pp = 0.88).

Table 2
Primers used in this study.

Locus	Primer	Sequence 5'–3'	Reference
Myoglobin	Myo-2	gcc acc aag cac aag atc cc	Slade et al. (1993)
	Myo-Par1-152R	cat tgg act tga ggg aga gc	This study
	Myo-Par2-84F	tgt gag agt tgg gct ttc att	This study
	Myo-Par2-290R	gca tgg gct ggt tta ttg tt	This study
	Myo-Par3-244F	cca cac aca gtc tga cca acc	This study
	Myo-Par3-446R	aag acg gca aat tca tgt cc	This study
	Myo-Par4-386F	tgc aga cca aga ccc ata aa	This study
	Myo-Par4-593R	cag cct ctg gag aga cag tg	This study
	Myo-Par5-530F	gcc cag tac ttc tgc cta gc	This study
	Myo-3F	ttc agc aag gac ctt gat aat gac tt	Heslewood et al. (1998)
ODC	ODC-6	gac tcc aaa gca gtt tgt cgt ctc agt gt	Allen and Omland (2003)
	ODC-Par1-167R	tca cat gca aag gca tct ct	This study
	ODC-Par2-123F	ttc ttc aaa tgg tct acc tga caa	This study
	ODC-Par2-320R	tcc cat atc gaa cac aca gc	This study
	ODC-Par3-274F	cct ttg ttc aag cca ttt ctg	This study
	ODC-Par3-444R	aaa aat tcc aac aac atg aag c	This study
	ODC-Par4-387F	caa ctg ttt tgg ggt taa aag c	This study
	ODC-Par4-573R	aac act tga aac aga gct aac aca	This study
	ODC-Par5-516F	ggc agc tta aac tca aat gac tg	This study
	ODC-8R	tct tca gag cca ggg aag cca cca cca at	Allen and Omland (2003)
ND2	ND2-L5215	tat cgg gcc cat acc ccg aaa at	Hackett (1996)
	ND2-Par1-176R	tat tta gtg gcg gct tcg at	This study
	ND2-Par2-116F	cca tcc tac ccc tca tct ca	This study
	ND2-Par2-320R	cct agt ttt att gct agg gct ga	This study
	ND2-Par3-272F	acc cag cat cct gca taa tc	This study
	ND2-Ceph3F	gga caa tga gac atc acc caa ct	This study
	ND2-Par3-459R	cag ggt ggg gtt aag tga tg	This study
	ND2-Par4-410F	cac caa tgc ccc tac tct tc	This study
	ND2-Par4-605R	aag gtg agt ttg ggg ttg tag a	This study
	ND2-Ceph4R	gta gaa gtt tag tag ggt gag ctt	This study
	ND2-Par5-553F	tcc cac cta ggc tga ata rcc	This study
	ND2-Par5-749R	gat agc agg gct agc agg ag	This study
	ND2-Par6-698F	cat gaa caa aaa ccc cat ca	This study
	ND2-Par6-889R	cga ggc gga ggt aga aga at	This study
	ND2-Par7-833F	cag caa caa tca tag ccc tac	This study
	ND2-Ceph7F	tcc aag aat taa cca aac agg aca t	This study
ND2-H6312	ctt att taa ggc ttt gaa ggc c	Cicero and Johnson (2001)	

4. Discussion

Monophyly of Paridae, including *Pseudopodoces humilis*, is strongly supported by the present study. Our results strongly suggest that the Fire-capped Tit (*Cephalopyrus flammiceps*), which presently is placed in Remizidae, forms part of Paridae and appears to be the sister-group of all other parid species (Fig 1; see also Tietze and Borthakur, 2012). The Fire-capped Tit shares some behavioral traits with the Remizidae, including a similar song and feeding behavior (Löhr, 1967, 1981; Madge, 2008), but like the tits, it nests in tree holes (Gosler and Clement, 2007).

The Yellow-browed Tit (*Sylviparus modestus*) and the Sultan Tit (*Melanochlora sultanea*), which are the morphologically most deviant species in Paridae, are the next sequential branches in tree (see also Tietze and Borthakur, 2012). A similar position of these two species was indicated by Gill et al. (2005) with mitochondrial cytochrome b sequences, but in their tree the two species were sisters, albeit without strong bootstrap support. *Cephalopyrus flammiceps*, *Sylviparus modestus* and *Melanochlora sultanea* all have distributions centered in the Himalayas, southern China and South-east Asia, and it is likely that parids originated in this region (Tietze and Borthakur, 2012).

The remaining parid species fall into two sister clades of approximately equal species richness. The first includes all the African tits, the Great Tit complex, *Pseudopodoces* and the *Cyanistes* species (clade A in Fig. 1). The second (clade B in Fig. 1) includes two clades of crested tits (*Baeolophus* and *Lophophanes*), the chickadees (*Poecile*) and the Coal Tit group (*Periparus*). Both these clades

were also supported by Tietze and Borthakur (2012) and recovered by the DNA–DNA hybridizations studies of Sheldon et al. (1992) and Slikas et al. (1996). However, in the mitochondrial study of Gill et al. (2005) only the latter clade was recovered. Instead, in this study, *Cyanistes* was placed either basal to all other parids or basal to the other species in these two clades but these alternative topologies were not strongly supported.

Species in clade B (Fig. 1) share two behavioral characteristics that are not found in any other group of parids. As far as is known, all species of parids nest in holes, but only the members of clade B are able to excavate their own nests, usually in soft, dead wood (Gosler and Clement, 2007; Harrap and Quinn, 1996). In addition, members of clade B are the only parids that cache food, particularly seeds but also insects (Gosler and Clement, 2007; Harrap and Quinn, 1996). Within this clade, *Periparus* appear to be basal relative to *Baeolophus*, *Lophophanes*, and *Poecile*, but with the exception of the proposed sister group relationships between *Lophophanes* and *Poecile*, interrelationships among these subclades are poorly supported. In contrast, the studies by Gill et al. (2005) and Tietze and Borthakur (2012) place the two clades of crested tits, the Nearctic *Baeolophus* and the Palearctic *Lophophanes*, as sister-groups, a relationship also suggested by the morphological similarity between *Baeolophus wollweberi* and *Lophophanes cristatus*. The DNA–DNA hybridization data (Slikas et al., 1996) is, on the other hand, in agreement with the results of the present study. The conflicting topologies, weak nodal support and short internodes are all suggestive of rapid divergence of these groups, and at present it is best to consider these relationships unresolved.

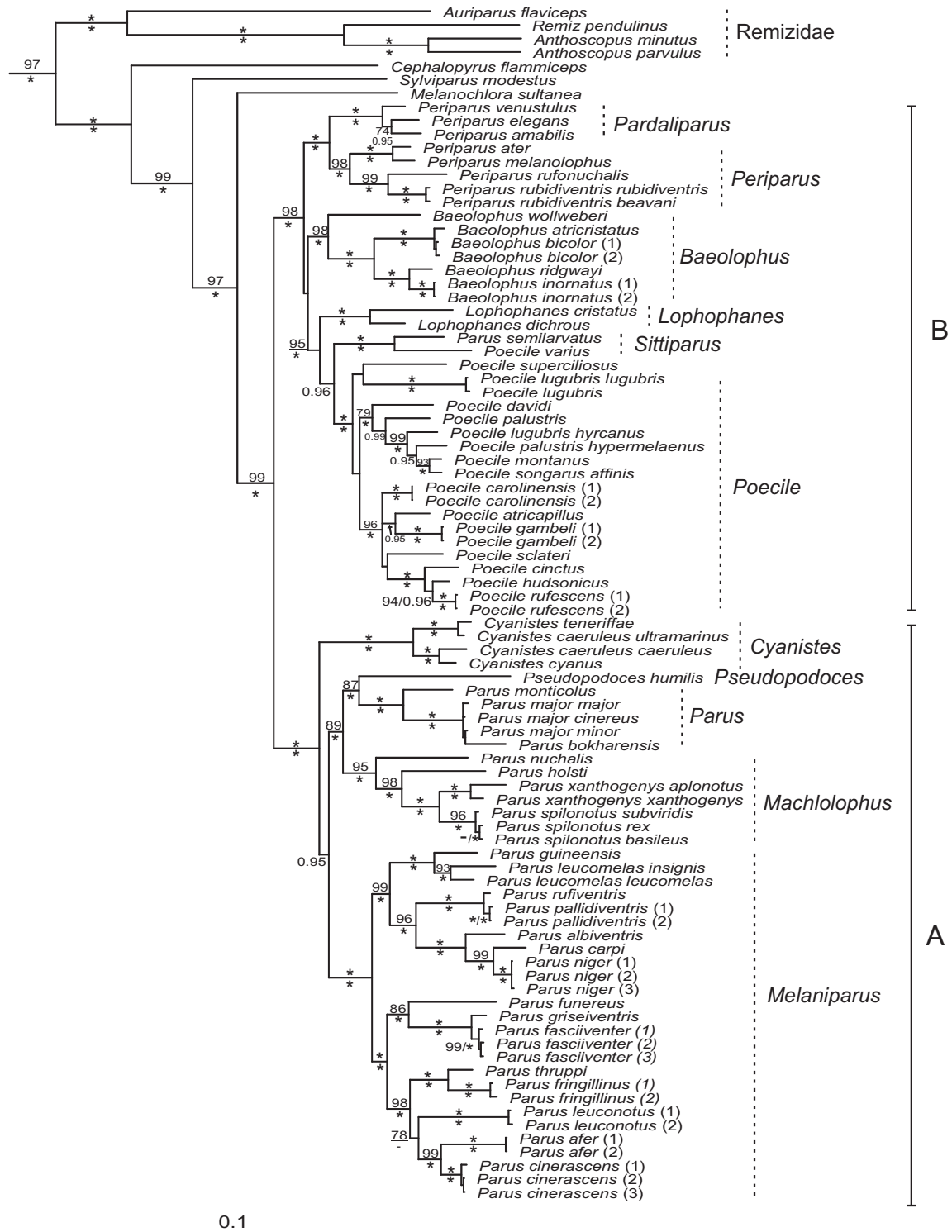


Fig. 1. Bayesian consensus tree of the combined, mixed-model analysis of three data partitions (total 2542 bp): myoglobin intron 2, ODC introns 6 and 7, along with exon 7 and ND2. Numbers below the branches indicate Bayesian posterior probabilities and numbers above bootstrap support from the maximum likelihood analysis. An asterisk * indicates a posterior probability of 1.0 or 100% bootstrap support.

4.1. *Periparus*

The seven species included in *Periparus* (Gosler and Clement, 2007) are monophyletic and the clade is divided into two lineages, one containing the primarily tropical/subtropical species Yellow-bellied Tit (*Periparus venustulus*), Elegant Tit (*Periparus elegans*) and Palawan Tit (*Periparus amabilis*), the other species with a primarily temperate distribution. The former clade is sometimes

referred to as *Pardaliparus* with *Periparus* restricted to the temperate clade (e.g. Harrap and Quinn, 1996). Both *Periparus elegans* and *P. amabilis* are restricted to the Philippines, the latter being endemic to Palawan, whereas *P. venustulus* is endemic to China. Cytochrome *b* data (Martens et al., 2006) suggests rapid divergence within these three lineages, and indicates that both *P. venustulus* and *P. amabilis* may be nested within *Periparus elegans*. Although Gill et al. (2005) did not include *Periparus venustulus*, their study

also indicated non-monophyly of *P. elegans* with respect to *P. amabilis*. Our study includes only one representative of each taxon but supports monophyly of the two Philippine species. It is clear that this group is in need of further investigation with respect to possible non-monophyly.

A similar situation has been observed in the temperate clade where the Spot-winged Tit (*Periparus melanolophus*) appears nested within the polytypic Coal Tit (*Periparus ater*) (Martens et al., 2006; Tietze et al., 2011). Our results confirm a close relationship between the two taxa, and they are in turn placed as sisters to the Black-breasted (*Periparus rufonuchalis*) and Rufous-vented (*Periparus rubidiventris*) tits. The same topology was also indicated by Gill et al. (2005); the sister group relationships between *P. ater* and *P. melanolophus*, as well as *P. rufonuchalis* and *P. rubidiventris* were recovered by Martens et al. (2006), although the relationships between these two and taxa *Pardaliparus* remained unresolved in that study.

4.2. *Poecile*, *Sittiparus*

Gosler and Clement (2007) recognize 13 species in *Poecile*. The taxonomic delimitation of several of these species remains uncertain. For example, some taxa included in the Sombre Tit (*Poecile lugubris*), Marsh Tit (*Poecile palustris*), and in the Willow Tit (*Poecile montanus*), are sometimes treated as distinct species (e.g. Harrap and Quinn, 1996). Our data strongly support monophyly of a clade containing the Rusty-breasted Tit (*Poecile davidi*), *P. palustris*, *P. montanus*, and their purported relatives, as well as a subspecies of the Sombre Tit (*P. lugubris hyrcanus*), but that species limits within this group need to be studied in more detail. *Poecile montanus* widely distributed across the Palearctic with several subspecies. This species also has several isolated populations in the south of the range (*songarus*, *affinis*, *stoetzeri* and *weigoldicus*) that are sometimes treated as a distinct species, the Songar Tit (*Poecile songarus*) (Harrap and Quinn, 1996). However, cytochrome *b* sequence data suggest that these four taxa are not monophyletic relative to *P. montanus*, but rather are sequential sister-taxa to that taxon, with *weigoldicus* basal relative to the others (Salzburger et al., 2002a). Of these taxa our study only includes the taxon *affinis*, which is placed as the sister to *P. montanus*. Our data also suggest that the Black-bibbed Tit (*Poecile hypermelaenus*) from western China is more closely related to this group than to the Marsh Tit (*Poecile palustris*) with which it has often been associated. The taxon *hyrcanus*, which has usually been considered to be a subspecies of *P. lugubris*, also appear to be closer to the *montanus*–*songarus* complex. This relationships has previously also been suggested based on similarities in voice and breeding biology (see Harrap and Quinn, 1996). It is clear that the entire *palustris*/*montanus* complex, as outlined in this study, is in need of an extensive study to clarify relationships and species boundaries.

The monophyly of North American chickadees is strongly supported and they are placed as the sister group of the *montanus*/*palustris* complex, in agreement with the results of Gill et al. (2005). Only two clades within this group receive strong support; the “brown-capped clade”, containing the Siberian Chickadee (*Poecile cinctus*), Boreal Chickadee (*Poecile hudsonicus*), and Chestnut-backed Chickadee (*Poecile rufescens*), and the group containing the Black-capped Chickadee (*Poecile atricapillus*) and Mountain Chickadee (*Poecile gambeli*). Relationships between these two taxa and the Carolina Chickadee (*Poecile carolinensis*) and Mexican Chickadee (*Poecile sclateri*) remain unresolved.

Poecile lugubris lugubris and *Poecile lugubris hyrcanus* are not sisters. As noted above, the Caspian Tit (*P. l. hyrcanus*) is part of the *montanus*/*palustris* complex, whereas the Sombre Tit (*P. l. lugubris*) appears in a more basal position within the *Poecile*. The Bayesian tree (Fig. 1) places the White-browed Tit (*Poecile superciliosus*) as

the sister of *Poecile lugubris*, but support for this association is weak. The placement of the Varied Tit (*Poecile varius*) as the sister taxon to the White-fronted Tit (*Parus semilarvatus*), as well as the placement of these two species as the sister group to the other *Poecile* species is strongly supported. *Poecile varius* is distributed in Northeastern China, on the Korean Peninsula and in the Japanese Archipelago, whereas *P. semilarvatus* is endemic to the Philippines. These two species are the only parids with white foreheads. They both have comparably long bills in comparison to other tits (Harrap and Quinn, 1996). As a consequence they have sometimes been placed together in their own subgenus, *Sittiparus* (Harrap and Quinn, 1996).

4.3. *Parus*, *Pseudopodoces*, *Machlolophus*

Within Clade A (Fig. 1), the African tits are monophyletic and placed as the sister group to a clade of predominately Asian species, including the Ground Tit (*Pseudopodoces humilis*), Great Tit (*Parus major*), Green-backed Tit (*Parus monticolus*), White-naped Tit (*Parus nuchalis*), Black-lored Tit (*Parus xanthogenys*), Yellow-cheeked Tit (*Parus spilonotus*) and the Yellow Tit (*Parus holsti*). *Cyanistes* is placed basal relative to these two clades (Fig. 1). This topology is strongly supported but is not congruent with the tree indicated by Gill et al. (2005). In that analysis only the African species, and Palearctic taxa *major*/*monticolus* and *spilonotus*/*xanthogenys*/*holsti* form a monophyletic group, whereas both *Pseudopodoces* and *Cyanistes* appear in a more basal position within the Paridae. However, that topology received only weak support (Gill et al., 2005).

Harrap and Quinn (1996) placed the Eurasian *major*, *monticolus*, *nuchalis*, *spilonotus*, *xanthogenys* in the subgenus *Parus*, whereas the African species were placed in the subgenus *Melaniparus*. Our data largely support this division, but the former clade also includes the Yellow Tit (*Parus holsti*), a species endemic to Taiwan that is sometimes placed in a monotypic subgenus, *Machlolophus*, and the Ground Tit *Pseudopodoces*, an aberrant ground-living tit from the Tibetan Plateau that has previously been placed in the Corvidae (crows, jays, magpies and their allies).

The placement of *Pseudopodoces* in this clade is strongly supported and within this group it is placed with strong support as the sister of the Great Tit complex and *P. monticolus*. In the studies by Gill et al. (2005) as well as Tietze and Borthakur (2012) *Pseudopodoces* appears in a more basal position relative to the two above mentioned clades. However, in both these studies the alternative placement of *Pseudopodoces* has weak support.

The White-naped Tit (*Parus nuchalis*) from the dry regions of northwestern India has previously not been included in any molecular study but has on morphological grounds been suggested to form a superspecies with *P. monticolus* from the Himalayas and the mountainous regions of western China (Eck, 1988). This relationship is not supported by the present study. Instead, it appears to be more closely related to a group that contains the Black-lored Tit (*Parus xanthogenys*), Yellow-cheeked Tit (*Parus spilonotus*) as well as *P. holsti*. Together with the White-naped Tit, which is the most basal member, this clade is the sister group of the *Pseudopodoces*/*major*/*monticolus* clade. From this topology it is apparent that the current classification of these species is inconsistent with recognition of the monotypic genus *Pseudopodoces* for the Ground Tit, unless *Parus*, as it is currently recognized (e.g. Gosler and Clement, 2007), is further subdivided. We favor the latter approach and propose that the name *Pseudopodoces humilis* be retained for the Ground Tit and that the *P. xanthogenys*, *P. spilonotus*, *P. holsti* and *P. nuchalis* are placed in the genus *Machlolophus*, Cabanis, 1851. For the African species, *Melaniparus*, Bonaparte, 1850 is available. *Parus* is thus restricted to the Great Tit complex (including *bokharensis*) and *P. monticolus*.

4.4. *Melaniparus*

All Afrotropical tits are strongly supported as monophyletic, indicating a single colonization of this region. This clade is divided into two main groups, and the phylogeny agrees in many respects with traditional classifications, although with some notable exceptions. Hall and Moreau (1970) placed *niger* (including *carpi*), *leucomelas* (including *guineensis*), *albiventris* and *leuconotus* in a superspecies *niger*, whereas Sibley and Monroe (1990) restricted this superspecies to include only the first four taxa (also followed by Fry et al., 2000). Our results do not support either concept of this superspecies. The Southern Black-Tit (*Parus niger*) and Carp's Black Tit (*Parus carpi*) are sister taxa, but instead of being allied with the other black-bellied species, the White-shouldered Black-Tit (*Parus guineensis*) and White-winged Black-Tit (*Parus leucomelas*), they are more closely related to the White-bellied Tit (*Parus albiventris*). Furthermore, the two rufous-bellied species, Rufous-bellied Tit (*Parus rufiventris*) and the Cinnamon-breasted Tit (*Parus pallidiventris*), which to our knowledge, have not been associated with these taxa before, form the sister clade to the *P. niger* clade. *P. guineensis* and *P. leucomelas* are basal relative to these two clades. The African gray tits fall in the second of the two main clades of African tits. Although they are morphologically very similar to each other and often considered a superspecies (Fry et al., 2000; Sibley and Monroe, 1990), they appear not to be monophyletic (Fig. 1). Rather the African gray tits form three distinct clusters, each with a morphologically deviating species as its sister taxon. The Dusky Tit (*Parus funereus*), another black species, which was included in the superspecies *niger* by Hall and Moreau (1970), is sister to the Miombo Tit (*Parus griseiventer*) and the Stripe-breasted Tit (*Parus fasciventer*). The White-backed Black-Tit (*Parus leuconotus*), is sister to the Gray Tit (*Parus afer*) and the Ashy Tit (*Parus cinerascens*), whereas the Red-throated Tit (*Parus fringillinus*) is sister to the Somali Tit (*Parus thruppi*).

4.5. *Cyanistes*

The western Palearctic Eurasian Blue Tit (*Cyanistes caeruleus*) and the mainly eastern Palearctic Azure Tit (*Cyanistes cyanus*) are morphologically quite similar and have traditionally been considered to be sisters (Vaurie, 1957). The two species, each with several subspecies, differ, however, in several respects but most notably in abdominal colors, which is yellow in the former species and white in the latter. However, molecular studies (Illera et al., 2011; Kvist et al., 2005; Päckert et al., 2013; Salzburger et al., 2002b) have demonstrated that the Blue Tit, as traditionally recognized (e.g. Snow, 1967; Vaurie, 1957), is paraphyletic and that the subspecies from North Africa and the Canary Islands (*teneriffae* and *ultramarinus* in our study) form a species that is the sister to the mainland Blue Tits and the Azure Tit. This is also strongly supported by our data (Fig. 1) as well as morphological and bioacoustic evidence (Kvist et al., 2005; Martin, 1991; Salzburger et al., 2002b).

5. Proposed classification

Cephalopyrus

Fire-capped Tit (*C. flammiceps*).

Sylviparus

Yellow-browed Tit (*S. modestus*).

Melanochlora

Sultan Tit (*M. sultanea*).

Periparus

Black-breasted Tit (*P. rufonuchalis*), Rufous-vented Tit (*P. rubidiventris*), Coal Tit (*P. ater*).¹

Pardaliparus

Yellow-bellied Tit (*P. venustus*), Elegant Tit (*P. elegans*), Palawan Tit (*P. amabilis*).

Lophophanes

European Crested Tit (*L. cristatus*), Gray-crested Tit (*L. dichrous*).

Baeolophus

Bridled Titmouse (*B. wollweberi*), Oak Titmouse (*B. inornatus*), Juniper Titmouse (*B. ridgwayi*), Tufted Titmouse (*B. bicolor*), Black-crested Titmouse (*B. atricristatus*).

Sittiparus

Varied Tit (*S. varius*), White-fronted Tit (*S. semilarvatus*).

Poecile

White-browed Tit (*P. superciliosus*), Sombre Tit (*P. lugubris*), Rusty-breasted Tit (*Poecile davidi*), Marsh Tit (*P. palustris*), Caspian Tit (*P. hyrcanus*)², Black-bibbed Tit (*P. hypermelaenus*)², Willow Tit (*P. montanus*)³, Carolina Chickadee (*P. carolinensis*), Black-capped Chickadee (*P. atricapillus*), Mountain Chickadee (*P. gambeli*), Mexican Chickadee (*P. sclateri*), Siberian Chickadee (*P. cinctus*), Boreal Chickadee (*P. hudsonicus*), Chestnut-backed Chickadee (*P. rufescens*).

Cyanistes

Canary Blue Tit (*C. teneriffae*), Eurasian Blue Tit (*C. caeruleus*), Azure Tit (*C. cyanus*).⁴

Pseudopodoces

Ground Tit (*P. humilis*).

Parus

Great Tit (*P. major*)⁵, Green-backed Tit (*P. monticolus*).

Machlolophus

White-naped Tit (*M. nuchalis*), Yellow Tit (*M. holsti*), Black-lored Tit (*M. xanthogenys*), Yellow-cheeked Tit (*M. sillonotus*),

Melaniparus

White-shouldered Black-Tit (*M. guineensis*), White-winged Black-Tit (*M. leucomelas*), Southern Black-Tit (*M. niger*), Carp's Black Tit (*M. carpi*), White-bellied Tit (*M. albiventris*), White-backed Black-Tit (*M. leuconotus*), Dusky Tit (*M. funereus*), Rufous-bellied Tit (*M. rufiventris*), Cinnamon-breasted Tit (*M. pallidiventris*), Red-throated Tit (*M. fringillinus*), Stripe-breasted Tit (*M. fasciventer*), Somali Tit (*M. thruppi*), Miombo Tit (*M. griseiventris*), Ashy Tit (*M. cinerascens*), Gray Tit (*M. afer*).

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² Tentatively recognized as a separate species pending further analyses.

³ Tentatively includes the taxa *songarus*, *affinis*, *stoetznieri* and *weigoldicus*.

⁴ Includes *flavipectus* (not sampled in this study).

⁵ Tentatively includes the taxa *minor*, *cinereus* and *bokharensis*.

¹ Includes *melanolophus*.

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