reduction and trichloroethylene oxidation were measured. The reduction and oxidation reactions were inversely sensitive to O_2 concentration (Fig. 2). Oxygen concentrations intermediate between atmospheric and anaerobic supported both reactions. Pentachloroethane metabolism to trichloroethylene and its subsequent disappearance was observed at 50% atmospheric oxygen. At atmospheric oxygen concentrations, trichloroethylene was a transient intermediate when anaerobic microenvironments were provided for pentachloroethane reduction as a result of incomplete mixing. Our results have implications for metabolic reactions in soil or bioreactors, where oxygen concentrations are typically subatmospheric.

Perhalogenated compounds containing chlorine and fluorine atoms are generally resistant to attack by microbial oxygenases, but 1,1,1-trichlorotrifluoroethane is reduced by cytochrome $P450_{cam}$ to yield 1,1,-dichlorodifluoroethylene[9]. The strains described here, which express toluene dioxygenase, oxidized 1,1-dichloro-2,2-difluoroethylene and 1,2-dichlorofluoroethylene (Table 1). Disappearance of 1,1-dichloro-2,2-difluoroethylene was accompanied by the formation of one equivalent of oxalic acid and two equivalents of fluoride (Table 2). Oxalic acid probably arises from substrate dioxygenation and subsequent *gem*[2] elimination and hydrolysis reactions. There were no products in incubations with *P. putida* G786, indicating that only toluene dioxygenase is responsible for substrate oxidation.

We have described the recruitment of enzymes and genes for catalysing dehalogenation reactions, complementing efforts to redesign the active site of cytochrome $P450_{cam}$ so that it can accommodate new substrates, including halocarbons[16,17]. Attempts to isolate a soil bacterium having the collection of genes found in *P. putida* G786 (pHG-2) were unsuccessful (E. Moe, M. S. P. Logan and L. P. W., unpublished results). This might reflect the comparatively recent entry of most polyhalogenated compounds into the natural environment. Evolutionary forces probably modify pre-existing bacterial genes to make new enzymes capable of metabolizing halocarbons. For example, 4-chlorobenzoate-coenzyme A dehalogenase, a hydrolytic dehalogenase, shares sequence similarity with the enoyl-coenzyme A hydratases that function in the β-oxidation of fatty acids[18]. Directed laboratory evolution, drawing on a knowledge of enzyme mechanisms and gene structure, can now be used to construct organisms for the breakdown of toxic and environmentally persistent organohalides. □

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Sympatric speciation suggested by monophyly of crater lake cichlids

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THE existence of sympatric speciation—that populations diverge into species in the absence of physical or ecological barriers—is controversial[1–6]. The East African Great Lakes harbour hundreds of cichlid species representing only a few monophyletic lineages[7,8], although palaeolimnological evidence[9–11] and local restrictions on species distribution[12] suggest that speciation in these lakes could have been allopatric[13,14]. The case for sympatry in restricted areas of Lakes Malawi and Tanganyika is stronger[15–17] but not unassailable. A better case might be made for cichlid species flocks in small, ecologically monotonous crater lakes. Here we present a mitochondrial DNA analysis of cichlid species flocks endemic to two such lakes in Cameroon. The results suggest that the flocks in each lake are monophyletic: the implication being that each lake was colonized once only, the size and shape of each lake being such that subsequent diversification would have been sympatric.

The two volcanic crater lakes, Barombi Mbo and Bermin in Cameroon (Fig. 1*a*), are extremely small (4.15 and 0.6 km^2) but harbour 11 and 9 endemic cichlid species, respectively. These species, which all belong to the tilapiines or tilapia-like cichlids, have been described on the basis of their morphological characters, including different breeding colorations[18,19]. Furthermore, the species status of most of these is supported by field observations documenting assortative mating, and by the failure to find intermediate morphotypes (hybrids) despite extensive sampling of the populations. Finally, the trophic and reproductive ecology are different for each of the species judged from field observations, stomach content analyses and specialized morphological features related to feeding (refs 18–20, and U.K.S., unpublished results).

Mitochondrial DNA sequences were used to differentiate between a polyphyletic and a monophyletic origin of the species flocks. Samples were collected from all 20 cichlid species inhabiting the lakes. To achieve an adequate representation of possible ancestral species, samples were also collected from all tilapiine species existing in the neighbouring river systems and lakes. Two species considered to be particularly likely to be related to species in the lake, *Sarotherodon galilaeus* and *Tilapia* (*Coptodon*) *guineensis*, were also collected from different riverine populations. Finally, *T. busumana*, which on morphological grounds represents an early divergence among tilapiine cichlids[21], was included in the analysis to provide an additional outgroup taxon.

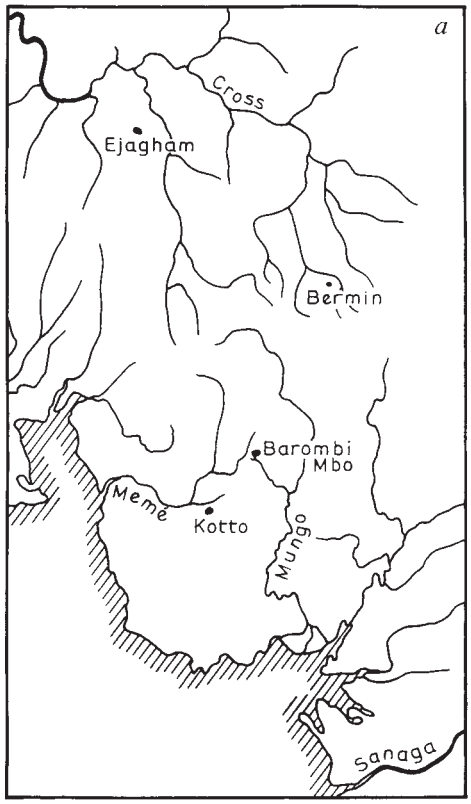
A 340-base-pair fragment of the mitochondrial cytochrome *b* gene was sequenced from each species. The distance matrix (Table 1) shows that the species of Barombi Mbo are closely related to one another, differing by 0–8 transitions and 0–4 transversions. These distances are within the range of differences found for the four individuals of *S. galilaeus* sampled from populations of the adjacent rivers. All other species exhibit more sequence differences when compared with the species of Barombi

TABLE 1 Pairwise comparisons of transitions/transversions in cichlid mtDNAs

mtDNAs	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1 *St. mariae*	—	0	2	1	2	1	1	1	0	0	0	0	0	4	10	10	10	11	10	9	9	3	10	10
2 *St. pindu*	3	—	2	1	2	1	1	1	0	0	0	0	0	4	10	10	10	11	10	9	9	3	10	10
3 *St. mongo*	3	2	—	3	4	3	3	3	2	2	2	2	2	6	12	12	12	13	12	11	11	5	10	10
4 *S. linnellii/caro*	4	5	3	—	1	0	0	2	1	1	1	1	1	5	11	11	11	12	11	10	10	4	11	11
5 *M. myaka*	3	4	2	1	—	1	1	3	2	2	2	2	2	6	12	12	12	13	12	11	11	5	12	12
6 *S. steinbachi*	5	6	4	3	2	—	0	2	1	1	1	1	1	5	11	11	11	12	11	10	10	4	11	11
7 *S. lohbergeri*	3	4	2	1	0	2	—	2	1	1	1	1	1	5	11	11	11	10	11	10	10	4	11	11
8 *P. maclareni*	7	8	6	7	6	8	6	—	1	1	1	1	1	5	9	9	9	10	9	8	8	4	9	11
9 *K. dikume*	7	8	6	7	6	8	6	8	—	0	0	0	0	4	10	10	10	11	10	9	9	3	10	10
10 *K. eisentrauti*	6	7	5	6	5	7	5	7	1	—	0	0	0	4	10	10	10	11	10	9	9	3	10	10
11 *S. gal. 'Meme/Cross'*	5	4	2	5	4	6	4	8	6	5	—	0	0	4	10	10	10	11	10	9	9	3	10	10
12 *S. gal. 'Ejagham'*	4	5	3	4	3	5	3	7	5	4	1	—	0	4	10	10	10	11	10	9	9	3	10	10
13 *S. gal. sanagaensi*	12	13	11	12	11	13	11	13	13	12	9	8	—	4	10	10	10	11	10	9	9	3	10	10
14 *S. m. melanother*	23	24	22	21	22	24	22	24	24	25	22	21	26	—	10	10	10	11	10	9	9	5	10	10
15 *T. Bermin A*	36	38	36	36	37	39	37	40	38	37	38	36	40	35	—	0	0	1	0	3	1	9	14	12
16 *T. Bermin B*	36	38	36	36	37	39	37	40	38	37	38	36	40	35	2	—	0	1	0	3	1	9	14	12
17 *T. guineensis 'Cross'*	35	35	33	35	36	38	36	39	37	36	35	35	39	34	5	3	—	1	0	3	1	9	14	12
18 *T. kottae*	34	36	34	34	35	37	35	38	38	37	36	34	38	33	8	6	5	—	1	4	2	10	15	13
19 *T. 'Ejagham'*	34	34	32	34	35	37	35	38	38	37	34	34	38	33	6	4	1	3	—	3	1	9	14	12
20 *T. zillii*	35	38	36	36	37	39	37	40	40	39	38	36	42	39	15	13	12	11	11	—	2	8	13	11
21 *T. g. 'Ivory Coast'*	33	35	33	33	34	36	34	37	35	36	35	33	35	35	23	21	20	19	19	22	—	8	13	11
22 *O. nil. vulcani*	27	28	28	27	28	30	28	26	28	27	28	26	28	32	34	34	33	32	32	31	31	—	11	11
23 *T. mariae*	32	33	32	33	32	34	32	34	36	35	35	33	33	32	41	41	40	37	39	40	38	33	—	12
24 *T. busumana*	41	42	42	40	41	43	41	45	40	41	42	41	46	41	42	44	43	42	44	44	38	43	41	—

The numbers of transversion differences are given above the diagonal and the numbers of transition differences below the diagonal for a 340-bp fragment of the mitochondrial cytochrome *b* gene. Species 1–10 from Barombi Mbo; 15 and 16 from Bermin.

Mbo (21–45 transitions, 3–13 transversions). In Bermin, all representatives of the nine species sampled show one of two cytochrome *b* sequences. These sequences differ from each other by two transitions and no transversions. When compared with populations of *Tilapia* (*Coptodon*), the Bermin species differ by 3–23 transitions and 0–3 transversions. For all other species, the Bermin sequences exhibit more differences (34–44 transitions, 9–14 transversions). Phylogenetic analysis of all species confirms that the Barombi Mbo species are closely related to one another and to the *S. galilaeus* taxa (Fig. 2*a*), and that the Bermin species



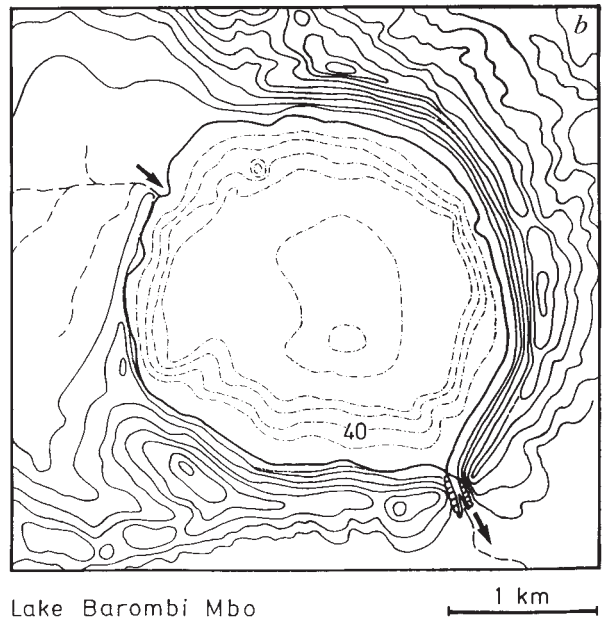
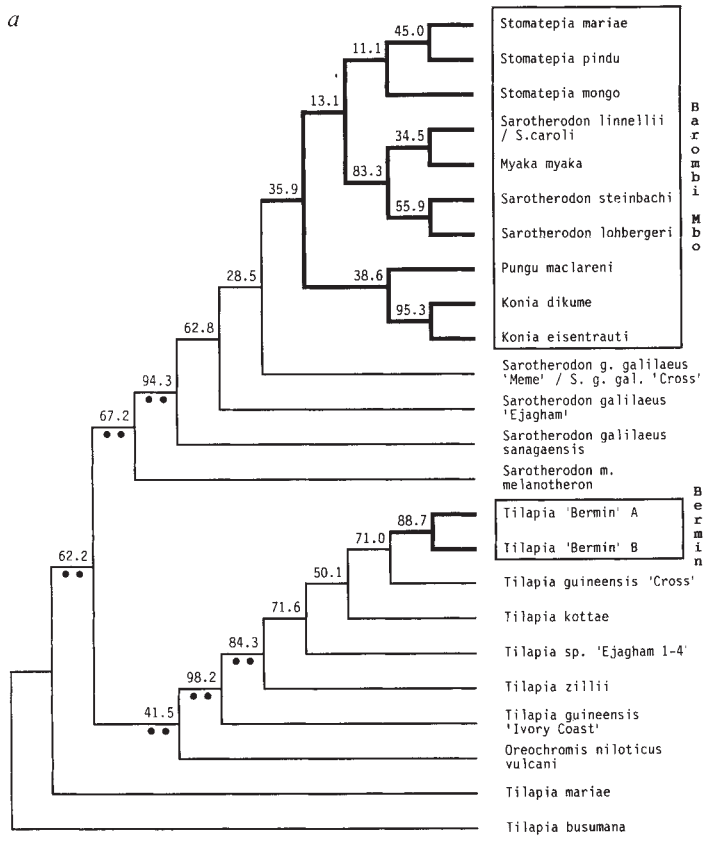
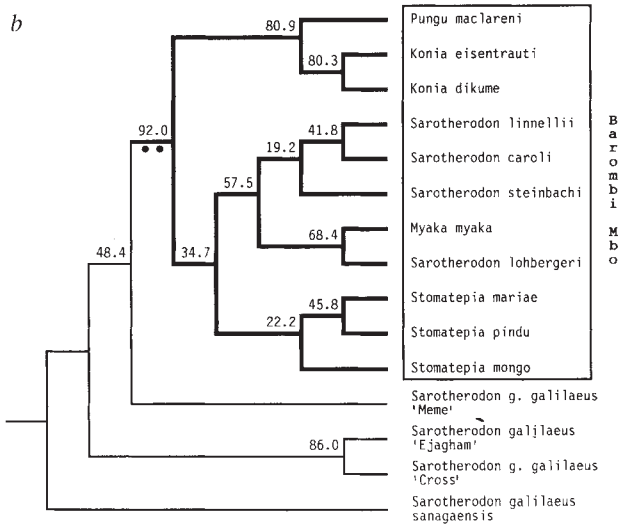


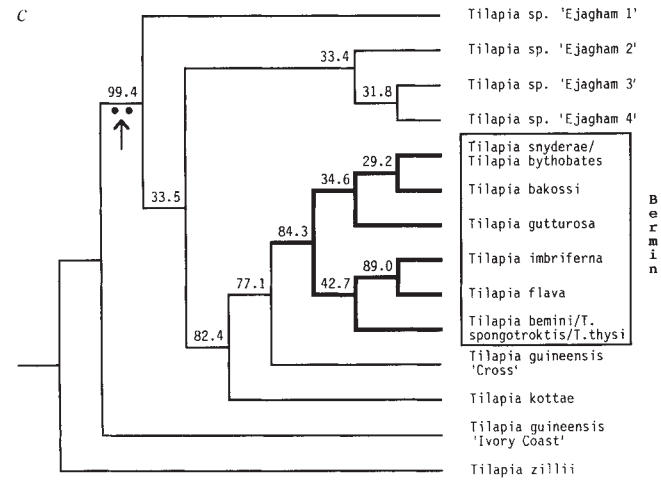
FIG. 1 Maps of *a*, southwestern Cameroon and *b*, Lake Barombi Mbo. The craterlakes from which cichlid samples were analysed are shown as well as river systems: Lake Barombi Mbo nowadays drains into the Mungo, but is believed to have drained into Memé River in the past when no connection to the Mungo existed[18]. Lake Bermin lacks an inflow and is connected through an outflow with the Cross River System, as is Lake Ejagham. Below, a topographical map of Lake Barombi Mbo shows the homogeneous shoreline below and above water, as well as the crater rims. The small inflow to Lake Barombi Mbo lacks connection to other river systems. The areas of Lake Barombi Mbo and Bermin are 4.15 km^2 and 0.6 km^2, respectively, and their depths are 110 m and 14.5 m, respectively[31]. But, in Lake Barombi Mbo there are fish only to ~40 m depth, where the oxygenated layer ends. Inflows and outflows are marked by arrows. Maps were drawn from the 1:50,000 topographical map of Cameroon and the depth from ref. 32 for Lake Barombi Mbo.

FIG. 2 Phylogenetic trees relating the tilapiine cichlids of Barombi Mbo and Bermin with all tilapiine cichlids of adjacent rivers and lakes and tilapiine outgroups from neighbouring regions[33,34]. *Sarotherodon galilaeus galilaeus* is represented by individuals from three populations ('Meme', 'Cross' and 'Ejagham'; despite several attempts, no S. *g. galilaeus* could be found in Mungo, where it is apparently not present). *Sarotherodon galilaeus sanagaensis* is the geographically closest subspecies different from S. *g. galilaeus*. S. *melanotheron* is the second *Sarotherodon* species in the region. The subgenus *Coptodon* is represented by *T. guineensis* from the Cross River and, as an outgroup, by a sample from Bia-Dam Lake in Ivory Coast; *T. kottae* from Lake Kotto in the Memé-River drainage, and all four tentative species from Lake Ejagham in the Cross River drainage (undescribed, except *T. deckerti*, which is *T. spec. 'Ejagham 4'*). *T. mariae* represents the single species of the subgenus *Pelmatolapia* in the area, *Oreochromis niloticus* is included because it inhabits the Niger river, which might have once been connected with the Cross River. *Tilapia busumana* from Lake Bosumtwi, Ghana, represents a basal tilapiine lineage for outgroup comparison. *a*, A tree relating all species based on 340 bp of cytochrome *b* sequence. Lineages shared among species in Barombi Mbo and Bermin are shown in bold. In Bermin, all nine species carried one of two cytochrome *b* sequences and in Ejagham all four tentative species carried one sequence. Further lineages found in more than one species are indicated by the localities the respective populations were sampled. *b*, Tree for Barombi Mbo and relevant outgroups. *c*, Tree for Bermin with other members of the *Coptodon* subgenus. *b* and *c* are based on the cytochrome *b* sequences as well as ~350 bp of control region sequences. Dots denote internal branches for which the likelihood confidence intervals are significantly positive ($P<0.01$) and exclude zero. The arrow denotes a 9-bp-deletion in the control region sequence which is shared by the Bermin species and *T. guineensis 'Cross'*, *T. kottae* and the Ejagham species. In addition, the species of Bermin share an amino-acid substitution at position 32 of cytochrome *b* which was not found in the other species analysed nor in any other cytochrome *b* sequence determined so far.

METHODS. Fishes were collected and stored in ethanol. DNA was extracted as described in ref. 35. Extractions with no tissue present were done to control for contamination by extraneous DNA. Amplifications were performed as in ref. 36. Primers used for cytochrome *b* were L14724 (ref. 7), H15149 (ref. 37), and for the control region: (Uli71L) 5′-TACCCCTAGCTCCCAAAGCT-3′ and (Uli70H) 5′-TGGTGGGCTCTTATC-ACATT-3′. The double-stranded amplification product was sequenced as in ref. 38. Sequences were analysed with the PHYLIP package[39]. The consensus of 1,000 minimum mutation trees constructed from bootstrap replications[40] of the alignable parts of the sequences are shown. Transitions and transversions were weighted equally. Numbers refer to the frequency with which the respective branches occurred among the bootstrap replications (alignments available from U.K.S.). When maximum likelihood trees for the same sequences were constructed, the only topological changes from the minimum mutation trees were three internal branches in the Barombi Mbo group.







are likewise closely related to one another and the subgenus *Coptodon*. Thus, in contrast to previous assumptions[18], the tree suggests that the species flocks of both lakes are monophyletic.

In addition, about 350 base pairs of the rapidly evolving mitochondrial control region were sequenced from all crater lake species as well as all available members of the *S. galilaeus* and *Tilapia* (*Coptodon*) groups. The control region data were included in the analysis and the most parsimonious trees reconstructed (Fig. 2*b* and *c*). The topologies of these trees are nearly identical to the one based on cytochrome *b* alone (Fig. 2*a*) and show the species of Barombi Mbo as well as Bermin to be monophyletic with respect to the river species. For Barombi Mbo, bootstrap replications as well as likelihood estimates support the monophyly of the species flock. The phylogenetic results agree with the generic designations introduced by Trewavas[18], except that *Pungu maclareni* was assumed[18] to be derived from a distantly related tilapiine lineage[18,22]. For Bermin, *T.* (*Coptodon*) *zillii* and *T.* (*C.*) *guineensis* '*Ivory Coast*' are excluded as sister taxa of the species flock by the tree analysis as well as by the occurrence of a deletion shared by the Bermin taxa and *T.* (*Coptodon*) *guineensis* '*Cross*' and *T.* (*C.*) *kottae*. The identification of *T.* (*Coptodon*) *guineensis* '*Cross*' as the sister taxon to the species flock is suggested by bootstrap as well as maximum likelihood analyses of the complete data set and agrees with expectations from biogeography (Fig. 1). On the other hand, *T.* (*C.*) *kottae* and the *Coptodon*-related cichlids of Lake Ejagham could not be excluded as sister-taxa. The monophyly of the Bermin lake flock is further supported by the presence of glycine at position 32 in the cytochrome *b* sequence, where all other species have an asparagine residue. This unusual amino-acid replacement, which involves a change in charge, has thus become fixed in the Bermin species flock, presumably in the small population that colonized the lake and which subsequently gave rise to the species radiation.

The molecular data show that the species flocks in each lake are monophyletic, and suggest that they evolved within each lake after a single colonization event. An alternative explanation—allopatric origin for all 20 species—implies the independent colonization of the lakes with subsequent extinction of the source river populations—an extremely unlikely scenario. The results might also be explained by the colonization of the lakes by several species, each with different mitochondrial (mt) DNA types. If these species could hybridize, selection for and fixation of a particular type in each lake is conceivable. We find this explanation unlikely for two reasons. First, that the same events would have occurred independently in each lake seems implausible. Second, the mtDNA types cluster in the phylogenetic trees according to the ecological characteristics of the species concerned.

There are good reasons to assume that any cichlid population in these lakes lives in sympatry *sensu ref. 1*, that is that individuals of the populations are physically capable of encountering one another with at least moderately high frequency. According to this definition, a population is sympatric even if individuals are ecologically segregated, as long as a fairly high proportion of them encounters the others along ecotones. Cichlid populations within the lakes live in sympatry, because, first, the lakes are too small to restrict gene flow to certain areas among highly mobile organisms such as tilapiine cichlids. Second, the habitats along the shorelines are uniform and free of physical barriers (Fig. 1*b*)[18,19]. Therefore no microgeographical barriers exist within the lakes which would have allowed separation of microallopatric subpopulations. Third, the calderas have a uniformly conical shape so that past lake-level fluctuations could not have produced separate basins. Fourth, the lakes are isolated from the surrounding river systems by crater rims, penetrated only by small creeks. Gene flow from outside the lake is thus restricted. The isolated nature of many crater lakes in Cameroon is impressively illustrated by the complete absence of fish in some lakes located at similar altitude as Barombi Mbo and Bermin, such as Lake Benakuma[23]. Finally, the species that are either benthically or pelagically oriented in their feeding behaviour both breed close to the lake bottoms. Therefore, even if there were microgeographical sorting of subpopulations based on feeding behaviour, mating would still take place in sympatry.

Most models for sympatric speciation[6,24–30] assume a genetically based ecological diversification as a prerequisite for the evolution of increased assortative mating among different ecotypes leading to reproductive isolation. Interestingly, the basal branches of the phylogenetic trees cluster ecological groups within the lake flocks. In Bermin, the two basal lineages of the flock separate the pelagic planktivorous species from the substrate oriented feeders. In Barombi Mbo, the sequence analysis identifies three ecological groups, one containing the predatory genus *Stomatepia*, another the fine-particle feeders of the genera *Sarotherodon* and *Myaka*, and a third specialists of the genera *Konia* and *Pungu*, the latter being a sponge-feeder, for example. Thus, ecological diversification may have been a key factor responsible for speciation after colonization. □

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