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Charting the course of reed-warblers across the Pacific islands

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ABSTRACT

Aim Deciphering the complex colonization history of island archipelagos is greatly facilitated by comprehensive phylogenies. In this study we investigate the phylogeny and biogeography of the insular reed-warblers (genus *Acrocephalus*) of the tropical Pacific Ocean, from Australia to eastern Polynesia.

Location Oceania.

Methods We used sequences of mitochondrial DNA (cytochrome *b*, ND2 and ATP8 genes) to infer the colonization patterns of reed-warblers endemic to Pacific islands and Australia. We sampled all known taxa of *Acrocephalus* in the Pacific except *A. luscinius nijoi*, for which no sample was available. Most taxa were represented by toe-pad samples from museum specimens collected in the 19th and 20th centuries. With a few exceptions, several specimens per taxon were sequenced independently in two institutions (Smithsonian Institution and Natural History Museum of Geneva).

Results Our data indicate that Pacific reed-warblers do not form a monophyletic group, because *A. luscinius luscinius* from Guam falls outside the main Pacific radiation. The remaining Pacific taxa are divided into two clades: one clade includes all the reed-warblers from Micronesia (except Guam) and Australia, and two Polynesian taxa from the Line Islands and the southern Marquesas; the other clade includes all remaining Polynesian taxa. The taxa endemic to three archipelagos (Mariana, Marquesas and Society islands) are polyphyletic, suggesting several independent colonizations.

Main conclusions Our results provide evidence for a complex pattern of colonization of the Pacific by reed-warblers. Calibration analyses suggest that reed-warbler lineages are much younger than the ages of the islands they occupy. Several remote archipelagos were colonized independently more than once. Consequently, we infer that the colonization of reed-warblers in the Pacific did not follow a regular, stepping-stone-like pattern. The phylogeny also suggests a previously undetected case of reverse colonization (from island to continent) for the Australian lineage and indicates that *A. luscinius*, as currently defined, is not monophyletic. We discuss the supertramp strategy of reed-warblers in the Pacific and show that, although Pacific reed-warblers meet some of the supertramp criteria in their aptitude for colonizing remote archipelagos, their life history characteristics do not fit the model.

Keywords

Acrocephalidae, *Acrocephalus*, back-colonization, colonization patterns, island biogeography, Micronesia, Pacific Ocean islands, phylogeny, Polynesia, reed-warblers.

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INTRODUCTION

Island biogeography is currently experiencing a change in perspective owing to the exponential increase of new data on insular colonization, based for the most part on molecular phylogenies. Synthetic hypotheses intended to provide a general framework for island biogeography (MacArthur & Wilson, 1967) are being challenged by the accumulation of complex colonization patterns that defy simpler or more parsimonious models (de Queiroz, 2005; Heaney, 2007). For instance, the regular and progressive colonization of oceanic islands by terrestrial species is central in the 'stepping-stone' hypothesis, in which islands are used in a stepwise fashion to reach the most isolated pieces of land (MacArthur & Wilson, 1967). In volcanic archipelagos, this colonization scenario posits a 'progression rule' whereby the age of divergence of endemic species is correlated with the appearance of islands. This progressive pattern has been observed in birds (Fleischer *et al.*, 1998; Vanderwerf *et al.*, 2009), mammals (Steppan *et al.*, 2003) and arthropods (Garb & Gillespie, 2006), suggesting that colonization of volcanic archipelagos can sometimes be predicted by the geological history of the islands. However, molecular phylogenies of insular organisms have also shown that colonization of islands is not always a regular phenomenon. For example, long-distance dispersal events have leap-frogged 'step' islands or archipelagos (Gillespie, 2002; Howarth *et al.*, 2003), islands and archipelagos have been colonized multiple times by a number of lineages (Gillespie *et al.*, 1994; Slikas *et al.*, 2002; Warren *et al.*, 2006), and insular lineages have colonized continental areas (the 'reverse colonization' of Bellemain & Ricklefs, 2008). Thus, a principal challenge in island biogeography is to determine whether colonization is a regular phenomenon with 'stepping-stone' patterns or if long-distance dispersal occurs more frequently than originally thought.

Deciphering the complexity of colonization over large distances and a multitude of islands requires comprehensive phylogenies for widely distributed taxa. In this context, the reed-warblers (genus *Acrocephalus*) of the tropical Pacific are ideal candidates for the study of insular colonization. The known distribution of these insectivorous passerines includes 12 archipelagos and isolated islands, as well as Australia. More importantly, reed-warblers are atypical among land birds in the Pacific in that they occur on remote islands in eastern Polynesia and Hawai'i but are absent from central Polynesia and all of southern Melanesia (Fig. 1). Other widely diversified groups of land birds in the tropical Pacific either exhibit a relatively uniform distribution from west (New Guinea or Melanesia) to east (central and eastern Polynesia) that avoids Hawai'i (e.g. *Ptilinopus* fruit-doves, *Ducula* pigeons, *Vini* lorikeets, *Aplonis* starlings and *Aerodramus* swiftlets), or cover all of the tropical Pacific except for small, dry islands and atolls (monarchs, Monarchidae) (Pratt *et al.*, 1987).

Pacific reed-warblers occupy a variety of habitats on a wide spectrum of island types, ranging from large humid mountainous islands such as Tahiti to small dry carbonate islands (e.g. Nauru) and low-lying atolls (e.g. Tuamotu and Line islands) where the flora and entomofauna are particularly depauperate. They are present on both old and young islands in some archipelagos (e.g. Society, Marquesas and Mariana islands) or only on the oldest islands of others, as in Hawai'i. All species are sedentary except in Australia, where local seasonal movements are known. Nine to thirteen endemic species are recognized on the basis of morphology and distribution (Dickinson, 2003; Bairlein *et al.*, 2006), but many queries remain concerning the taxonomic status of Pacific reed-warblers (Kennerley & Pearson, 2010).

World-wide, the genus *Acrocephalus* includes more than 35 species in the Palaearctic, Africa and Australasia. Previous molecular phylogenies of reed-warblers (Leisler *et al.*, 1997; Helbig & Seibold, 1999; Fregin *et al.*, 2009) suggested that the Pacific species formed a monophyletic group closely related to three species with a large distribution: the clamorous reed warbler *A. stentoreus* (Hemprich and Ehrenberg, 1833) from the Middle East, Asia to Melanesia; the oriental reed warbler *A. orientalis* (Temminck and Schlegel, 1847) from Asia; and the great reed warbler *A. arundinaceus* (Linnaeus, 1758) from Eurasia. However, these studies treated only a subset of Pacific taxa. In addition, phylogenetic studies of three Polynesian endemics (from Hawai'i, the Marquesas and Society islands) have been conducted recently. In the Hawai'i archipelago, the two reed-warbler populations from Nihoa and Laysan were found to be sister-taxa (Fleischer *et al.*, 2007), whereas the Marquesas were colonized independently by two distinct lineages of reed-warblers (Cibois *et al.*, 2007a). A complex pattern of colonization was also found in the Society Islands, where the reed-warblers do not form a monophyletic group (Cibois *et al.*, 2008). These results, albeit partial in terms of taxon sampling and geographical coverage, suggest that the colonization history of reed-warblers in the Pacific might be more complex than previously thought.

In the present study, we used mitochondrial DNA (mtDNA) sequence data to infer the colonization patterns of all species of reed-warblers endemic to the Pacific islands and Australia. Our phylogeny, which includes eight extinct taxa known only from museum specimens, permits us to address the following questions. (1) How many lineages colonized the Pacific Ocean? (2) Do reed-warblers show a stepping-stone pattern of colonization, at least in volcanic archipelagos? (3) Alternatively, how many long-distance dispersal events are required to explain the current distribution of reed-warblers in the Pacific? Finally, we discuss our findings in relation to Diamond's (1974) supertramp hypothesis of colonization.

MATERIALS AND METHODS

DNA sequencing

The taxonomic classification used in this paper follows Bairlein *et al.* (2006) except for taxa endemic to the Society, Marquesas and Gambier islands, for which we follow Cibois *et al.* (2007a, 2008, 2011). We sampled all reed-warbler taxa in the Pacific
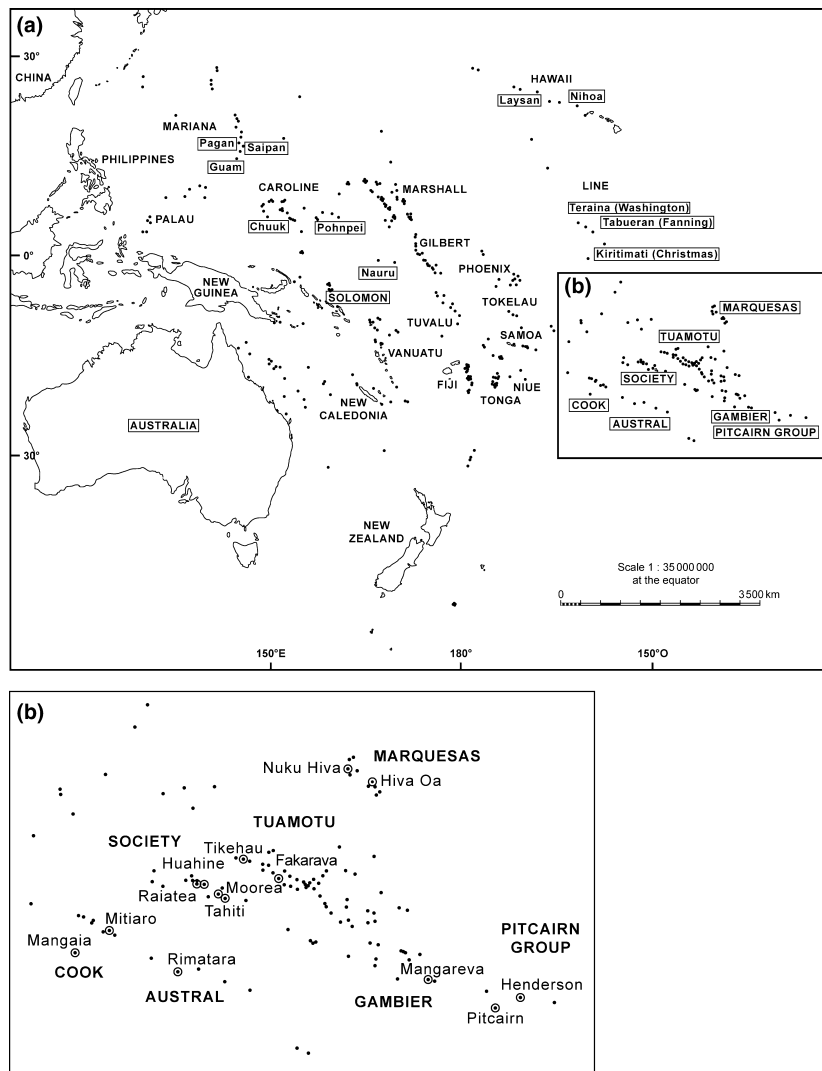
Figure 1 Maps showing the sampling site locations for the Pacific reed-warblers (*Acrocephalus* spp.) studied. (a) Map of Oceania. The islands or archipelagos sampled in this study are indicated by boxes. (b) Details of the eastern Polynesia section. The islands sampled are outlined.

except *A. luscinius nijoi* (Yamashina, 1940), for which no sample was available (see Appendix S1 in Supporting Information). More than half of the samples were toe-pads from specimens collected in the 19th or 20th century and held in museums. With a few exceptions, several specimens per taxon were sequenced independently in our two laboratories (Smithsonian Institution and Natural History Museum of Geneva). Blood or tissue samples were used for several extant species, mainly from Australia, Hawai'i and French Polynesia. Sequences already deposited in GenBank from previous studies on Pacific reed-warblers were also included. Three outgroup species were used based on the results of previous molecular phylogenies on reed-warblers: *Acrocephalus arundinaceus*, *A. stentoreus* and *A. orientalis* (see Appendix S1 for GenBank accession numbers). DNA extraction, amplification and sequencing were conducted following the procedures described in Cibois *et al.* (2007a) and Fleischer *et al.* (2007). Regions of three mtDNA subunits were amplified: ATP synthase subunit 8 (ATP8), cytochrome *b* (cyt *b*) and NADH dehydrogenase subunit 2 (ND2). An additional primer was specifically designed for this study: ND2-ACH2 5′-CCATGCCGGTTAG TACTG-3′. Island names follow Bier (2009), and island elevation is indicated in metres above sea-level (m a.s.l.).

Phylogenetic analyses and divergence

The data were first subjected to Bayesian inference (BI) using MRBAYES 3.1.2 (Ronquist & Huelsenbeck, 2003), with models selected using MRMODELTEST 2.3 (Nylander, 2004). We performed two independent runs of four Markov chains for 10 million generations each. Markov chains were sampled every 1000 generations, with a 10% burn-in period. We ran analyses using all data in a single partition (combined analysis), or partitioned the data by gene and codon position. Results for the various partitioning schemes were evaluated with Bayes factors (Kass & Raftery, 1995; Brandley *et al.*, 2005), computed using the harmonic mean from the *sump* command in MRBAYES. We used TRACER 1.4.1 (Rambaut & Drummond, 2007) to check that we reached convergence for the posterior distributions of the parameter estimates. We then performed

phylogenetic analyses under the maximum likelihood (ML) criterion using RAxML 7.0.3 (Stamatakis, 2006), with the same partitions (combined, partitioned by gene or codon position) and 1000 bootstrap iterations. We compared the resulting topologies and nodal support for all generated trees; nodes were treated as supported if posterior probabilities were ≥ 0.95 and bootstrap values were ≥ 70% (see Alfaro *et al.*, 2003, for a discussion on the interpretation of support values). Because specimens from Guam (Mariana Islands) were basal to the Pacific reed-warbler clade in all analyses, we also conducted a broader analysis of cyt *b* sequences that included more outgroup species.

We used molecular clocks to estimate divergence dates within this group because no calibration points (fossil or biogeographical) were available for Pacific reed-warblers. We tested whether a strict molecular clock could be appropriately applied to our data with Tajima's relative rate test (Tajima, 1993) in mega 4.0 (Tamura *et al.*, 2007). Because a global clock hypothesis could not be rejected, we first applied a strict clock to our data, combined in one partition or partitioned by gene or codon position. We also submitted the same partitions to a relaxed clock with an uncorrelated lognormal model that allowed rate variation, and checked whether the two models provided different divergence-time estimates. We used a mean rate of evolution of 1.6% sequence divergence per million years (Myr), estimated for Hawaiian honeycreepers, based on the fact that the Hawaiian islands were ordered linearly by age, and based on the assumption that an island's formation date provides a maximum age for taxa inhabiting the island (Fleischer *et al.*, 1998). Of course, this calibration may not hold if species dispersed to one or more islands after speciation but did not survive on the island of origin. We employed this rate as a proxy for the global rate of sequence divergence for mitochondrial protein-coding genes (the '2% rate', range 1.5–3%; Ho, 2007) in order to allow comparison with previous divergence-time estimates for passerine radiations in the Pacific region (Cibois *et al.*, 2004; Vanderwerf *et al.*, 2009). Although a general 2% rate has typically been found within birds (Weir & Schluter, 2008), it has also been suggested that rates of molecular evolution are time-dependent and that saturation effects could lead to an underestimation of molecular rates (Ho *et al.*, 2007). Therefore we also analysed our data using a time-independent neutral evolutionary rate, based on synonymous sites, which has recently been proposed for birds (Subramanian *et al.*, 2009). This rate of 0.073 substitutions per site per million years was estimated from fourfold-degenerated sites in Adélie penguins (*Pygoscelis adeliae*). It has also recently been used for closely related species of *Picus* in comparison with a biogeographical calibration point (Pons *et al.*, 2010). In our data set, 292 fourfold-degenerated sites were selected by mega. All calibration analyses were conducted using the package beast 1.5.2 (Drummond & Rambaut, 2007). We used the models selected by ModelTest for the various partitions. The Markov chain Monte Carlo (MCMC) chain length was 10 million, with a 10% burn-in period and parameters logged every 1000 generations. The same run was performed several times to ensure the convergence of models, and Tracer was used to analyse the results of the MCMC runs.

The occurrence of reed-warblers in remote archipelagos in the Pacific demonstrates their great aptitude for over-water dispersal, but isolation could influence gene flow between islands. We tested the correlation between isolation and genetic distances with Mantel tests (Mantel, 1967; Sokal, 1979) between the pairwise genetic distances (Kimura two-parameter model, computed using mega) and the geographic distances between islands (in kilometres, $\log_{10}$-transformed).

RESULTS

The final data set included 67 individuals representing 25 taxa, with 36 individuals represented by toe-pad samples from museum specimens. For most samples, we obtained partial sequences for cyt *b* (921 bp), ND2 (600 bp) and ATP8 (195 bp). Shorter sequences were obtained for a few samples. New sequences were deposited in GenBank under accession numbers HQ844270–HQ844429. The alignments were straightforward with no indels, as expected for protein-coding genes. We detected no contamination in the negative controls. We translated the nucleotide sequences to proteins using mega and found no stop-codons or indels, suggesting that we did not amplify pseudo-genes (Allende *et al.*, 2001). The total matrix contained 1716 bp, of which 17.3% were variable (10.4%, 4.9% and 2.0% for cyt *b*, ND2 and ATP8, respectively) and 13.6% were parsimony-informative (7.7%, 4.2% and 1.7%). Results from the Akaike information criterion (AIC) values in ModelTest supported the following models for the individual genes or codon positions: GTR + I + G for cyt *b*, HKY + I for ND2 and GTR + I for ATP8; HKY + I + G for first codon position, HKY + I for second codon position and GTR + G for third codon position. When combined, the supported model was GTR + I + G. Estimated likelihood harmonic means were −5556.93 for the combined data set, −5460.89 for the partition by gene, and −5055.58 for the partition by codon position. Differences in Bayes factors were significant for both partitions compared with the non-partitioned model. However, all Bayesian analyses resulted in the same phylogenetic tree (Fig. 2), and no posterior probability below our significance threshold (i.e. < 0.95) exceeded that value when more complex partitioning schemes were used. Similarly, all analyses in RAxML produced the same topology as illustrated in Fig. 2, except for the position of *A. caffer* (Sparrman, 1786), which was unresolved in the Bayesian topology (i.e. posterior probability < 0.50) but was securely placed in ML topologies (nodal support > 70%). In addition, support values differed for the ML and BI analyses in three other nodes but all these internodes were short, and differences could be linked to the sensitivity of the two methods when few character changes occurred along a branch (Alfaro *et al.*, 2003).

Our primary finding is that Pacific reed-warblers do not form a monophyletic group because the reed-warblers from Guam [*A. luscinius luscinius* (Quoy and Gaimard, 1830)] fall
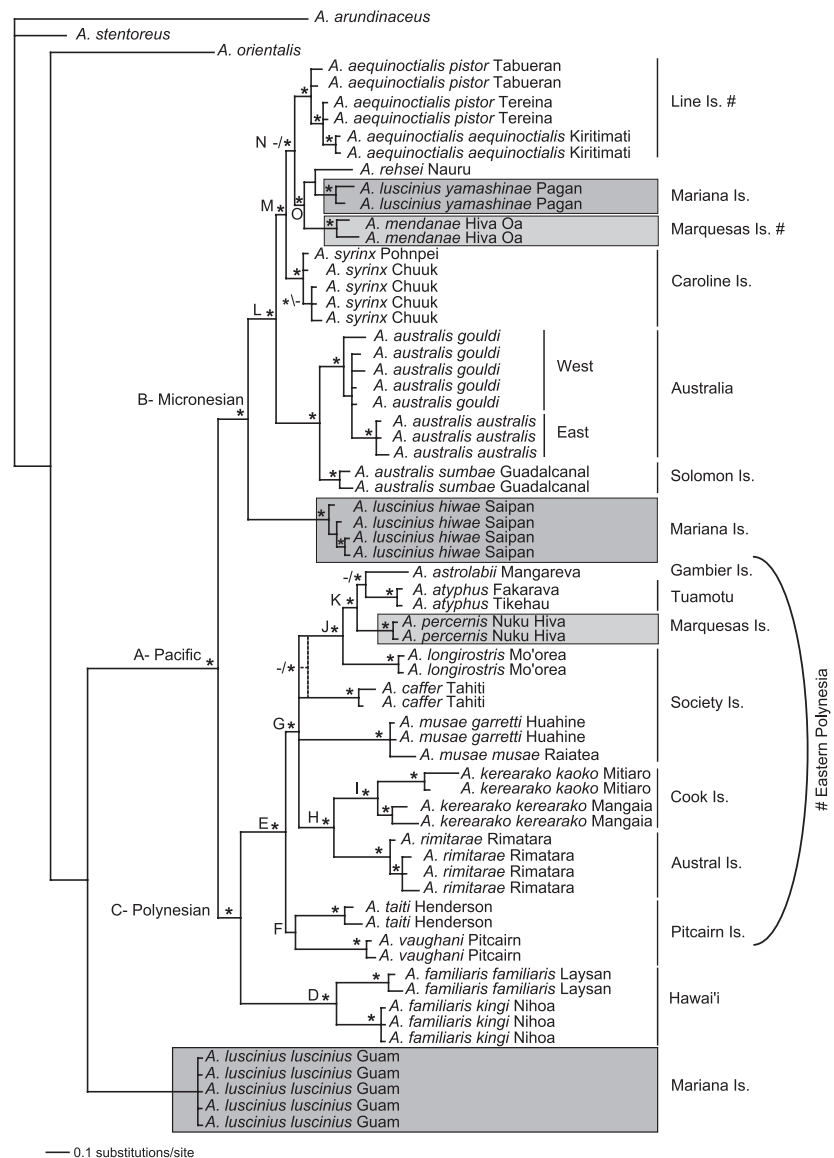
Figure 2 Phylogenetic tree of Pacific reed-warblers (*Acrocephalus* spp.), based on the cytochrome *b*, ND2 and ATP8 sequence data, estimated using Bayesian and maximum likelihood inferences. Asterisks indicate nodes supported by posterior probabilities (PPs) ≥ 0.95 and bootstrap (BT) values ≥ 70%. When support differs between methods, PPs are indicated first and BT values second, with support below significance labelled by a dash. A # indicates the isolated taxa from eastern Polynesia in the Micronesian clade. Letters from A to O refer to clades in Table 1 (the calibration analysis). Grey boxes show the polyphyletic Mariana and Marquesas archipelagos.

outside the main Pacific radiation. Additional searches with a denser *Acrocephalus* sampling suggested that reed-warblers from Guam belong to the group that includes the Pacific taxa, *A. stentoreus*, *A. orientalis* and *A. arundinaceus*, but with no clear resolution between the taxa (see Appendix S2 in Supporting Information). This surprising result was confirmed by the independent extraction and sequencing of five specimens of reed-warblers from Guam from two museums in our two laboratories (Smithsonian Institution and Natural History Museum of Geneva; Appendix S1). In each case, we obtained identical sequences for each of the three genes. The remaining Pacific taxa were divided into two clades, which we will subsequently refer to as the 'Polynesian' and 'Micronesian' clades. The Polynesian clade included all Polynesian taxa except the reed-warblers from two archipelagos, the Line Islands [*A. aequinoctialis* (Latham, 1790)] and the southern Marquesas Islands [*A. mendanae* Tristram, 1883]. The taxa endemic to Hawai'i [*A. familiaris familiaris* (Rothschild, 1892)

and *A. f. kingi* (Wetmore, 1924)] formed a monophyletic group that was the first to branch off from all other eastern Polynesian endemics. The taxa from Pitcairn Island [*A. vaughani* (Sharpe, 1900)] and Henderson Island (*A. taiti* Ogilvie-Grant, 1913) formed a clade but with weak support. The remaining phylogenetic relationships among eastern Polynesia taxa were not well supported except for two cases. The Rimatara reed-warbler [*A. rimitarae* (Murphy and Mathews, 1929)] is related to the reed-warblers of the Cook Islands (*A. kerearako* Holyoak, 1974), and the reed-warblers from Mo'orea in the Society Islands [*A. longirostris* (Gmelin, 1789)], the northern Marquesas Islands [*A. percernis* (Wetmore, 1919)], the Tuamotu Islands [*A. atyphus* (Wetmore, 1919)] and Mangareva in the Gambier Islands (*A. astrolabii* Holyoak and Thibault, 1978; see Cibois *et al.*, 2011, for a full review of the origin of this specimen) form a well-supported clade. Finally, the positions of the Tahiti reed-warbler (*A. caffer*) and populations from Raiatea and Huahine in the Society Islands

[*A. m. musae* (Forster, 1844) and *A. musae garretti* Holyoak and Thibault, 1978] were not fully resolved in this phylogeny.

The Micronesian clade includes all the reed-warblers from Micronesia (except Guam) and Australia, and two Polynesian taxa from the Line Islands and the southern Marquesas. Interestingly, the birds from the Mariana Islands did not form a monophyletic group: individuals from Saipan [*A. luscinius hiwae* (Yamashina, 1942)] were the first to branch off, and the extinct population from Pagan [*A. luscinius yamashinae* (Taka-Tsukasa, 1931)] was more closely related to individuals from Nauru [*A. rehsei* (Finsch, 1883)], an isolated island in central Micronesia, and the southern Marquesas (*A. mendanae*) in eastern Polynesia. The Australian reed-warbler (*A. australis*) was embedded among Pacific taxa, suggesting that its progenitors had an insular origin. Disjunct populations in eastern [*A. australis australis* (Gould, 1838)] and western (*A. a. gouldi* Dubois, 1901) Australia formed a monophyletic group and exhibited little genetic difference (0.75% mean divergence for the three mitochondrial genes). Individuals from the Solomon Islands (*A. a. sumbae* Hartert, 1924) were closely related to the Australian clade. Populations sampled from Pohnpei and Chuuk in the Caroline Islands [classified as *A. syrinx* (Kittlitz,

1835)] formed a monophyletic group with little genetic difference (0.28% mean divergence for the three mitochondrial genes).

Sequence data confirmed a single origin for three geographically separated populations of reed-warblers in the Line Islands of western Polynesia, with little genetic difference between islands (0.16% mean divergence for the three mitochondrial genes). The topology suggests that the subspecies *A. aequinoctialis pistor* Tristram, 1883 is paraphyletic, but a more detailed morphological and genetic analysis of the extant populations would be necessary to clarify the systematics of this taxon.

Divergence-time estimates for the supported nodes of the phylogenetic tree are indicated in Table 1. Global and relaxed clock models using a 1.6% per Myr divergence rate and different partitioning schemes yielded similar results. However, analyses using the molecular clock rate from the fourfold-degenerated sites yielded more recent estimates. Several short branches were not recovered in this analysis (nodes F, G, K and N), probably because fewer characters were used. The 95% highest posterior density intervals were broad in both cases, and intervals obtained with the different rates barely overlapped. Nevertheless, all calculated divergence times were

Table 1 Divergence times of Pacific reed-warblers (*Acrocephalus* spp.) in million years with the 95% highest posterior density obtained using two molecular clock rates (a global mitochondrial protein-coding genes rate and a rate based on fourfold-degenerated sites). For the mitochondrial (mt) rate, global or relaxed clocks were used. Results for the various partitioning schemes were similar, and we show here only the partition by codon. The last column provides the ages of the islands in million years.

Node	Divergence times (Ma) mt rate Global clock	Relaxed clock	Divergence times (Ma) Fourfold-degenerated sites rate	Island age (Ma)
A – Pacific clade	2.22 (1.24–3.44)	2.42 (1.24–3.94)	0.79 (0.51–1.12)	–
B – Micronesian clade	1.58 (0.84–2.46)	1.74 (0.84–2.96)	0.57 (0.32–0.85)	Saipan (41)[1]
C – Polynesian clade	2.01 (1.12–3.12)	2.12 (1.04–3.47)	0.70 (0.44–0.99)	–
D – *familiaris/kingi*	0.75 (0.34–1.24)	0.90 (0.26–1.76)	0.17 (0.04–0.33)	Nihoa (7.2)[2]
				Laysan (19.9)[3]
E – Eastern Polynesia	1.54 (0.85–2.39)	1.66 (0.78–2.70)	0.54 (0.34–0.77)	–
F – *vaughani/taiti*	1.23 (0.59–1.94)	1.18 (0.37–2.10)	–	Pitcairn (0.4–0.9)[4]
				Henderson (0.4–13)[5]
G – French Polynesia and Cook Islands	1.41 (0.80–2.17)	1.45 (0.73–2.42)	–	Huahine (2.1–2.7)[6]
				Raiatea (2.4–2.8)[6]
				Tahiti (0.2–1.4)[6]
H – *rimitarae/kerearako*	1.04 (0.55–1.65)	1.02 (0.43–1.74)	0.36 (0.19–0.56)	Rimatara (*c.* 15)[7]
I – *kerearako/kaoko*	0.57 (0.26–0.94)	0.60 (0.19–1.09)	0.13 (0.05–0.23)	Mangaia (19)[8]
				Mitiaro (12)[8]
J – *longirostris*/clade K	0.78 (0.41–1.26)	0.79 (0.31–1.38)	0.19 (0.09–0.30)	Mo'orea (1.4–1.7)[6]
K – *percernis/atyphus-astrolabii*	0.60 (0.30–0.96)	0.57 (0.21–1.05)	–	North Marquesas (2–5)[9]
				Mangareva (5.2–7.2)[4]
				Tuamotu (30–65)[1]
L – *australis*/clade M	1.03 (0.54–1.64)	1.22 (0.54–2.07)	0.35 (0.19–0.53)	–
M – *syrinx*/clade N	0.76 (0.41–1.24)	0.95 (0.42–1.61)	0.23 (0.22–0.36)	Pohnpei (5.2)[11]
				Chuuk (4.7–13.9)[11]
N – *aequinoctialis*/clade O	0.65 (0.33–1.03)	0.78 (0.33–1.32)	–	Line Islands (44–93)[10]
O – *mendanae/yamashinae/rehsei*	0.57 (0.29–0.92)	0.65 (0.26–1.10)	0.17 (0.07–0.27)	South Marquesas (1–4)[9]
				Pagan (1)[1]
				Nauru (*c.* 40)[12]

[1]Gillespie & Clague, 2009; [2]Dalrymple *et al.*, 1974; [3]Dalrymple *et al.*, 1981; [4]Duncan *et al.*, 1974; [5]Blake, 1985; [6]Guillou *et al.*, 2005; [7]Dickinson, 1998; [8]Bonneville *et al.*, 2006; [9]Legendre *et al.*, 2006; [10]Schlanger *et al.*, 1984; [11]Keating *et al.*, 1984; [12]Jacobson *et al.*, 1997.

much more recent than the age of the corresponding island (Table 1). Because of the uncertainty in the molecular clock results, we focused mainly on relative divergence-time estimates among taxa. Thus the oldest divergence between lineages was found between the reed-warblers from Hawai'i (*A. familiaris*) and the other Polynesian taxa (node C). The second lineage in the Pacific reed-warblers, the Micronesian clade (node B), is younger, and diversification within the lineage occurred at approximately the same time as the diversification of the eastern Polynesia clade (node E). Interestingly, the two independent colonizations of the Marquesas archipelago (nodes O and K) occurred almost simultaneously, a result also found when fewer taxa were analysed (Cibois *et al.*, 2007a).

A Mantel test performed for the entire Pacific radiation (not including Guam) showed that pairwise genetic distance was correlated with geographic distance ($P = 0.026$). A similar correlation was found within the Polynesian clade ($P = 0.036$); however, the null hypothesis that there was no association between the elements of the two matrices could not be rejected for the Micronesian clade ($P = 0.71$).

DISCUSSION

Colonizations did not follow a stepping-stone pattern ...

This phylogeny strongly suggests that all reed-warblers in the tropical Pacific Ocean share a common ancestor, except for birds from Guam, the largest and one of the oldest islands of the Mariana archipelago (32–43 Ma; Reagan & Meijer, 1984). The reed-warblers of the Mariana Islands exhibit a brownish plumage with indistinct pattern, a widespread character in *Acrocephalus*, but they are particularly long-billed compared

with other Micronesian taxa (Baker, 1951). Our molecular phylogeny suggested that the long bill is a convergent character among taxa and that the archipelago was independently colonized at least three times, in Guam, Pagan and Saipan. Two other extant populations on Alamagan (*A. l. luscinius*) and Aguijan (*A. l. nijoi*) in the Mariana Islands have yet to be sampled.

Several eastern Polynesian lineages are embedded in the Micronesian clade (Fig. 2), suggesting that the colonization of reed-warblers in the Pacific did not follow a regular, stepping-stone-like pattern, contrary to the hypothesis of a west-to-east progression proposed previously (Mayr, 1941; Baker, 1951). The Mantel test confirmed that geographic and genetic distances were uncorrelated in the Micronesian clade.

Probably the most puzzling example of complex colonization patterns was found in the Marquesas, a remote archipelago of eastern Polynesia, where a previous study showed that two distinct lineages, *percernis* in the north and *mendanae* in the south, were separated by only 41 km (Cibois *et al.*, 2007a). The additional taxa in this study showed that the southern Marquesas lineage is closely related to two Micronesian taxa (from Nauru and Pagan), whereas the lineage endemic to the northern part of the archipelago is closely related to the reed-warblers from the Tuamotu and Gambier islands. A study at the population level with both mitochondrial and nuclear markers is now under way to test whether introgression occurred between the two lineages in the Marquesas Islands (A. Cibois, E. Pasquet & J.-C. Thibault, in prep.). The two lineages that colonized the archipelago originated from different parts of the Pacific Ocean, as shown in Fig. 3. The fact that they reached the the Marquesas at roughly the same time (around 0.6 Ma according to a mitochondrial molecular clock) suggests that specific conditions, perhaps linked to climatic change during the Pleistocene, favoured the colonization of the archipelago.
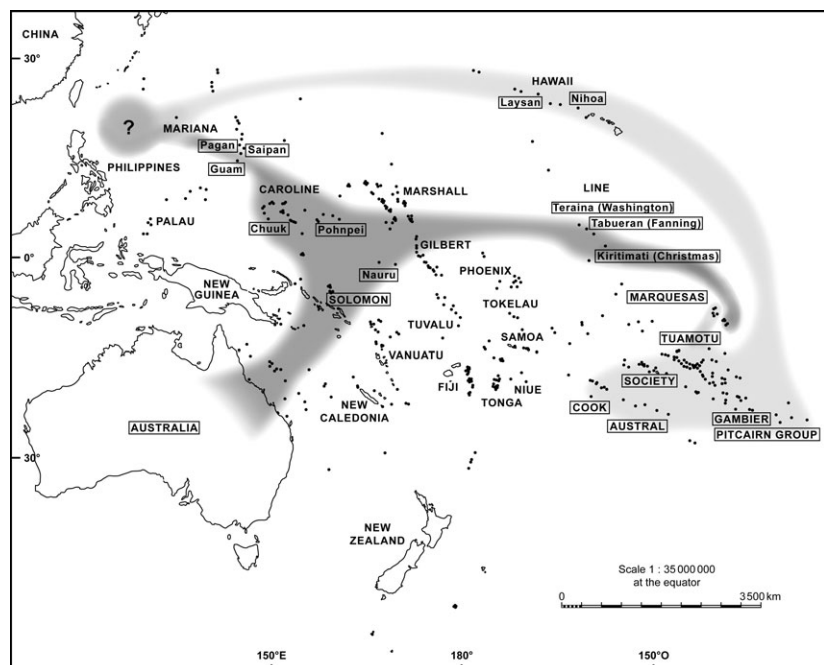
Figure 3 Map indicating the two main lineages of reed-warblers (*Acrocephalus* spp.) in the Pacific Ocean. The question mark indicates the unknown common ancestor of the Pacific radiation (not including Guam).

The situation is almost equally complex in the Society Islands. Although the islands are quite linearly disposed, owing to their sequential appearance over a single hotspot in the Pacific Plate (Guillou *et al.*, 2005), the reed-warblers do not form a monophyletic group. The Mo'orea reed-warbler (*A. longirostris*, extinct) is closely related to the northern Marquesas/Tuamotu/Gambier clade with good support. Taxa from the Leeward Islands in the Society group (*A. m. musae* from Raiatea and *A. m. garretti* from Huahine, both extinct) form a clade, but their position relative to the other taxa, including the Tahiti reed-warbler (*A. caffer*), is unresolved. In sum, results from the Mariana, Marquesas and Society islands provide no support for the 'progression rule' colonization of volcanic archipelagos.

… but islands were successfully colonized only once

Although multiple colonizations occurred in three archipelagos, species from different lineages are not known to co-occur on any island. This situation contrasts with other insular colonizers in the tropical Pacific, such as white-eyes or fruit-doves, which exhibit quite different life history characteristics. Social behaviour (van Balen, 2008) is probably one of the key characters that has enabled several lineages to successively colonize the same island and allowed their coexistence. In the Mariana Islands, for instance, two species of white-eyes coexist on Yap (*Rukia oleaginea* and *Zosterops hypolais*) and on Saipan (*Cleptornis marchei* and *Zosterops conspicillatus saypani*), each belonging to a distinct lineage that colonized independently, probably at different times (Slikas *et al.*, 2000). Similar situations probably occur in the Caroline Islands, where two white-eye species co-occur on Pohnpei and Chuuk, and in the Marquesas Islands, where two sympatric fruit-doves coexisted on Nuku Hiva and Hiva Oa (Ripley & Birckhead, 1942). Analyses of the sub-fossil record also suggest other possible cases of sympatry on eastern Polynesian islands for rails, pigeons and lorikeets (Steadman, 2006). Phylogenetic hypotheses, however, are pending for these taxa.

In contrast to the gregarious fruit-doves, reed-warblers on the Pacific islands live in pairs or trios that defend year-round territories (Holyoak & Thibault, 1984; Mosher, 2006; Thibault & Cibois, 2006). This territorial behaviour, combined with their propensity for high population levels, which may saturate available habitat (Craig, 1992; Graves, 1992; Morin *et al.*, 1997), probably impedes the establishment and survival of new immigrants. On the other hand, distance between islands does not appear to be a limiting factor to colonization over geological time. Indeed, the phylogeny suggests that several long-distance dispersal events occurred in the Pacific (Figs 2 & 3).

Timing of reed-warbler diversification in the Pacific

According to our calibration analyses, reed-warblers colonized Hawai'i approximately at the same time as did the 'elepaio (*Chasiempis* spp.) (*c.* 2.3 Ma; Vanderwerf *et al.*, 2009). In contrast, *Pomarea* monarchs colonized the Mar-

quesas earlier than the reed-warblers (*c.* 3 Ma; Cibois *et al.*, 2004). Pacific reed-warbler lineages are apparently much younger than the islands they currently occupy (Table 1). The only known exceptions are the reed-warblers on two relatively young islands, Pitcairn Island (0.4–0.9 Ma; Duncan *et al.*, 1974) and Tahiti (0.2–1.4 Ma; Guillou *et al.*, 2005). The analysis based on fourfold-degenerated sites could not resolve the phylogenetic positions of these taxa, but analyses using the global mitochondrial rate suggested that reed-warblers may have colonized these islands not long after their emergence.

The dating analyses suggest that the diversification of the main Pacific reed-warbler lineages took place between the Mid-Pleistocene and the Late Pleistocene, a period that corresponds to an increase of glacial/interglacial contrasts and to a rearrangement of ocean circulation (Head & Gibbard, 2005). In the New Zealand region, evidence suggests that the southeast trade winds decreased and the westerlies increased during the Last Glacial Maximum, 20,000 years ago (Shulmeister *et al.*, 2004). Similar conditions during previous Pleistocene glacial periods could have facilitated the dispersion of organisms to the eastern parts of Oceania (Wright *et al.*, 2000).

Reverse colonization and the origin of Australian passerines

Until recently, the colonization of Pacific islands was predicted to be unidirectional from Australasia, even for organisms that did not lose the ability to disperse long distances (Mayr, 1941). This hypothesis was based on the assumptions that (1) small island populations produce fewer potentially dispersing individuals than larger continental populations, and (2) highly diverse mainland communities are more difficult to invade than islands with many 'empty' ecological niches (Elton, 1958; MacArthur & Wilson, 1967; Lodge, 1993; Mayr & Diamond, 2001). However, a number of recent phylogenies show evidence of reverse colonization (from island to continent), revealing continental lineages nested within insular clades (reviewed in Bellemain & Ricklefs, 2008). Within the Pacific region, two passerine lineages exhibit a reverse colonization of Australia. First, the monarchs (Monarchidae), a group that diversified in the Asian continent, dispersed to the Pacific archipelagos and subsequently recolonized Australia from the Solomon Islands (Filardi & Moyle, 2005). Second, the whistlers (Pachycephalidae) probably diverged *in situ* in Australia/New Guinea, then dispersed to Asia and to the Pacific archipelagos and then recolonized Australia from Melanesia (Jønsson *et al.*, 2009). The endemic reed-warbler of Australia nested within the insular Micronesian clade of *Acrocephalus* represents a third case of reverse colonization (Fig. 2).

Distribution of reed-warblers in the Pacific and the supertramp hypothesis

The distribution of reed-warblers in the Pacific is unique among land birds for including remote islands in eastern

Polynesia and Hawai'i but excluding the more species-rich archipelagos in southern Melanesia and central Polynesia. A large gap in their distribution is observed between the Caroline and Line islands, as reed-warblers are absent in the Marshall (a mention in Spennemann (2006) based on oral tradition is very speculative), Gilbert (except Nauru), Tuvalu, Phoenix and Tokelau islands. Two main hypotheses can be invoked to explain this gap. First, reed-warblers may have been extirpated on low small islands during high sea-level stands in the Pleistocene and Holocene, in the same way as certain reed-warbler populations have vanished in the Tuamotu Islands (Cibois *et al.*, 2010). Indeed, several atolls (maximum elevation 4 m a.s.l.) in the Gilbert, Phoenix and Tuvalu islands show traces of a 2.4-m highstand in the late Holocene, whereas in the Tuamotu Islands the same highstand did not reach more than 1 m a.s.l. (Grossman *et al.*, 1998; Dickinson, 1999). Such a vulnerability to high sea level could explain the absence of reed-warblers and more generally the low number of landbirds on these low-lying atolls (Baker, 1951). The alternative scenario for the absence of reed-warblers is extirpation by anthropic factors. Fossil or sub-fossil remains are known from only two localities, Tinian in the Mariana Islands (Steadman, 1999) and Majuro Atoll in the Marshall Islands (bones in deposits dated from 1st–11th centuries AD; M. Weisler, University of Queensland Australia, pers. comm., identified and cited in Steadman, 2006). These populations were never encountered by naturalists, suggesting extinction coincident with the arrival of humans in the islands. In historical times, local extinctions have been recorded in the Mariana, Line, Hawai'i, Society, Tuamotu and Gambier islands. However, investigations of 19th century naturalists were quite random, and many additional extinctions in the post-European era may have gone undetected. The known causes of extinction include habitat destruction in Hawai'i (Morin *et al.*, 1997) and in the Tuamotu archipelago (Cibois *et al.*, 2007b), and the introduction of predators such as the brown tree snake (*Boiga irregularis*) in Guam (Savidge, 1987). The black rat (*Rattus rattus*) is a threat to Pacific birds (Atkinson, 1985). Mosher (2006) recorded rat predation on songbird nests in the Mariana Islands, but in the Marquesas the introduction of the black rat more than a century ago on several islands, some small, has not resulted in a noticeable decline of reed-warblers (Thibault & Meyer, 2001; A. Cibois & J.-C. Thibault, pers. obs.). The impact of the Polynesian rat (*Rattus exulans*) on reed-warbler populations is uncertain, as the two coexist in large numbers on several islands, for instance on Henderson (Graves, 1992). In the Society Islands the causes of reed-warbler extinction remain unknown (Cibois *et al.*, 2008).

Notwithstanding known local extinctions, the distribution of reed-warblers in the Pacific displays some characteristics of the 'supertramp' concept introduced by Diamond (1974) to describe the ability of some bird species to colonize small and isolated islands. As noted above, reed-warblers occur on some of the most remote islands in the Pacific but are absent from archipelagos of the central Pacific with more diverse avifaunas (e.g. Fiji and Tonga). Diamond characterized supertramp

species by their high reproductive potential ('*r*-selection' using the terminology of MacArthur & Wilson, 1967), broad habitat tolerance (niche shifts; Diamond, 1970), and high over-water colonization rates 'enabling them to maintain populations (through frequent recolonisations) on islets too small to support self-sustaining populations in isolation, and also enabling them to be among the first species to colonise islands defaunated by volcanic eruptions' (Diamond *et al.*, 2009, p. 173). At a larger scale, Diamond (1975) and Mayr & Diamond (2001) defined species as supertramps on the basis of their island distributional pattern, that is, their absence from species-rich or large islands. The capability of the Pacific reed-warblers to thrive on very small and isolated islands, where resources are sparse, is consistent with the supertramp definition – they are indeed the only land bird on numerous low-lying atolls. However, Pacific reed-warblers also exhibit *K*-selected life history characteristics (MacArthur & Wilson, 1967) in that they have notably long life spans and are territorial, living in pairs or trios (Holyoak & Thibault, 1984; Mosher, 2006; Thibault & Cibois, 2006). In all likelihood, colonizations of remote islands by reed-warblers are rare events, occurring over long periods of time. It is also unlikely that small island populations are sustained by frequent recolonizations from large source populations under a 'source–sink' scenario. Thus, although Pacific reed-warblers meet some of the 'supertramp' criteria in their aptitude for colonizing remote archipelagos, their life history characteristics do not fit the model.

Taxonomic conclusions and perspectives

Results of the phylogenetic analyses indicate that *A. luscinius*, as currently defined (Dickinson, 2003; Bairlein *et al.*, 2006), is not monophyletic. We suggest recognizing three distinct species in the Mariana Islands: *A. luscinius* from Guam (the type locality); *A. hiwae* from Saipan (named in honour of Yamashina's assistant Minori Hiwa and erroneously indicated as *A. hivae* in Watson *et al.*, 1986); and *A. yamashinae* from Pagan. The taxon *nijoi* on Agiguan is probably best treated as a separate species but additional information is needed. Our genetic results also support the recognition of *A. rehsei* and *A. syrinx* as distinct species, as suggested earlier by Pratt *et al.* (1987) based on morphological characters. Although this phylogeny sheds light on the colonization pattern of reed-warblers in the Pacific, the mainland origin of the Pacific clade remains to be fully investigated. This will require the study of the numerous reed-warbler populations in the Indonesian and Melanesian region that have not yet been sampled, as well as additional populations of *A. orientalis* from Indonesia to Japan.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 *Acrocephalus* samples used in this study.
Appendix S2 Additional analysis with a denser sampling of *Acrocephalus* species to infer the position of the reed-warbler from Guam.

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BIOSKETCH

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Editor: Lawrence Heaney

Charting the course of reed-warblers across the Pacific islands

Alice Cibois, Jon S. Beadell, Gary R. Graves, Eric Pasquet, Beth Slikas, Sarah A. Sonsthagen, Jean-Claude Thibault and Robert C. Fleischer

Journal of Biogeography

Appendix S1 *Acrocephalus* samples used in this study. The classification follows Bairlein *et al.* (2006) except for the taxa endemic to the Society, Marquesas and Gambier that have been reviewed recently (Cibois *et al.*, 2007, 2008, 2011).

Sample number [a]	Taxon [b]	GenBank accession number			Locality
		ATP8*	ND2	cyt *b*	(island, archipelago)
AMNH 190.549	*A. aequinoctialis aequinoctialis*	HQ844271	EF156314	EF156277	Kiritimati (Christmas), Line Is.
AMNH 190.540	*A. aequinoctialis aequinoctialis*	HQ844270	EF156315	EF156278	Kiritimati (Christmas), Line Is.
USNM 493398	*A. aequinoctialis pistor*	HQ844272	HQ844381	HQ844333	Teraina (Washington), Line Is.
USNM 493399	*A. aequinoctialis pistor*	HQ844273	HQ844382	HQ844334	Teraina (Washington), Line Is.
NML 6092	*A. aequinoctialis pistor †*	HQ844274	HQ844383	HQ844335	Tabuaeran (Fanning), Line Is.
NML 6094	*A. aequinoctialis pistor †*	-	HQ844384	HQ844336	Tabuaeran (Fanning), Line Is.
MNHN 1982-769	*A. astrolabii †*	HQ844275	HQ844385	HQ844337	Mangareva, Gambier Is.
USNM 212496	*A. atyphus atyphus*	HQ844276	HQ844386	HQ844338	Fakarava, Tuamotu
MHNG PO2-52	*A. atyphus atyphus*	HQ844277	HQ844387	HQ844339	Tikehau, Tuamotu
USNM 33226	*A. australis australis*	HQ844278	HQ844388	HQ844340	East Australia
USNM 33227	*A. australis australis*	HQ844279	HQ844389	HQ844341	East Australia
USNM 33228	*A. australis australis*	HQ844280	HQ844390	HQ844342	East Australia
USNM RW1	*A. australis gouldi*	HQ844281	HQ844391	HQ844343	West Australia
USNM RW2	*A. australis gouldi*	HQ844283	HQ844393	HQ844345	West Australia
USNM RW16	*A. australis gouldi*	HQ844282	HQ844392	HQ844344	West Australia
USNM RW7	*A. australis gouldi*	HQ844285	HQ844395	HQ844347	West Australia
USNM RW34	*A. australis gouldi*	HQ844284	HQ844394	HQ844346	West Australia
KU 19430	*A. australis sumbae*	HQ844286	HQ844396	HQ844348	Guadalcanal, Solomon Is.
KU 19431	*A. australis sumbae*	HQ844287	HQ844397	HQ844349	Guadalcanal, Solomon Is.
MHNG PO0-1	*A. caffer*	HQ844288	EF156347	EF156307	Tahiti, Society Is.

MHNG PO0-3	*A. caffer*	HQ844289	EF156348	EF156308	Tahiti, Society Is.
USNM 189465	*A. familiaris familiaris †*	HQ844290	EU119963	HQ844350	Laysan, Hawai'i
USNM 231198	*A. familiaris familiaris †*	HQ844291	EU119964	HQ844351	Laysan, Hawai'i
USFWS 1600-19327	*A. familiaris kingi*	HQ844294	HQ844399	HQ844354	Nihoa, Hawai'i
USFWS 1600-19328	*A. familiaris kingi*	HQ844292	HQ844398	HQ844352	Nihoa, Hawai'i
USFWS 1600-19333	*A. familiaris kingi*	HQ844293	EU119962	HQ844353	Nihoa, Hawai'i
AMNH 594.897	*A. musae garretti †*	HQ844295	HQ844400	EU303306	Huahine, Society Is.
ZMH 7915	*A. musae garretti †*	HQ844296	HQ844401	EU303307	Huahine, Society Is.
UMB 7947	*A. musae musae †*	HQ844297	HQ844402	EU303310	Raiatea, Society Is.
UWBM 42807	*A. kerearako kerearako*	HQ844300	HQ844404	HQ844356	Mangaia, Cook Is.
AMNH 823.953	*A. kerearako kerearako*	HQ844301	EF156332	EF156292	Mangaia, Cook Is.
USNM 582291	*A. kerearako kaoko*	HQ844299	HQ844403	HQ844355	Mitiaro, Cook Is.
AMNH 823.954	*A. kerearako kaoko*	HQ844298	EF156331	EF156291	Mitiaro, Cook Is.
AMNH 190.398	*A. longirostris †*	HQ844302	HQ844405	EU303308	Mo'orea, Society Is.
AMNH 199.963	*A. longirostris †*	HQ844303	HQ844406	EU303309	Mo'orea, Society Is.
USNM 383057	*A. luscinius luscinius †*	HQ844305	HQ844408	HQ844358	Guam, Mariana Is.
USNM 383058	*A. luscinius luscinius †*	HQ844307	HQ844410	HQ844360	Guam, Mariana Is.
USNM 383059	*A. luscinius luscinius †*	HQ844304	HQ844407	HQ844357	Guam, Mariana Is.
USNM 385077	*A. luscinius luscinius †*	HQ844306	HQ844409	HQ844359	Guam, Mariana Is.
MNHN 1888-504	*A. luscinius luscinius †*	HQ844308	HQ844411	HQ844361	Guam, Mariana Is.
USNM 8101-37579	*A. luscinius hiwae*	HQ844309	HQ844412	HQ844362	Saipan, Mariana Is.
USNM 8101-37501	*A. luscinius hiwae*	HQ844310	HQ844413	HQ844363	Saipan, Mariana Is.
USNM 8101-37580	*A. luscinius hiwae*	HQ844311	HQ844414	HQ844364	Saipan, Mariana Is.
USNM 8101-37506	*A. luscinius hiwae*	HQ844312	HQ844415	HQ844365	Saipan, Mariana Is.
MNHN 1888-496	*A. luscinius yamashinae †*	HQ844313	HQ844416	HQ844366	Pagan, Mariana Is.
MNHN 1888-503	*A. luscinius yamashinae †*	HQ844314	HQ844417	HQ844367	Pagan, Mariana Is.
MHNG PO0-39	*A. mendanae mendanae*	HQ844315	EF156327	EF156287	Hiva Oa, Marquesas Is.
MHNG PO0-40	*A. mendanae mendanae*	HQ844316	EF156328	EF156288	Hiva Oa, Marquesas Is.
USNM GRG401	*A. percernis percernis*	HQ844318	HQ844418	HQ844368	Nuku Hiva, Marquesas Is.
MHNG PO0-25	*A. percernis percernis*	HQ844317	EF156339	EF156299	Nuku Hiva, Marquesas Is.
BMNH 1888.2.20.750	*A. rehsei*	HQ844319	-	HQ844369	Nauru Is.
USNM RR35	*A. rimitarae*	HQ844323	HQ844420	HQ844371	Rimatara, Austral
USNM RR30	*A. rimitarae*	HQ844322	HQ844419	HQ844370	Rimatara, Austral
MHNG PO0-22	*A. rimitarae*	HQ844320	EF156345	EF156305	Rimatara, Austral
MHNG PO0-23	*A. rimitarae*	HQ844321	EF156346	EF156306	Rimatara, Austral
USNM 212523	*A. syrinx*	HQ844327	HQ844424	HQ844375	Chuuk (Uala), Caroline Is.
USNM 212520	*A. syrinx*	HQ844326	HQ844423	HQ844374	Chuuk (Uala), Caroline Is.

USNM 212522	*A. syrinx*	HQ844328	HQ844425	HQ844376	Chuuk (Uala), Caroline Is.
USNM 212524	*A. syrinx*	HQ844324	HQ844421	HQ844372	Pohnpei (Ponape), Caroline Is.
USNM 388659	*A. syrinx*	HQ844325	HQ844422	HQ844373	Chuuk (Truk), Caroline Is.
USNM GRG312	*A. taiti*	HQ844329	HQ844426	HQ844377	Henderson Isl
USNM GRG345	*A. taiti*	HQ844330	HQ844427	HQ844378	Henderson Isl
USNM GRG380	*A. vaughani*	HQ844331	HQ844428	HQ844379	Pitcairn Isl
USNM GRG390	*A. vaughani*	HQ844332	HQ844429	HQ844380	Pitcairn Isl

Outgroups

	A. orientalis	-	-	AJ004785	
	A. stentoreus	-	-	AJ004253	
	A. arundinaceus	-	-	AJ004784	

[a] AMNH = American Museum of Natural History, New York, USA (specimen numbers); KU = Kansas University (specimen numbers); MHNG = Muséum d'Histoire Naturelle de Genève, Switzerland (tissue numbers); MNHN = Muséum National d'Histoire Naturelle, Paris, France (specimen numbers) ; NML = National Museums Liverpool, England (specimen numbers); BMNH = British Museum (Natural History), Tring, UK (specimen numbers); UMB = Übersee-Museum, Bremen, Germany (specimen numbers); USFWS = U.S. Fish and Wildlife Service (band numbers) USNM = U.S. National Museum, Washington, USA (specimen or tissue numbers); UWBM = University of Washington Burke Museum (specimen numbers); ZMH = Zoologisches Museum, Hamburg, Germany (specimen numbers).

[b] † extinct taxa

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Appendix S2 Additional analysis with a denser sampling of *Acrocephalus* species to infer the position of the reed-warbler from Guam. The phylogenetic tree is based on cytochrome *b* sequences only and estimated using Bayesian inference. Posterior probabilities > 0.95 are indicated with an asterisk. GenBank numbers are provided for all taxa (refer to Appendix S1 for the individual from Guam).