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Systematics of spiny-backed treefrogs (Hylidae: *Osteocephalus*): an Amazonian puzzle

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Spiny-backed tree frogs of the genus *Osteocephalus* are conspicuous components of the tropical wet forests of the Amazon and the Guiana Shield. Here, we revise the phylogenetic relationships of *Osteocephalus* and its sister group *Tepuihyla*, using up to 6134 bp of DNA sequences of nine mitochondrial and one nuclear gene for 338 specimens from eight countries and 218 localities, representing 89% of the 28 currently recognized nominal species. Our phylogenetic analyses reveal (i) the paraphyly of *Osteocephalus* with respect to *Tepuihyla*, (ii) the placement of '*Hyla*' *warreni* as sister to *Tepuihyla*, (iii) the non-monophyly of several currently recognized species within *Osteocephalus* and (iv) the presence of low (<1%) and overlapping genetic distances among phenotypically well-characterized nominal species (e.g. *O. taurinus* and *O. oophagus*) for the 16S gene fragment used in amphibian DNA barcoding. We propose a new taxonomy, securing the monophyly of *Osteocephalus* and *Tepuihyla* by rearranging and redefining the content of both genera and also erect a new genus for the sister group of *Osteocephalus*. The colouration of newly metamorphosed individuals is proposed as a morphological synapomorphy for *Osteocephalus*. We recognize and define five monophyletic species groups within *Osteocephalus*, synonymize three species of *Osteocephalus* (*O. germani*, *O. phasmatus and O. vilmae*) and three species of *Tepuihyla* (*T. celsae*, *T. galani and T. talbergae*) and reallocate three species (*Hyla helenae* to *Osteocephalus*, *O. exophthalmus* to *Tepuihyla* and *O. pearsoni* to *Dryaderces* gen. n.). Furthermore, we flag nine putative new species (an increase to 138% of the current diversity). We conclude that species numbers are largely underestimated, with most hidden diversity centred on widespread and polymorphic nominal species. The evolutionary origin of breeding strategies within *Osteocephalus* is discussed in the light of this new phylogenetic hypothesis, and a novel type of amplexus (gular amplexus) is described.

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Introduction

Treefrogs of the genus *Osteocephalus* constitute an important component of the amphibian fauna of the Amazonian and Guianan regions of South America. Their distributions range from the coastal areas of Venezuela and the Guianas south to central Brazil (Mato Grosso) and central Bolivia and from north-eastern Brazil (Piauí) west to the eastern Andean slopes from Colombia to Bolivia. Their altitudinal distribution ranges from sea level up to about 2000 m a.s.l., although most species are restricted to the lowlands (Trueb & Duellman 1971; Jungfer 2010, 2011; La Marca *et al.* 2010a,b; Ron *et al.* 2010). They are inhabitants of primary or old secondary forests in most of their range (with a few species also present in human-modified landscapes), entering some gallery forests in the drier habitats especially at the edge of their ranges (Trueb & Duellman 1971; Lynch 2006; La Marca *et al.* 2010b; Valdujo *et al.* 2012).

Species of *Osteocephalus* are diverse in their use of different kinds of water bodies for reproduction and exhibit a variety of reproductive modes. There are species that deposit eggs in lentic water, others in lotic waters, and some deposit their eggs in water-holding plant leaf axils and tree holes and exhibit various degrees of parental care (Jungfer & Weygoldt 1999; Jungfer *et al.* 2000; Moravec *et al.* 2009; KHJ, personal observation). Males of most species exhibit tuberculate dorsal skin, with tubercles often tipped by dark, heavily keratinized spicules in breeding males (hence, spiny-backed treefrogs), while the females exhibit a more or less smooth dorsum. Generalized omnivorous stream and pond-dwelling tadpoles of *Osteocephalus* share similar numbers of tooth rows (Trueb & Duellman 1970; Henle 1981; Hero 1990; Ron *et al.* 2010; Menin *et al.* 2011), while phytotelm adapted larvae, at least in two cases, are highly specialized feeders of conspecific nutritive eggs and have reduced numbers of labial tooth rows (Jungfer & Schiesari 1995; Schiesari *et al.* 1996; KHJ, personal observation).

The genus *Osteocephalus* was originally coined by Fitzinger (1843) in combination with the specific epithet *taurinus*, but he neither designated nor illustrated any specimen, so both names are *nomina nuda*. Steindachner (1862) reused both names, described *Osteocephalus taurinus*, and defined *Osteocephalus*. Cope (1867, 1874), Goin (1961) and Trueb (1970) included more species. The first and only thorough revision of the genus performed to date is that of Trueb & Duellman (1971). Subsequently, numerous species were described and named, rearranged to or from other genera, or resurrected (Duellman 1974; Henle 1981, 1992; Martins & Cardoso 1987; Duellman & Mendelson 1995; Jungfer & Schiesari 1995; Ron & Pramuk 1999; Jungfer *et al.* 2000; Jungfer & Lehr 2001; Smith & Noonan 2001; Jungfer & Hödl 2002; Lynch 2002, 2006; Faivovich *et al.* 2005; MacCulloch & Lathrop 2005; Wiens *et al.* 2006; Moravec *et al.* 2009; Jungfer 2010, 2011). Six species (*O. aecii*, *O. edelcae*, *O. galani*, *O. luteolabris*, *O. rimarum* and *O. rodriguezi*) from the Venezuelan Guiana Shield were transferred to a new genus, *Tepuihyla* (Ayarzagüena *et al.* 1993). Although a combination of characters distinguishes *Tepuihyla* from *Osteocephalus*, no putative phenotypic synapomorphy has yet been reported. Thus, the generic assignment of several *Osteocephalus*-like species from the Guiana Shield has been problematic (e.g. *O. exophthalmus* Smith & Noonan, 2001; *Tepuihyla talbergae* Duellman & Yoshpa, 1996). More recently, Salerno *et al.* (2012) have shown that *Osteocephalus* might not be monophyletic because *O. exophthalmus* is the sister taxon of *Tepuihyla*.

Osteocephalus is plagued with species-level taxonomic problems. Several reasons make species taxonomy in this genus particularly difficult. One of them is a drastic ontogenetic colour change between recently metamorphosed juveniles and adults, with most juveniles having bright red eyes and light areas on upper arm, elbow, knee and heel (Appendix S1), while adults have golden to brown irises with ornamentation consisting of venation or radiating lines. As a result of these differences, *Osteocephalus mimeticus* (Melin, 1941) was named twice as new species of *Hyla* on the basis of juveniles (Jungfer 2010). Apart from the ontogenetic changes in *Osteocephalus*, sexual dimorphism in dorsal skin structure makes males and females look distinctly different in some species. In collections, we have frequently seen females of different species grouped under one name and males under a different one. A third source of misidentification is the considerable morphological variation within apparently widespread species, for example in *O. taurinus* (Trueb & Duellman 1971). On the other hand, diagnostic morphological characters useful to distinguish some species, such as amount of webbing, tuberculation or maximum snout–vent length (SVL), appear to be insufficient to identify putative closely related species such as those of the *Osteocephalus buckleyi* complex (Jungfer 2010). This taxonomic conundrum casts doubts on our ability to develop an accurate and complete taxonomy of *Osteocephalus* on the basis of currently available morphological data alone and encourages the use of other types of data. Indeed, the use of different lines of evidence (e.g. behaviour, bioacoustics, ecology, morphology, molecules) in alpha taxonomy, although not new (see the works of Myers & Daly 1976a,b, 1979, 1980), has recently gained attention because of its potential to solve long-standing taxonomic problems (e.g. Köhler *et al.* 2010; Padial & De la Riva 2010; Brown *et al.* 2011; Castroviejo-Fisher *et al.* 2011).

The phylogenetic relationships within *Osteocephalus* remain poorly understood. Faivovich *et al.* (2005) presented a phylogenetic analysis of the hylid subfamily Hylinae. Their results indicated the polyphyly of *Osteocephalus*, inasmuch as *O. langsdorffii*, the only species of the genus present in the Atlantic Forest of south-eastern Brazil, was not related to the other four species. On the basis of these results, Faivovich *et al.* (2005) erected the new monotypic genus *Itapotihyla* for *O. langsdorffii* and continued with the recognition of *Osteocephalus* for the remaining species. *Tepuihyla edelcae* was found to be sister taxon to *Osteocephalus* and that clade in turn was found to be sister to *Osteopilus*. Two species in this analysis (*O. leprieurii* and *O. oophagus*) turned out to be misidentified (Moravec *et al.* 2009), although this fact was irrelevant for their conclusions.

Wiens *et al.* (2006) combined most sequences generated by Faivovich *et al.* (2005) with those of Wiens *et al.* (2005), added 12S sequences of a few other hylid species and obtained a clade composed of *Osteocephalus cabrerai*, *O. buckleyi*, *Osteocephalus mutabor* and *Osteocephalus verruciger*, a clade composed of *O. oophagus* + *O. taurinus* and a clade composed of *O. leprieurii* and *Osteocephalus planiceps*. An important point of their results was that '*Hyla*' *alboguttata*, a species considered *incertae sedis* by Faivovich *et al.* (2005), was embedded within *Osteocephalu*s. The contributions by Wiens *et al.* (2006) and Moen & Wiens (2009) represented increasingly stringent tests of the monophyly of *Osteocephalus*.

Phylogenetic analyses presented by Moravec *et al.* (2009), Ron *et al.* (2010) and Wiens *et al.* (2010) and Pyron & Wiens (2011) added more information on the relationships of *Osteocephalus*. Salerno *et al.* (2012) recently presented a phylogenetic analysis of *Tepuihyla* and included several species of *Osteocephalus* as outgroups. A more in-depth analysis of genetic diversity among tepui dwelling vertebrates, including frogs currently referred to *Osteocephalus* and *Tepuihyla*, additionally sheds light on their genetic diversity (Kok *et al.* 2012). Ron *et al.* (2012) presented an analysis of north-western Amazonian stream-breeding species called the *O. buckleyi* species complex by them and described three new species. In this paper, we present the first densely sampled phylogenetic analysis of *Osteocephalus*. The goals of this study are to (i) test the monophyly of *Osteocephalus* and identify its sister group, (ii) explore relationships among its species, (iii) elucidate the potential number of unnamed species in the genus on the basis of molecular data, (iv) develop a monophyletic taxonomy and (v) study the evolution of the various reproductive modes observed in the group in the context of our phylogenetic hypothesis.

Materials and methods

Taxon sampling and terminology

In a combined effort by numerous colleagues, we tried to obtain as many samples as possible from throughout the range of the genus. We obtained tissue samples from Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Peru and Venezuela; thus covering most of the Guiana Shield, Amazonia and the north-western part of the Brazilian Cerrado where the genus occurs. Nevertheless, not all of the countries have been sampled sufficiently yet. Data are lacking especially for parts of Colombia, Amazonian Venezuela and Brazil. All in all, we included sequences of up to 6134 bp of nine mitochondrial and one nuclear gene from 338 individuals, including 25 of the 28 presently known species of *Osteocephalus*, plus a large number of unidentified specimens. The species for which tissues are unavailable for this study are *Osteocephalus duellmani*, *Osteocephalus inframaculatus* and *Osteocephalus vilarsi* which are known only from their type material. We also included six species of *Tepuihyla* (*T. aecii*, *T. edelcae*, *T. galani*, *T. rodriguezi*, *T.* sp. and *T. talbergae*), and '*Hyla*' *warreni*, a species that could not be associated with any of the genera recognized by Faivovich *et al.* (2005), because preliminary analyses and morphological data suggest that it could be related to the *Osteocephalus* + *Tepuihyla* clade (e.g. Kok *et al.* 2012). '*Hyla*' *warreni* was recently considered a species of *Tepuihyla* by Aubrecht *et al.* (2012) without mentioning any supporting evidence. As outgroups, we included exemplars of several genera of Lophiohylini, and *Myersiohyla kanaima*, of Cophomantini, was used to root the trees, following the results of a previous phylogenetic analysis (Faivovich *et al.* 2005). Newly generated sequences are deposited in GenBank under accession numbers KF001880-KF002004 and KF002006-KF002249. For a list of species, voucher specimens, localities and detailed GenBank accession numbers of the data used in the phylogenetic analyses, see Appendix S2. Unnamed species are referred to as candidate species following the terminology of Vieites *et al.* (2009): for unnamed species for which evidence is conclusive, we use the term Confirmed Candidate Species (CCS), and when evidence is inconclusive (i.e. mostly molecular data only), we use the term Unconfirmed Candidate Species (UCS). We follow the system of Padial *et al.* (2010) to refer to particular candidate species, where the candidate species name results from the combination of the binomial species name of the most similar or closely related nominal species, followed (in square brackets) by the abbreviation 'Ca' (for candidate) with an attached numerical code referring to the particular candidate species (e.g. Ca1, Ca2), and terminating with the GenBank accession numbers of one of the sequences that revealed the putative species.

Character sampling and laboratory protocols

The mitochondrial gene sequences produced for this project include portions of cytochrome oxidase I (COI), cytochrome *b* (cyt*b*), 12S, two non-overlapping fragments of the 16S, NADH dehydrogenase subunit 1 (ND1) and the intervenings tRNA^{Val}, tRNA^{Leu} and tRNA^{Ile}. The primers employed are the same used by Faivovich *et al.* (2005), with the addition of AnF1 (ACHAAYCAYAAAGAYATYGG) - AnR1 (CCRAARAATCARAADARRTGTTG) for cytochrome oxidase I designed by MLL, and 16S-frog and tMet-frog for the fragment containing the downstream fragment of 16S, RNA^{Leu}, ND1 and tRNA^{Ile} (Wiens *et al.* 2005). We also included sequences of these genes and of the mitochondrial control region and the nuclear gene pro-opiomelanocortin (POMC) recently produced by Kok *et al.* (2012), Ron *et al.* (2012), Salerno *et al.* (2012), and for outgroups, Faivovich *et al.* (2005, 2010), Wiens *et al.* (2005).

DNA extraction, amplification and sequencing methods are those described in a recent paper by Blotto *et al.* (2013). All samples were sequenced in both directions. Chromatograms obtained from the automated sequencer were read and contigs made using the sequence editing software SEQUENCHER 3.0. (Gene Codes, Ann Arbor, MI, USA). Complete sequences were edited with BioEdit (Hall 1999).

Phylogenetic analyses

The phylogenetic analyses included treatment of sequences both as dynamic homologies (simultaneous tree searches and alignment) and as static homology hypotheses. The consideration of sequences as dynamic homologies simultaneously with tree searches has been discussed and justified by Wheeler (1996, 2002) and De Laet (2005). Static alignments (multiple alignments) independent of tree searches are the most common procedure in molecular phylogenetics, regardless of the omnipresent and always ignored problem of the lack of an optimality criterion to choose among competing alignments. We also performed a multiple sequence alignment and analysed it using both maximum parsimony (MP) and likelihood (ML) inference.

The rationale for using parsimony as an optimality criterion was advanced by Farris (1983) and discussed, among others, by Goloboff (2003), Goloboff & Pol (2005) and Kluge & Grant (2006), Grant & Kluge (2009) and Wheeler (2012) for its conceptualization in a dynamic homology framework. Within this framework, the phylogenetic analysis under direct optimization was performed with POY4.1.1 (Varon *et al.* 2009), using equal weights for all transformations (substitutions and insertion/deletion events). Sequences of 12S, 16S and intervening tRNA^{Val} were preliminarily delimited in sections of putative homology (Wheeler *et al.* 2006), and equal-length sequences of protein-coding genes were considered as static alignments to accelerate the searches. Searches were performed using the command ‘Search’. This command implements a driven search building Wagner trees using random addition sequences (RAS), Tree Bisection and Reconnection (TBR) branch swapping followed by Ratchet (Nixon 1999), and Tree Fussing (Goloboff 1999). The command (Search) stores the shortest trees of each independent run and performs final tree fusing using the pooled trees as a source of topological diversity. Two 144-h runs of Search were implemented in parallel at the American Museum of Natural History Cluster using 32 processors. The resulting trees were submitted to a final round of swapping using iterative pass optimization (Wheeler 2003a). Parsimony Jackknife (Farris *et al.* 1996) absolute frequencies were estimated from the implied alignment (Wheeler 2003b) with T.N.T., Willi Hennig Society Edition (Goloboff *et al.* 2008), generating 50 RAS + TBR per replicate, for a total of 1000 replicates. Tree edition was performed with Winclada (Nixon 2002), and character optimizations and reconstructions with T.N.T.

We performed a multiple sequence alignment with CLUSTALW (Thompson *et al.* 1997) under default parameters. For the phylogenetic analysis using parsimony, we employed T.N.T. Willi Hennig Society Edition (Goloboff *et al.* 2008). Tree searches were performed with a driven new technology search, using 100 as the initial level. The strategy included sectorial searches, tree drift and tree fusing (Goloboff 1999). The driven search was requested to hit the minimum length 500 times. Gaps were considered as a fifth state. Parsimony Jackknife estimation was performed as performed with the implied alignment. Trees were edited with Winclada (Nixon 2002).

Maximum likelihood analyses were performed on the static alignment with 16 partitions (see Appendix S3). All partitions were analysed simultaneously and unlinked, and model parameters were optimized during tree search. We used the program JMODELTEST 1.0 (Posada 2008) under ML to select the model of nucleotide evolution for each partition according to the Akaike Information Criterion (Akaike 1974). Maximum likelihood analyses were performed in GARLI 2.0 (Zwickl 2006; available at http://www.nescent.org/informatics/download.php?software_id=4). The following parameter values differ from default conditions and were modified to improve tree search intensity following Zwickl (2006): 5 000 000 generations each replicate (stopgen = 5 000 000), with random starting addition (streefname = random), 677 attachment per taxon (attachmentspertaxon = 677), a threshold of 40 000 generations without topology improvement for termination (genthreshfortopoterm = 40 000), and a threshold of 30 for the maximum number of branches away from its original location for a branch to be reattached during subtree pruning and regrafitting (limsprrange = 30). We did a total of 100 independent searches to reduce the probability of inferring a suboptimal likelihood solution. Node support was assessed by 1000 bootstrap pseudoreplicates under the same search conditions explained above. Sequence variation of the static alignment of 551 characters of 16S gene, corresponding to the most used DNA barcode in amphibians (e.g. Vieites *et al.* 2009; Crawford *et al.* 2010), was assessed with uncorrected proportional distances (*p*-distances) calculated in PAUP* 4.0b10 (Swofford 2002).

Inferences on species numbers

We consider a species as the single lineage segment of ancestor-descendant populations or metapopulations delimited by one splitting event. Under this theoretical perspective, species exist and evolve regardless of our ability to discover them and are discoverable to the degree that footprints of their evolutionary history allow us to infer their existence (Ghiselin 1975; Hull 1976; Wiley 1978; Frost & Kluge 1994). We used two criteria to infer the existence of distinct species using DNA data and to guide the recognition of candidate species: monophyly and genetic distances. Reciprocal monophyly supported by the congruent phylogenetic optimization of neutral and unlinked molecular character states can be considered evidence of species divergence (e.g. Vences & Wake 2007), because the recovered congruent pattern will reflect the shared history of gene genealogies among populations (the species history) rather than the history of particular gene genealogies (gene trees) (Avise & Ball 1990; reviewed by Sites & Marshall 2004). In addition, fixed diagnostic traits across populations are indicative of lineage divergence, because character fixation across populations requires limited or absent gene flow (see review by Padial *et al.* 2010). Therefore, reciprocally monophyletic groups recovered by the total evidence analysis of DNA sequences, and for which distinct phenotypic characters have been described, are herein considered distinct species. Paraphyly of species inferred by total evidence analyses of DNA sequences that, yet, include morphologically distinct groups is considered indicative of the presence of more than one species. The second criterion, based on genetic divergences, assumes that genetic divergence among populations within a species tends to be relatively small because of gene flow, whereas divergence among species increases with time due to lack of gene flow (reviewed by Avise 2000). When large gaps in genetic divergences were detected between populations of the same nominal species, morphological and behavioural evidence was revised to determine whether genetic divergences were indicative of otherwise overlooked divergence in phenotypic traits and hence of the presence of unnamed species. However, for the reasons exposed by Padial *et al.* (2009) and Padial & De la Riva (2010), we refrain from using thresholds of genetic divergences to avoid creating artificially established species (or candidate species) numbers.

Results

Phylogenetic relationships

The combined data set included 338 terminals and 6134 aligned characters (ClustalW alignment), of which 3509 were constant, 533 were variable but parsimony uninformative, and 2092 were parsimony informative. No ambiguously aligned regions were detected. (Data deposited in the Dryad Repository http://dx.doi.org/10.5061/dryad.j04vf). Models of sequence evolution for each partition used in the ML analyses are in Appendix S3.

All optimality criteria produced quite similar results and no strongly supported conflicting topologies were recovered (Figs 1–4, Appendices S5–S6). Maximum parsimony with direct optimization recovered four most parsimonious trees (length = 12 865), see Figs 1–4. For the static alignment, the MP new technology search hit 500 times the best length. It recovered 4232 MPT (length = 13 254), see Appendix S6. Further TBR revealed that there were more MPT (>10 000), but successive strict consensus converged on the same topology as that obtained with the initial 4232 MPTs, and so we considered that further effort to find equally parsimonious trees was unnecessary (Goloboff 1999). The ML analysis recovered one tree with ln Likelihood = −54492.326409. Most of the conflict among the MPT (both from dynamic and static alignments) and the ML analysis occurs among shallow clades of closely related terminals of *Osteocephalus*, and among the poorly supported relationships between the five major clades that we recognize in this paper as species groups of *Osteocephalus*.

Relationships among outgroups (not shown in Fig. 1; see Appendices S5 and S6) differ from previous analyses (Faivovich *et al.* 2005; Wiens *et al.* 2006, 2010; Moen & Wiens 2009). However, outgroup relationships in general are poorly supported. This analysis has not been designed (in terms of character and taxon sampling) to assess internal relationships of Lophiohylini, and the results involving them should not be interpreted as a test of previous hypotheses.

The strict consensus of the best hypotheses indicates that *Osteocephalus* as currently defined is paraphyletic. The most basal clade in the ingroup (Fig. 1) includes species of

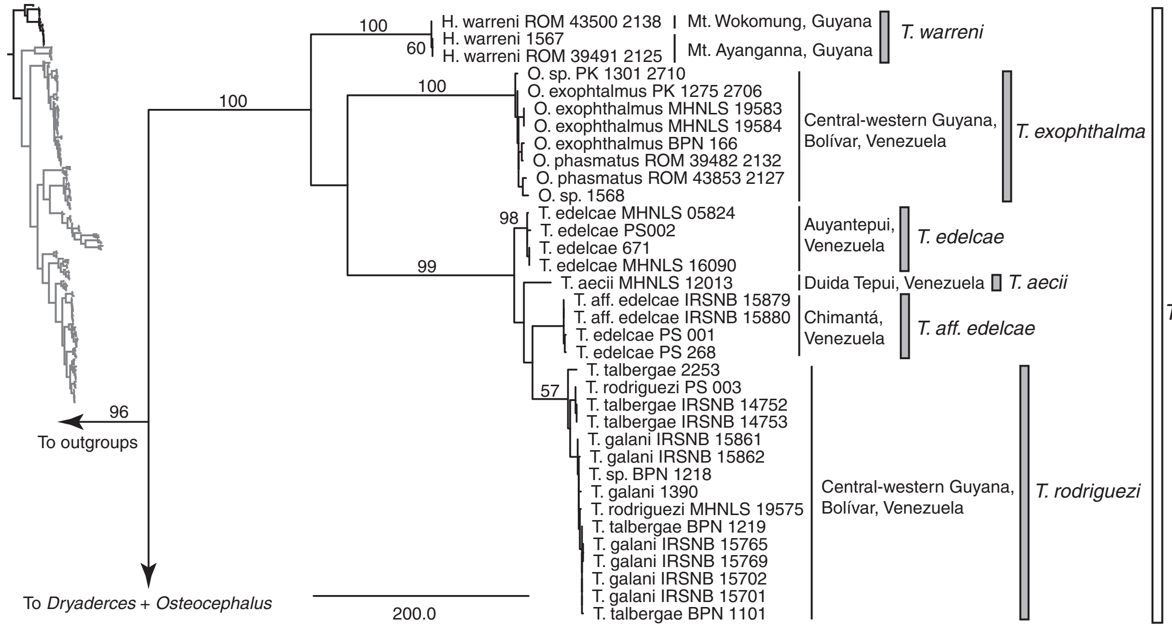

Fig. 1 Phylogenetic relationships of *Osteocephalus* and *Tepuihyla* inferred from maximum parsimony analysis under dynamic homology in the program POY4.1.1. This topology reflects one of the four most parsimonious trees (length 12 865 steps) with branch lengths proportional to the number of unambiguous transformations and black dots on nodes indicate clades collapsed in the strict consensus tree; not all loci are available for all terminals. For brevity, the outgroups are not shown. Their relationships are identical to those shown in Appendix S5 for the static parsimony analysis. The taxonomy proposed in this work and distribution of clades mentioned in the main text is shown. Numbers on branches are parsimony jackknife absolute frequencies calculated for the static parsimony analysis and shown only for nodes in common between optimal trees of both analyses. A skeletal topology for reference, with the magnified section marked in black, is shown on the left side.

Tepuihyla, the Guiana Shield species of *Osteocephalus* (*O. exophthalmus* and *O. phasmatus*), and '*Hyla*' *warreni*. This clade is the sister taxon of a clade composed of *Osteocephalus pearsoni* and a candidate species from Amazonian Brazil (see below) plus a clade composed of all remaining species of *Osteocephalus* (Figs 1–4). To remediate the paraphyly of *Osteocephalus* and *Tepuihyla*, we transfer *O. exophthalmus*, and *O. phasmatus* to *Tepuihyla* and '*Hyla warreni*' is placed in *Tepuihyla* (see Systematics section).

The bulk of species of *Osteocephalus* are divided into five main clades: (i) a clade including *O. taurinus*, *O. oophagus* and five candidate species that we call the *O. taurinus* Species Group (Fig. 2), (ii) a clade composed of *O. alboguttatus*, *O. heyeri*, and *O. subtilis* that we call the *O. alboguttatus* Species Group (Fig. 3), (iii) a clade composed of *O. leprieurii*, *O. yasuni* and two candidate species that we call the *O. leprieurii* Species Group (Fig. 3), (iv) a clade composed of *O. castaneicola*, *O. deridens*, *O. fuscifacies*, *O. leoniae*, *O. planiceps* and a candidate species that we call the *O. planiceps* Species Group (Fig. 3) and (v) a clade composed of *O. buckleyi*, *O. cabrerai*, *O. cannatellai*, *O. helenae*, *O. mimeticus*, *O. mutabor*, *O. verruciger* and one candidate species that we call the *O. buckleyi* Species Group (Fig. 4). Each of these five clades is in general well supported, but the relationships among most of them received poor support (Figs 1–4, Appendices S5 and S6). Furthermore, in both the static parsimony and ML analyses, the relationships among these clades differ from that obtained in the dynamic homology analysis. The *O. alboguttatus* Group is the sister taxon of all other species groups of *Osteocephalus*, while in the latter analysis, this is one of the possible positions, the other being the sister taxon of the *O. planiceps* Group. In the three analyses, the alternative positions of the *O. alboguttatus* Group are poorly supported. We also observed that in most cases, clades received higher node support in the ML analysis. This should be interpreted with caution because several recent studies have reported a pathological inflation of bootstrap values in ML analyses (Simmons & Freudenstein 2011; Simmons 2012; Simmons & Norton 2013 and references therein). See Systematics section for definitions and diagnoses of these groups.

Species diversity

Non-monophyly of nominal species was inferred for *O. buckleyi*, *O. leprieurii*, *O. planiceps*, *O. taurinus* and most

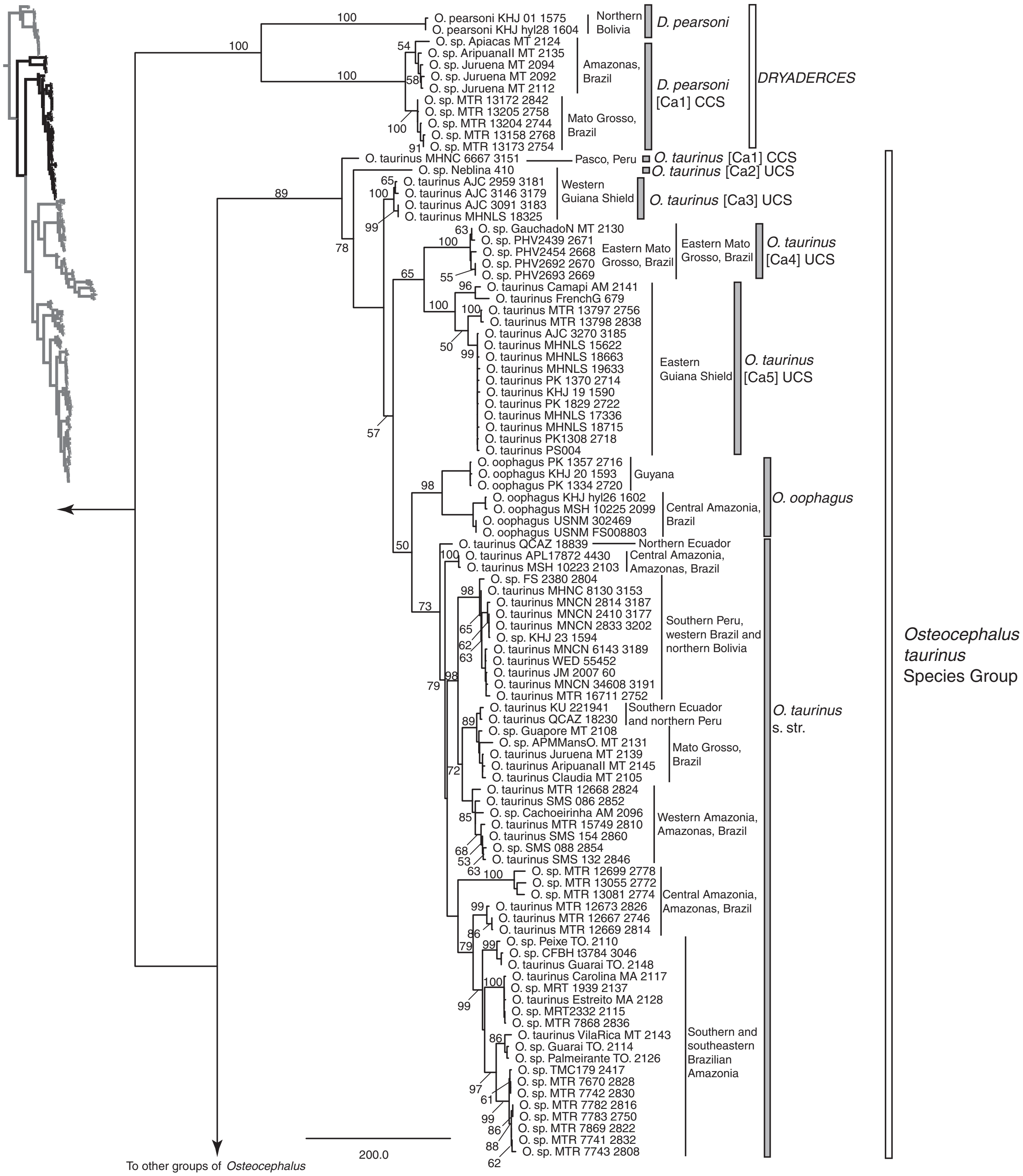

Fig. 2 Continuation of the phylogenetic tree of Fig. 1. Phylogenetic relationships of *Dryaderces* gen. n. and the *Osteocephalus taurinus* Species Group. For details, see Fig. 1.

likely for *O. mutabor* (Figs 2–4). Several geographically restricted and well-supported lineages were inferred within *O. taurinus*, *O. buckleyi* and *O. leprieurii*, which are the most widely distributed nominal taxa in the Amazon Basin. Specimens identified as *O. planiceps* were recovered as a monophyletic group, although without support in the ML analysis

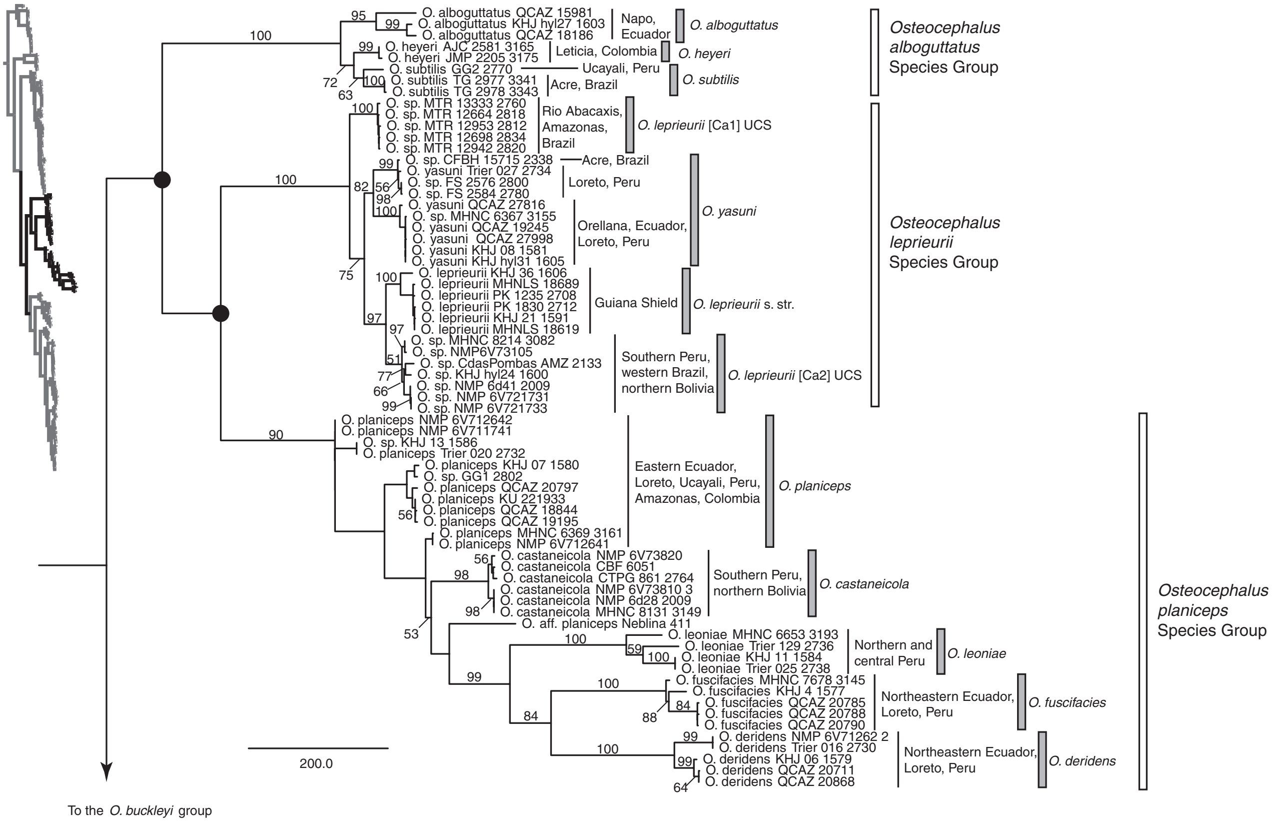

Fig. 3 Continuation of the phylogenetic tree of Fig. 1. Phylogenetic relationships of the *Osteocephalus alboguttatus*, *Osteocephalus leprieurii* and *Osteocephalus planiceps* Species Groups. For details, see Fig. 1.

(Appendix S5), but as a paraphyletic group with respect to *O. castaneicola*, *O. deridens*, *O. fuscifacies* and *O. leoniae* in the maximum parsimony trees (Fig. 3, Appendix S6).

Within species placed in *Tepuihyla* (Fig. 1), '*Hyla warreni*' samples are monophyletic. Samples of '*O. phasmatus*' from Mt. Ayanganna and Mt. Wokomung in Guyana cluster together and are sister to '*O. exophthalmus*' from Kaieteur, Guyana. Interspecific genetic divergences ranged from 0.0% to 0.2% between '*O. exophthalmus*' and '*O. phasmatus*', to 6.1–6.2% between '*Hyla warreni*' and '*O. phasmatus*'. Sequence divergence between *T. edelcae* and *T. galani* was 0.9%. The 16S barcode fragment was missing for *T. talbergae* in this study, but Kok *et al.* (2012) found a sequence divergence ranging from 0.0% to 0.7% between different and geographically distant populations (some from different tepui summits) of *T. galani*, *T. rodriguezi* and *T. talbergae*, although no taxonomic decision was taken. The same authors found relatively low sequence divergence (0.9–1.6%) between populations of the *T. galani/rodriguezi/talbergae* clade and *T. edelcae* from its type locality (Auyantepui, Bolívar, Venezuela) and found *T. edelcae* to be non-monophyletic suggesting that specimens previously identified as *T. edelcae* from the Chimantá Massif (Bolívar, Venezuela) belong to a distinct, unnamed species (*T.* aff. *edelcae*).

Two samples of *O. pearsoni* from near the type locality in northern Bolivia cluster together and are sister to a highly supported monophyletic lineage from Mato Grosso and Amazonas (Brazil) (Fig. 2), which is morphologically distinct, and show large genetic divergences with respect to *O. pearsoni* (3.9–4.2%). This lineage is considered a CCS and is referred here to as *O. pearsoni* [Ca1_MTR13158_2768]. Within this lineage, two distinct geographically restricted clades are recovered (Mato Grosso and Amazonas), showing genetic divergences of 2.0%.

Within the *O. alboguttatus* Species Group, the three species were all monophyletic (although with low support for *O. subtilis* in the MP trees). Genetic divergences are 4.2% between *O. alboguttatus* and *O. heyeri*, 3.1% between *O. alboguttatus* and O. *subtilis* and 1.8% between *O. subtilis* and *O. heyeri*. No candidate species are proposed for this group.

Within the *O. taurinus* Species Group (Fig. 2), *O. taurinus* is found paraphyletic with respect to *O. oophagus*.

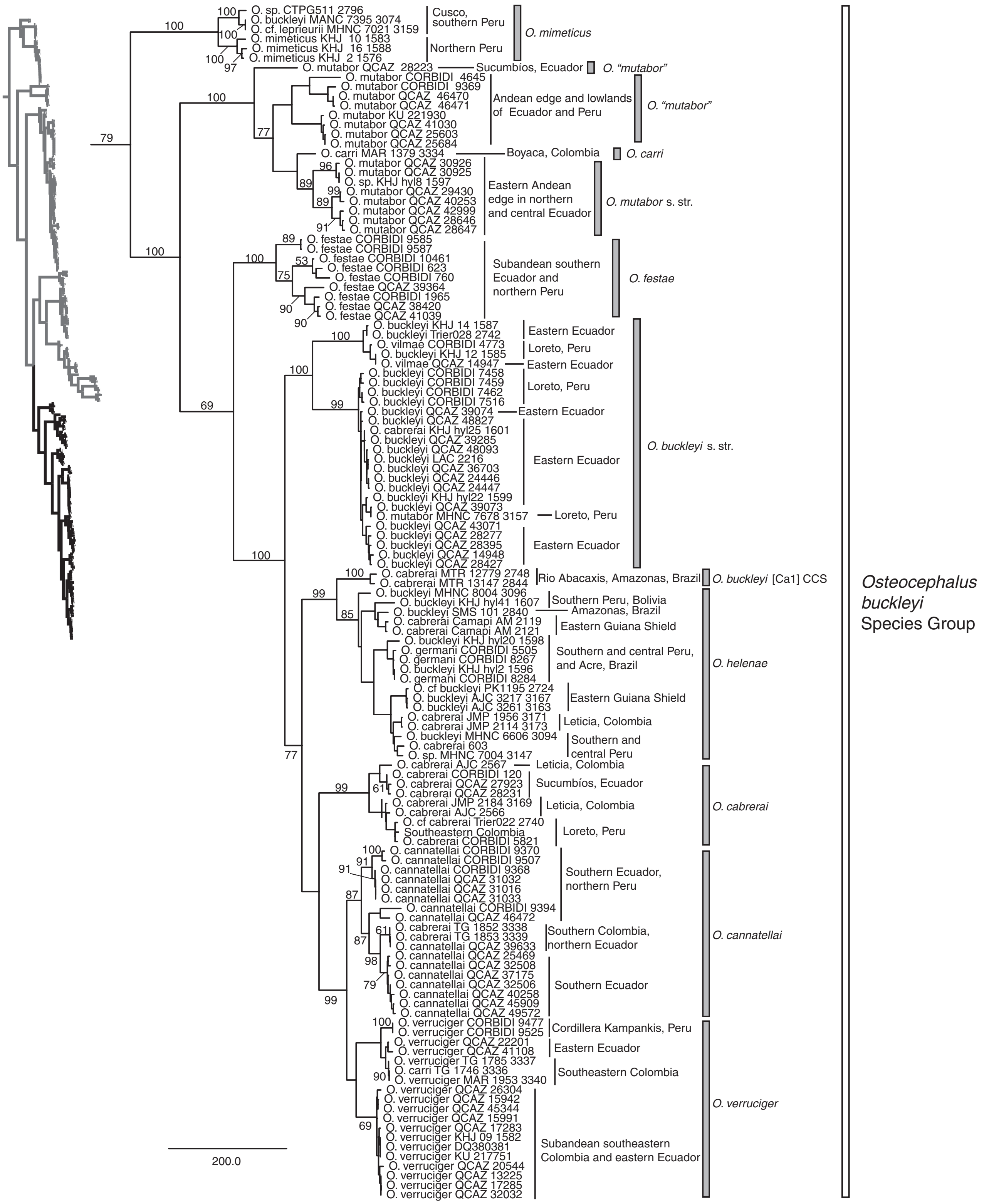

Fig. 4 Continuation of the phylogenetic tree of Fig. 1. Phylogenetic relationships of the *Osteocephalus buckleyi* Species Group. For details, see Fig. 1.

Samples from the type locality of *O. oophagus* from Reserva Ducke in central Amazonia cluster together and are sister to a clade containing samples from the eastern Guiana Shield, with genetic divergences among populations reaching 2.0%. The genetic structuring of *O. taurinus* and its paraphyly with respect to *O. oophagus* – an easily distinguishable species with single vocal sac and breeding in bromeliads in contrast to the paired lateral sacs and pond breeding of nominal *O. taurinus* and allies (Appendices S9 and S10) – suggest the existence of unnamed species within this species group. Interestingly, despite large morphological divergences, genetic distances between these two species range between 0.9% and 2.2%, overlapping with the range of their respective intraspecific divergences (Appendix S4a). These results support bioacoustical data suggesting the existence of multiple species-level lineages within nominal *O. taurinus* (De la Riva *et al.* 1995). However, analyses of call data are hampered by misidentifications, for example by Duellman & Lescure (1973) and Schlüter (1979) with *O. planiceps*. On the basis of phylogenetic position (Fig. 2) and genetic divergences, we flag five candidate species for four geographically restricted and supported clades within the large clade including nominal *O. taurinus* and *O. oophagus*.

The type locality of *O. taurinus* is 'Barra do Rio Negro', an old name for Manaus, in central Amazonia. Therefore, we consider our sample from Conjunto Pedro, a central-western urban district of Manaus close to the ancient Barra do Rio Negro, as nominal *O. taurinus*, and refer to it as *O. taurinus sensu stricto* (s. str. hereafter). *Osteocephalus taurinus* [Ca1_MHNC6667_3151] from Pasco, Central Peru, represents a lineage that is basal to all other lineages within *O. taurinus* and *O. oophagus*. Genetic divergences (Appendix S4a) range between 1.5–2.2% and 2.1–2.6% with respect to *O. taurinus* s. str. and *O. oophagus*, respectively. This Central Peruvian lineage is considered herein as a CCS for being basal to all other lineages in the *O. taurinus* Group. *Osteocephalus taurinus* [Ca2_Neblina410] from Cerro de la Neblina (the southern tip of Venezuela bordering Brazil) shows genetic divergences of 0.9–1.8 and 2.0–2.2 with respect to *O. taurinus* s. str. and *O. oophagus*, respectively. This species is recovered in the ML tree as sister to *O. taurinus* [Ca3_AJC2959_3181] from the western lowlands of the Guiana Shield, but as sister to all *O. taurinus* and *O. oophagus* included in the analyses but *O. taurinus* [Ca1_MHNC6667_3151] in the MP trees. Due to incomplete evidence and uncertain phylogenetic position, we consider it a UCS. The three other candidate species within *O. taurinus* are considered UCSs. Although there is some morphological evidence suggesting that they may be different species (KHJ, personal observation), evidence at hand is still fragmentary and we refrain from proposing them as CCS at this time (see Discussion for details of an analysis of available morphological evidence). *Osteocephalus taurinus* [Ca3_AJC2959_3181] is endemic to the lowlands of the western portion of the Guiana Shield in Venezuela. Genetic divergences range between 0.9–2.2% and 1.8–2.6% with respect to *O. taurinus* s. str. and *O. oophagus*, respectively. *Osteocephalus taurinus* [Ca4_PHV2439_2671] is restricted to eastern Mato Grosso (Brazil). Genetic divergences range between 1.1–2.2% and 2.2–2.8% with respect to *O. taurinus* s. str. and *O. oophagus*, respectively. It is sister to *O. taurinus* [Ca5_SMNS12063] from the eastern Guiana Shield, east of the Sierra de Maigualida. Both lineages are allopatric, showing genetic divergences between 1.3 and 2.2%, and intervening areas are occupied by *O. taurinus* s. str. *Osteocephalus taurinus* [Ca5_SMNS12063] shows genetic divergences between 1.1–2.6% and 1.3–3.1% with respect to *O. taurinus* s. str. and *O. oophagus*, respectively. Interestingly, this lineage is not sister to *O. taurinus* [Ca3_AJC2959_3181], the other Guiana Shield lineage, inhabiting the area west of the Sierra de Maigualida, but to *O. taurinus* [Ca4_PHV2439_2671] from eastern Mato Grosso.

Osteocephalus taurinus s. str. shows variable interpopulational genetic divergences (0.0–1.7%) partially associated with particular geographic regions (Fig. 2), although haplotypes from central Amazonia are intermixed with haplotypes of other geographic areas. One of the main clades within *O. taurinus* s. str. contains haplotypes from eastern Brazilian Amazonia in the states of Amazonas, Mato Grosso, Tocantins, Piauí and Maranhão. Members of this lineage occur in sympatry or almost so with two other haplotypes from central Amazonia grouped under other lineages, both from Amazonas, Brazil. Likewise, members of a lineage from the central-western Amazon Basin in Amazonas, Brazil, occur in sympatry with a relatively densely sampled lineage occurring in southern Peru, northern Bolivia and north-westward to the Rio Purus, Brazil. The northernmost lineage, represented by a single specimen from the Cordillera del Cóndor, southern Ecuador (Ron *et al.* 2010), occurs at an unusually high elevation (940 m). Poor resolution of relationships among phylo-groups nonetheless hampers a detailed phylogeographic interpretation.

Nominal species within the *O. planiceps* Species Group are inferred as monophyletic except *O. planiceps* (Fig. 3). *Osteocephalus planiceps* is retrieved as non-monophyletic in MP analyses, but as monophyletic in ML (Fig. 3, Appendices S5–S6). Neither of those analyses shows high support values for their respective topologies. Given the relative homogeneity of the habitat, the clustering of samples of *O. planiceps* from near its type locality in Loreto (Peru) with other samples from nearby areas of Colombia, Ecuador and Peru, the low resolution of the trees, the relatively low and greatly overlapping genetic distances (0.0–1.3%, Appendix S4b) between the MP clades of *O. planiceps* and, to the best of our knowledge, the absence of phenotypic data supporting the split of this lineage, we prefer to maintain *O. planiceps* as a single nominal species until more evidence is collected. Samples from the type locality of *O. castaneicola* in northern Bolivia cluster with other samples from the area and with samples from Madre de Dios in southern Peru. Samples of *O. leoniae* from near the type locality in Pasco, northern Peru, cluster with other samples from San Martín and Amazonas in north-western Peru. Samples of *O. deridens* from the lowlands of Ecuador near the type locality cluster with samples from adjacent Loreto in Peru. Samples from the type locality of *O. fuscifascies* cluster with samples from nearby areas in Ecuador and adjacent Peru. A divergent lineage from the western Guiana Shield (from the Cerro de la Neblina area) does not cluster with any of the nominal species. We consider this lineage as a CCS due to its basal position to three supported nominal species and refer to it as *O. planiceps* [Ca1_Neblina411]. Genetic divergences among this lineage and other nominal species in the *O. planiceps* Group range between 2.6% and 5.9%, while intraspecific genetic divergences within species of the group range between 0.0% and 4.0% (Appendix S4b).

Within the *O. leprieurii* Species Group, *O. leprieurii* is paraphyletic with respect to *O. yasuni* (Fig. 3). Samples of *O. leprieurii* from the eastern Guiana Shield in Guyana cluster together and are considered herein as representative for the type locality (Cayenne, French Guiana), and as *O. leprieurii* s. str. Samples of *O. yasuni* from the type locality in Orellana, Ecuador, cluster together with a sample from adjacent Loreto, Peru, and are sister to a clade containing samples from western Amazonia in Acre, Brazil and Loreto, Peru. Genetic divergences between *O. leprieurii* s. str. and *O. yasuni* range between 0.7% and 1.8%, while intraspecific divergences in the *O. leprieurii* Group range between 0.7% and 2.0%, taking into account the candidate species proposed below (Appendix S4c). Nonetheless, as *O. leprieurii* s. str. and *O. yasuni* are morphologically distinct (Ron & Pramuk 1999), we interpret the paraphyly of *O. leprieurii* as indicative of the putative existence of multiple unrecovered species-level lineages. A lineage composed of samples from Rio Abacaxis in Amazonas, Brazil, is inferred as basal to the lineage including *O. leprieurii* s. str. and *O. yasuni*. Due to its basal position, allopatry and genetic divergences with respect to *O. leprieurii* s. str. and *O. yasuni* (1.7–1.8% and 0.9–1.3%, respectively), we consider this lineage as an UCS pending additional evidence and refer to it as *O. leprieurii* [Ca1_MTR12698_2834]. A second well-supported lineage is sister to *O. leprieurii* s. str. This lineage contains samples from eastern Bolivia, northern Bolivia, southern Peru and adjacent Brazil and is allopatric to *O. leprieurii*. Both lineages show genetic divergences between 0.9% and 1.8%. Published morphological data are not available, and despite the large geographic gap between nominal *O. leprieurii* and this clade, we prefer to consider it a UCS pending further research. We refer to this lineage as *O. leprieurii* [Ca2_NMP6d41/2009].

Four of the nominal species included in the *O. buckleyi* Species Group, *O. cabrerai*, *Osteocephalu festae*, *O. mimeticus* and *O. verruciger*, are monophyletic and well supported (Fig. 4). Interspecific genetic distances within this clade are the highest recorded within *Osteocephalus* (Appendix S4d), up to 6.1% between *O. helenae* and *O. mimeticus*. Samples of *O. mimeticus* from near the type locality in San Martín, Peru, group with other samples from the Amazonian foothills of the Andes in San Martín and Huánuco, Peru. Three samples from Cusco, in southern Peru, cluster together and are sister to topotypic and Huánuco samples. A large geographic distance and large genetic divergences (2.8%) separate these two clades. Nonetheless, given the lack of samples from intervening areas and the morphological similarity of specimens from both areas, we refrain from flagging any candidate species within *O. mimeticus*. Samples of *O. festae* from northern Peru and Ecuador included in this analysis are those used by Ron *et al.* (2010). This lineage is morphologically distinct from other members in the group. Unfortunately, sequences of the 16S barcode were not available for representatives of this lineage.

A sample of *O. mutabor* from the type locality at Volcan Sumaco, Ecuador, clusters together with samples from Napo, Orellana and Pastaza in Ecuador. We refer to this clade as *O. mutabor* s. str. There are several more lineages phenotypically referable to *O. mutabor* distributed from northern Ecuador to northern Peru along the Andean edge and adjacent lowlands (Appendix S8.3b). Surprisingly, one of them is sister to *O. carri* (Fig. 4), rendering several lineages of '*O. mutabor*' outside the clade including sequences of topotypes. Although we clearly identified a picture of the specimen of *O. carri* used here from Boyacá, Colombia, as belonging to that species, we consider it premature to postulate several candidate species of *O. mutabor*, but rather refer to them as '*O. mutabor*' in the trees and the map until additional material is available to us.

Unfortunately, sequences of the 16S barcode were only available for representatives of *O. mutabor* s. str. and *O. carri;* these show a genetic divergence of 2.6% (Appendix S4d).

The type locality of *O. buckleyi* is Canelos, Ecuador. Multiple samples assignable to *O. buckleyi* from the lowlands of Ecuador and adjacent Peru group together in a well-supported clade that we consider here as nominal *O. buckleyi* and refer to it as *O. buckleyi* s. str. This clade shows genetic divergences ranging between 0.0% and 1.5% and is subdivided in two well-supported subclades sharing haplotypes from the same regions. One of these subclades is considered a distinct species by Ron *et al.* (2012). We do not follow their taxonomic arrangement for reasons given in the Appendix. Recognizing this clade as nominal *O. buckleyi* leaves a large and well-supported clade, which is part of the more inclusive clade containing *O. buckleyi* s. str., *O. cabrerai*, *O. cannatellai* and *O. verruciger*. This clade includes samples from the eastern Guiana Shield (Guyana, Delta Amacuro in Venezuela and Amapá in Brazil) across central Amazonia in Brazil and eastern Colombia, to south-western Amazonia in Acre (Brazil), Cusco (Peru) and the southern limit of Amazonia at Mataracú (Santa Cruz, Bolivia). Intra-lineage divergences are high among geographically restricted lineages (up to 2.6%), but absence of sufficient phenotypic information from parts of its range and the presence of shared haplotypes among distant populations prevent us from recognizing more than two species. Two samples from the Abacaxis River south of the Amazon River in Amazonas, Brazil, are basal to a larger and well-supported subclade including samples from all other areas. We consider the Abacaxis population as a distinct candidate species, *O. buckleyi* [Ca1_MTR12779_2748] with morphological characters sufficient to consider it a CCS. The relationships of geographically restricted lineages within the other subclade are not resolved. However, some interesting patterns are observable. Samples from Amazonian Colombia cluster with distant samples from southern Peru and Acre. Also, samples from the Guiana Shield (Amapá, Delta Amacuro) cluster together, although support for this relationship is low (Fig. 4). For the time being and given the evidence at hand, we consider this whole subclade as a single species referred here to *O. helenae* nov. comb.

A sample of *O. cabrerai* from south-eastern Colombia, the one closest to the type locality on the Río Apapóris, clusters with samples from adjacent Loreto, Peru, and Sucumbíos, Ecuador (Fig. 4), and shows no genetic divergences. We consider this well-supported lineage as nominal *O. cabrerai*.

The type locality of *O. verruciger* is 'Ecuador'. Multiple samples from Ecuador assigned to this taxon cluster together in a well-supported clade (Fig. 4) that is subdivided into four main subclades showing large variation in genetic divergences (0.0–1.7%).

Systematics

On the basis of our results, we provide a new monophyletic taxonomy where we redefine *Osteocephalus* and *Tepuihyla* and describe a new genus for *O. pearsoni* and an unnamed species considered here as CCS. Additionally, we comment on the species diversity of these genera, propose and define five species groups within *Osteocephalus*, and discuss the distribution and taxonomic situation of species and candidate species in these groups.

Genus *Dryaderces* gen. n.
Type species. *Hyla pearsoni* Gaige, 1929, by original designation.

Diagnosis. No phenotypic synapomorphies are known for this genus. A number of molecular synapomorphies are listed in Appendix S7. *Dryaderces* species differ from most *Osteocephalus* in sexual dimorphism of dorsal skin structure. Albeit being pond breeders (SR, personal observation), males have only scattered non-spinous tubercles on the dorsum (heavily tuberculate dorsa and tips of tubercles keratinized in pond breeding *Osteocephalus*). Females have smoother backs. Recently metamorphosed juveniles of *Dryaderces pearsoni* are coloured like the adults and lack the juvenile coloration typical of *Osteocephalus* (see below and Appendix S1). Frogs in the genus *Dryaderces* are medium-sized with males attaining SVLs of 43–50 mm and females 53–68 mm. Odontophores are oblique to angular. The distal subarticular tubercle on Finger IV is single to bifid. Posterior surfaces of thighs are mottled. The iris of adults is a deep dark reddish brown or light with fine dark venation and a broad dark horizontal band.

Content. One described species, *Dryaderces pearsoni* (Gaige, 1929) new combination. A second species from Brazilian Amazonia is currently being described.

Etymology. A noun of feminine gender derived from Ancient Greek *dryad* (tree) and *aderces* (unseen, invisible) in the sense of 'unseen in a tree'.

Distribution. Lowlands of the states of Amazonas and Mato Grosso, Brazil, south of the Amazon to northern La Paz, Bolivia (Appendix S8.1).

Remarks. The only described species has its type locality on the upper Río Beni below the mouth of Río Mapiri, Beni, Bolivia (Gaige 1929) and was originally placed in *Hyla* until Goin (1961) removed it and placed it in *Osteocephalus*. In their review of the genus, Trueb & Duellman (1971) used (and illustrated) one specimen of *O. mimeticus* among *O. pearsoni* (Jungfer 2011). Subsequent reports might therefore include misidentifications as well. A picture of an adult is found in the study by De la Riva *et al.* (2000) and Padial *et al.* (2000).

Genus *Osteocephalus*
Osteocephalus Fitzinger, 1843 (nomen nudum)

Osteocephalus Steindachner, 1862
Type species: *Osteocephalus taurinus* Steindachner, 1862, by subsequent designation of Kellog (1932).

Diagnosis. The only known putative phenotypic synapomorphy of *Osteocephalus* is the distinctive juvenile colouration (see discussion below and Appendix S1). A number of molecular synapomorphies are listed in Appendix S7.

Content. Twenty-four species: *Osteocephalus alboguttatus* (Boulenger, 1882), *O. buckleyi* (Boulenger, 1882), *O. cabrerai* (Cochran & Goin, 1970), *O. cannatellai* Ron, Venegas, Toral, Read, Ortiz & Manzano, 2012, *O. carri* (Cochran & Goin, 1970), *O. castaneicola* Moravec, Aparicio, Guerrero-Reinhard, Calderón, Jungfer & Gvoždík, 2009, *O. deridens* Jungfer, Ron, Seipp & Almendáriz, 2000, *O. duellmani* Jungfer, 2011, *O. festae* (Peracca, 1904), *O. fuscifacies* Jungfer, Ron, Seipp & Almendáriz, 2000, *O. helenae* (Ruthven, 1919), *O. heyeri* Lynch, 2002, *O. inframaculatus* (Boulenger, 1882), *O. leoniae* Jungfer & Lehr, 2001, *O. leprieurii* (Duméril & Bibron, 1841), *O. mimeticus* (Melin, 1941), *O. mutabor* (Jungfer & Hödl, 2002), *O. oophagus* Jungfer & Schiesari, 1995, *O. planiceps* Cope, 1874, *O. subtilis* Martins & Cardoso, 1987, *O. taurinus* Steindachner, 1862, O. *verruciger* (Werner, 1901), *O. vilarsi* (Melin, 1941), *O. yasuni* Ron & Pramuk, 1999.

Osteocephalus vilmae Ron, Venegas, Toral, Read, Ortiz & Manzano, 2012 is placed in the synonymy of *O. buckleyi* (Boulenger, 1882) (see Appendix) and *O. germani* Ron, Venegas, Toral, Read, Ortiz & Manzano, 2012 in the synonymy of *O. helenae* (Ruthven, 1919) nov. comb., a species previously not assigned to the genus (see Appendix).

Distribution. From coastal northern South America in Venezuela and the Guianas to the mouth of the Amazon and north-eastern Brazil (Piauí) in the East, to central Brazil (Mato Grosso) and central Bolivia in the south and to the eastern Andean slopes from Bolivia to Colombia up to about 2000 m a.s.l. (Appendix S8.2–8.6).

Remarks. Thirteen species of *Osteocephalus* for which data were available show a similar pattern in the colouration of juveniles that radically differs from adult colouration. Ten species share a red iris colour, white elbow and distal part of upper arm, small white knee spot and large white heel spot in juveniles. *Osteocephalus verruciger* and *O. buckleyi* lack the red iris (iris black and reddish golden, respectively) and *O. mutabor* lacks white markings on limbs (Appendix S1), but they all show a similar juvenile colour pattern that differs from that of adults. The *O. alboguttatus* Species Group is the only one of which we have not seen any recently metamorphosed juveniles. A photograph, however, of a subadult *O. subtilis* taken by A. J. Cardoso and deposited at the Biodiversity Institute, University of Kansas, has the typical intermediate colouration with orange iris and black venation (uniform, deep dark brown in adults) and faded cream elbow, knee and heel spot (absent in adults) that leaves no doubt that juveniles are coloured like the other *Osteocephalus* as described above. *Dryaderces pearsoni* differs from *Osteocephalus* in that it already exhibits adult colouration immediately after metamorphosis. Most other Lophiohylini also lack an ontogenetic colour change, for example *Aparasphenodon brunoi* (CFBH, personal observation), *Argenteohyla siemersi* (D. Baldo, personal communication), *Itapotihyla langsdorffii* (Appendix S1), *Nyctimantis rugiceps*, *Osteopilus crucialis*, *O. wilderi* (KHJ, personal observation), *Phyllodytes luteolus* (MTR, personal observation), while in others juvenile patterns remain, but only slightly dissolve, as in *Trachycephalus hadroceps* (KHJ, personal observation) and *T. resinifictrix* (Jungfer & Proy 1998). Juvenile *T. typhonius* are as polymorphic in colouration and pattern as their adult phases (KHJ, personal observation). Distinctly deviating juvenile colourations are only known to us in *Trachycephalus jordani* (green with white dorsolateral and supralabial stripes, iris golden) and *Osteopilus ocellatus* (green, transversal bands on limbs, iris coppery red), both of which lack the light limb spots of *Osteocephalus*. In general, with respect to colouration, juveniles of most *Osteocephalus* species resemble one another more than their interspecific adult stages, so that we consider the juvenile colouration a synapomorphy of *Osteocephalus sensu* this work. With the exception of the *O. buckleyi* Group (Ron *et al.* 2012), species groups in *Osteocephalus* so far have not been recognized. According to the results of phylogenetic analyses and morphological data, we recognize five species groups within *Osteocephalus*; these are defined below.

Osteocephalus alboguttatus *Species Group*.
Content. *Osteocephalus alboguttatus* (Boulenger, 1882), *O. heyeri* Lynch, 2002, and *O. subtilis* Martins & Cardoso 1987.

Diagnosis. Males in this clade are small (<40 mm SVL) and females are medium-sized (<50 mm). The dorsum in males and females is smooth or, in males of *O. heyeri*, nearly so. Hand webbing is reduced or absent. Vocal sacs are subgular and single or slightly bilobed. Nuptial excrescences are present in breeding males. Information on breeding sites and reproduction is missing. Amplexus is axillary in *O. subtilis* and unknown in the other members of the group. Iris colouration is golden with irregular black reticulation, dark grey or deep dark brown in adults (Duellman 1978; Martins & Cardoso 1987; Lynch 2002).

Distribution. *Osteocephalus alboguttatus* is known from sites along the Andean edge in north-eastern Ecuador below 600 m in elevation (Almendáriz *et al.* 2004a); *O. heyeri* from the upper Amazon in Loreto, Peru, and Amazonas, Colombia (Lynch 2002). *Osteocephalus subtilis* occurs in Acre, Brazil and adjacent Ucayali, Peru (Martins & Cardoso 1980; this study). See Appendix S8.2 for sampled localities.

Remarks. No morphological synapomorphies are known for this group. There is little variation within species in this group and the advertisement calls are unknown.

Osteocephalus buckleyi *Species Group*.

Content. *Osteocephalus buckleyi* (Boulenger, 1882), *O. cabrerai* (Cochran & Goin, 1970), *O. cannatellai* Ron, Venegas, Toral, Read, Ortiz & Manzano, 2012, *O. carri* (Cochran & Goin, 1970), *O. festae* (Peracca, 1904), *O. helenae* (Ruthven, 1919), *O. mimeticus* (Melin, 1941), *O. mutabor* (Jungfer & Hödl, 2002), *O. verruciger* (Werner, 1901). *Osteocephalus duellmani* Jungfer, 2011 and *O. inframaculatus* (Boulenger, 1882) are also included tentatively here on the basis of their morphological similarity, as no synapomorphies involving adult morphology are known for the group, and no tissues of these two species were available for this study.

Diagnosis. Stream breeding is a putative synapomorphy of this group. Reproduction is usually associated with streams in all species for which data are available, although we found *O. buckleyi* (GGU, personal observation) and *O. mutabor* (KHJ, personal observation) exceptionally breeding in stagnant pools of water. Dorsal skin texture in males varies from strongly tuberculate with tubercles bearing keratinized tips (e.g. in *O. verruciger*) to granulate (*O. duellmani*), while females are smoother. Vocal sacs are paired, situated laterally, with a subgular expansion (Appendix S9). Amplexus is axillary (Appendix S11). Nuptial pads are brown.

Distribution. As currently defined, *O. buckleyi* covers a huge area from the coastal Guianas to southern Venezuela, most of Amazonian Colombia, Ecuador and Peru and in the east from the mouth of the Amazon to Santa Cruz, Bolivia (La Marca *et al.* 2010a; Appendix S8.3a). However, *O. buckleyi* s. str. is restricted to Amazonian Ecuador and north-western Loreto, Peru (type locality restricted to Canelos, Provincia Pastaza, Ecuador by Trueb & Duellman 1971). *Osteocephalus festae* is known from montane sites from Morona Santiago, Ecuador, to Amazonas and Cajamarca, Peru (Jungfer 2010 as *Osteocephalus* sp., Ron *et al.* 2010). The CCS *O. buckleyi* [Ca1_MTR12779_2748] is known from a few sites along the Rio Abacaxis in Amazonas, Brazil, where it might occur in sympatry with *O. helenae*. The latter is a widely distributed species from the Delta Amacuro in Venezuela and the eastern Guiana Shield, through central Amazonia across Amapá, Amazonas, Acre, and Mato Grosso, Brazil, reaching the Andean foothills in central and northern Bolivia and southern and northern Peru.

Osteocephalus cabrerai has been reported from Amazonas, Colombia (Cochran & Goin 1970; Lynch 2002), Loreto and Ucayali, Peru (Jungfer 2010; Ron *et al.* 2012), Sucumbíos, Ecuador (Ron *et al.* 2011), and also from the Delta Amacuro and the Guiana Shield area in Venezuela, French Guiana and Amazonas, Brazil (Gorzula & Señaris '1998' [1999]; Lescure & Marty 2000; Lima *et al.* 2006 as *O. buckleyi*; Jungfer 2010; Menin *et al.* 2011). Frogs in this study are all from the upper Amazon in Sucumbíos, Ecuador, Amazonas, Colombia and Loreto, Peru (Appendix S8.3a).

Apart from *O. festae*, several species are known from the eastern Andean slopes and foothills (Appendix S8.3b): *O. carri* in Colombia (Cochran & Goin 1970; Lynch 2006; Jungfer 2010), *O. verruciger* in southern Colombia and northern Ecuador (Angulo *et al.* 2004), *O. duellmani* from the Cordillera del Cóndor in Ecuador (Jungfer 2011), *O. mimeticus* from San Martín southward to Cusco, Peru (Jungfer 2010). *Osteocephalus mutabor* occurs along the eastern Andean foothills of central and northern Ecuador, while phenetically similar frogs, here referred to as '*O. mutabor*', are known from lowland Amazonian Ecuador in Sucumbíos, Ecuador, the eastern Andean edge of southern Ecuador and northern Peru and adjacent lowlands as far east as the Iquitos and Leticia areas in Peru and Colombia (Jungfer *et al.* 2000; Ron *et al.* 2012; this study). *Osteocephalus inframaculatus* is known from the type locality, Santarem, Pará, Brazil on the Amazon River (Boulenger 1882; Jungfer 2010).

Remarks. As currently defined, *O. buckleyi* is a widespread and largely polymorphic taxon. Jungfer (2010) included only specimens from north-western Amazonia for comparisons with related species to avoid confounding morphological characters without first assessing the possible existence of putative new species. Populations vary in degree of tuberculation and webbing, SVL, especially in females, and other characters. Some populations are also difficult to distinguish from *O. cabrerai* despite a recent redescription of the latter (Jungfer 2010). La Marca *et al.* (2010a) assumed that *O. buckleyi* might be a complex of more than one species. Ron *et al.* (2012) attempted to resolve parts of this complex in Ecuador and Peru and described three new species, only one of which we consider valid: *Osteocephalus* *cannatellai* occurs in eastern Ecuador and north-eastern Peru. It also occurs in Caquetá, southern Colombia (this study). The second one, *O. germani*, is preoccupied by *O. helenae* (Ruthven, 1919). We do not recognize the third one, *O. vilmae*, as distinct from *O. buckleyi* for reasons given in the Appendix. Morphological characters of *O. cabrerai* are also polymorphic and partially overlap with those of *O. buckleyi*. Probably for this reason, specimens listed as *O. buckleyi* by Ron *et al.* (2010) were later used to report the first record of *O. cabrerai* for Ecuador by Ron *et al.* (2011) on the basis of molecular data. Jungfer (2010) also refrained from using material from north of the Amazon for a redescription of this species. The rest of species in this group, and those with more restricted distributions, are morphologically more uniform.

Osteocephalus leprieurii *Species Group*.

Content. *Osteocephalus leprieurii* (Duméril & Bibron, 1841) and *O. yasuni* Ron & Pramuk, 1999.

Diagnosis. Males and females in this clade are highly dimorphic in dorsal skin structure with females being smooth or bearing few low tubercles and males being heavily tuberculate. During breeding, tubercles bear keratinized tips. Keratinized excrescences, apart from the large dark brown nuptial pad, are also present on parts of the limbs, hands, feet and lower jaw in some populations of *O. leprieurii* (Jungfer & Hödl 2002). Vocal sacs are paired, lateral with subgular expansion (Appendix S9). Amplexus is axillary (Appendix S11). Both species are explosive breeders congregating at ponds or flooded areas. During that time, males become yellow dorsally and light yellow ventrally (Ron & Pramuk 1999; Lescure & Marty 2000; Jungfer & Hödl 2002; Deichmann & Williamson 2007; Kok & Kalamandeen 2008). Irises of adult frogs in the *O. leprieurii* clade are golden to golden brown with fine irregular dark venation and a broad dark brown horizontal midline.

Distribution. As currently defined, *O. leprieurii* is widely distributed from the Guianas and northern Venezuela to eastern Colombia, eastern Peru, northern Bolivia and the northern and eastern Brazilian Amazon including Amazonas and Amapá (La Marca *et al.* 2010c; this study). The nominal species s. str. occurs in the Guianas and southern Venezuela (Appendix S8.4); its type locality is 'Cayenne', French Guiana. The UCS *O. leprieurii* [Ca1_MTR12698_2834] occurs allopatrically from *O. leprieurii* s. str. in Brazilian central Amazonia. Our samples are from three localities on the Rio Abacaxis (Appendix S8.4), but there are also specimens from further south-east in Pará, Brazil. The UCS *O. leprieurii* [Ca2_NMP6d41/2009] has long been considered a distinct unnamed species occurring in northern Bolivia and south-western Amazonas, Brazil, apparently in allopatry from all other lineages within the *O. leprieurii* Group. *Osteocephalus yasuni* is known from lowland sites in north-eastern Ecuador, northern Peru, Acre, Brazil, and north of the Amazon in Amazonas, Colombia (Ron & Pramuk 1999; Lynch 2002; this study). Sampled localities are shown in Appendix S8.4.

Remarks. No morphological synapomorphies are known for this group. *Osteocephalus leprieurii* is a polymorphic species with considerable variation especially in SVL, colouration and degree of keratinized structures in breeding males. The advertisement calls of this species are highly complex and not as stereotyped as in most frogs (Jungfer & Hödl 2002), making bioacoustic comparisons among populations difficult. That *O. leprieurii* might be a composite of several species has already been hypothesized by Jungfer & Hödl (2002) and Moravec *et al.* (2009). The latter authors also presented some molecular evidence to support this.

Osteocephalus planiceps *Species Group*.

Content. *Osteocephalus castaneicola* Moravec, Aparicio, Guerrero-Reinhard, Calderón, Jungfer & Gvoždík, 2009, *O. deridens* Jungfer, Ron, Seipp & Almendáriz, 2000, *O. fuscifacies* Jungfer, Ron, Seipp & Almendáriz, 2000, *O. leoniae* Jungfer & Lehr, 2001, and *O. planiceps* Cope, 1874.

Diagnosis. Phytotelm breeding (see below) and a single, subgular vocal sac are putative synapomorphies of this group. All species in this clade breed in phytotelmata such as leaf axils, fruit capsules, bamboo and tree holes (Jungfer *et al.* 2000; Moravec *et al.* 2009; KHJ, personal observation). Except for *O. planiceps*, dorsal skin is not sexually dimorphic and more or less smooth in both sexes. Breeding males identified as *O. planiceps* vary from tuberculate to almost smooth. Nuptial excrescences in breeding males are dark brown (*O. planiceps*), light brown (*O. castaneicola*) or white (*O. deridens*, *O. fuscifacies*, *O. leoniae*). The vocal sac is white, single and subgular. Amplexus is axillary or gular (Appendix S11).

Distribution. Sampled localities are in Appendix S8.5. *Osteocephalus planiceps* has a wide distribution in the western Amazon Basin from Meta in south-western Colombia through Ecuador to Madre de Dios in south-western Peru (Lehr 2001; Lynch 2008; this study). There is also a record from the central Amazon in Amazonas, Brazil (Gordo & Neckel-Oliveira 2004). The other species have smaller ranges. *Osteocephalus castaneicola* occurs in Pando, Bolivia and adjacent Madre de Dios, Peru (Moravec *et al.* 2009); *O. deridens* ranges from north-eastern Ecuador, south-eastward through northern Loreto, Peru, to the Leticia area, Amazonas, Colombia, and adjacent Amazonas, Brazil (Moravec *et al.* 2002; Almendáriz *et al.* 2004b; this study). *Osteocephalus fuscifacies* is known from north-eastern Ecuador in Orellana, Sucumbíos and Napo, and adjacent Loreto, Peru (Jungfer *et al.* 2000; this study). *Osteocephalus leoniae* occurs from the Rio Marañon area in Amazonas, Peru southward to Cusco, Peru (Jungfer & Lehr 2001; Chávez *et al.* 2008; this study). An allopatric population from the western Guiana Shield is considered herein a CCS (*O. planiceps* [Ca1_Neblina411]).

Remarks. There is little morphological variation in those species with small ranges. *Osteocephalus planiceps*, however, exhibits considerable variation in SVL, dorsal skin structure in breeding males, and call structure (Duellman & Lescure 1973 as *O. taurinus*; Schlüter 1979 as *O. leprieurii*; KHJ, personal observation). The latter may be due to variation in types of calls or the presence of cryptic species.

Osteocephalus taurinus *Species Group*.

Content. *Osteocephalus oophagus* Jungfer & Schiesari, 1995, *O. taurinus* Steindachner, 1862, and *O. vilarsi* (Melin, 1941). The latter is tentatively associated with this group because of its close external similarity with *O. taurinus* (Jungfer 2010).

Diagnosis. Frogs in the group are medium-sized to large with frontoparietal ridges variably present (extremely pronounced to inconspicuous), iris golden with uniformly radiating dark lines and a broad dark horizontal midline, sexually dimorphic tuberculate and spiculate dorsum present or absent, nuptial excrescences dark, vocal sacs paired, lateral, with subgular expansion, or single, subgular (Appendix S9) and oviposition in pools or phytotelmata (Appendix S10). Amplexus is axillary (Appendix S11).

Distribution. *Osteocephalus oophagus* is primarily Guianan, ranging from central Guyana, Suriname and northern French Guiana southward to the Amazon and Rio Negro and slightly beyond into Pará, Brazil. To the west, it reaches Guainía, Colombia (Hoogmoed & Avila-Pires 1991; Jungfer & Schiesari 1995; Lescure & Marty 2000; Lynch & Vargas-Ramírez 2000; Ernst *et al.* 2005; Azevedo-Ramos 2010). As currently defined, the nominal species *O. taurinus* occupies a large range, from Amazonian and Guianan Venezuela throughout Amazonia to Bolivia and Mato Grosso, Tocantins, Piauí and Maranhão, Brazil (Trueb & Duellman 1971; La Marca *et al.* 2010b). However, as explained above, in addition to *O. taurinus* s. str., we recognize five candidate species (Fig. 2). Our data suggest that these have restricted non-overlapping ranges. However, we have not included samples from the western Amazon Basin yet (Appendix S8.6). The CCS *O. taurinus* [Ca1_MHNC6667_3151] is a large species only known from one site in Pasco, Peru, that does not appear to be in sympatry with any other lineage within *O. taurinus*. The UCS *O. taurinus* [Ca2_Neblina410] is known from an isolated site in the Pico de Neblina area in extreme southern Venezuela. The UCS *O. taurinus* [Ca3_AJC2959_3181] occurs in lowlands at the western edge of the Guianan region and is not known to occur sympatrically with any other lineage within the *O. taurinus* group either. The UCS *O. taurinus* [Ca4_PHV2439_2671] occurs in eastern Mato Grosso, Brazil, where it might occur in sympatry with *O. taurinus* s. str. to the both east and west. The UCS *O. taurinus* [Ca5_SMNS12063] occurs in the Guianan region from eastern Venezuela to the Guianas and the mouth of the Amazon in Brazil. There is a large collection gap for samples between Ca5 and *O. taurinus* s. str., although *O.* '*taurinus*' is known to occur there (La Marca *et al.* 2010b).

Remarks. As currently defined, *O. taurinus* is an extremely polymorphic species. SVL of mature females ranges from 56.4 to 109.8 mm and from 50.3 to 91.1 mm in breeding males, with neighbouring populations sometimes exhibiting extreme size differences. The presence of frontoparietal ridges, sexually dimorphic dorsal skin and an iris with radiating dark lines in all populations assigned to this species apparently led Trueb & Duellman (1971) to consider *O. taurinus* a single polymorphic species. The fact that *O. oophagus*, a species with single subgular vocal sac and other distinct characters, is nested within *O.* '*taurinus*', which has paired lateral sacs, suggests the existence of several unrecognized species. A detailed morphological and bioacoustic study of populations within *O.* '*taurinus*' is needed to unravel species diversity within this taxon.

Genus *Tepuihyla* Ayarzagüena, Señaris & Gorzula, 1993

Type species. *Hyla rodriguezi* Rivero, 1968, by original designation.

Diagnosis. No phenotypic synapomorphies are known for *Tepuihyla* (see discussion below). A number of molecular synapomorphies are listed in Appendix S7. Recently metamorphosed juveniles of *T. rodriguezi* (population from Guadacapiapu-tepui, Venezuela), *T.* aff. *edelcae* (undescribed species from the Chimantá Massif, Venezuela) and *Tepuihyla rimarum* (Ptari-tepui, Venezuela) lack the juvenile coloration typical of *Osteocephalus* and usually have granular skin (PJRK, personal observation, see below and Appendix S1);

likewise Myers & Donnelly (2008) do not report ontogenetic colour change between recently metamorphosed juveniles and adults of *T. edelcae* from Auyantepui, Venezuela.

Content. Seven species: *Tepuihyla aecii* (Ayarzagüena, Señaris & Gorzula, 1993), *T. edelcae* (Ayarzagüena, Señaris & Gorzula, 1993), *Tepuihyla exophthalma* (Smith & Noonan, 2001) new combination, *T. luteolabris* (Ayarzagüena, Señaris & Gorzula, 1993), *T. rimarum* (Ayarzagüena, Señaris & Gorzula, 1993), *T. rodriguezi* (Rivero, 1968) and *T. warreni* (Duellman & Hoogmoed, 1992).

Tepuihyla galani (Ayarzagüena, Señaris & Gorzula, 1993) and *T. talbergae* Duellman & Yoshpa, 1996 are considered junior synonyms of *T. rodriguezi*, *Tepuihyla celsae* Mijares-Urrutia, Manzanilla-Puppo & La Marca, 1999 a junior synonym of *T. luteolabris*, and *O. phasmatus* MacCulloch & Lathrop, 2005 a junior synonym of *T. exophthalma*; see Appendix.

Distribution. Eastern and south-eastern Venezuela and western Guyana (Appendix S8.7).

Remarks. Two alternatives were considered to remediate the non-monophyly of *Osteocephalus*; placing *Tepuihyla* in the synonymy of *Osteocephalus* or including *O. exophthalmus*, *O. phasmatus*, and '*Hyla*' *warreni* in *Tepuihyla*. We chose the latter. *Tepuihyla* was originally defined by Ayarzagüena *et al.* ('1992' [1993]) by a number of character states, whose polarity was uncertain at the time. Of these, the reduction of webbing between toes I and II has been suggested as a putative synapomorphy by Faivovich *et al.* (2005), who also noticed instances of homoplasy in the Lophiohylini. The exposition of the frontoparietal fontanelle noted by Ayarzagüena et al. (1992) also is a putative synapomorphy overlooked by Faivovich *et al.* (2005), with several instances of homoplasy in the Lophiohylini as well (e.g. some species of *Osteocephalus* and *Osteopilus*; Trueb & Tyler 1974). Our redefinition of *Tepuihyla* dissociates the genus from these putative synapomorphies because '*Hyla*' *warreni*, '*O*'. *exophthalmus* and '*O*'. *phasmatus* have webbing between toes I and II (Duellman & Hoogmoed 1992; Smith & Noonan 2001; MacCulloch & Lathrop 2005), and at least '*O.*' *exophthalmus* has the frontoparietal fontanelle covered by the frontoparietals (Smith & Noonan 2001). The modification of content and concept of *Tepuihyla* is the less disruptive alternative in terms of deviation from the current taxonomy. The phylogenetic relationships of *T. luteolabris* and *T. rimarum* remain unknown, as tissues of these species are still unavailable.

Little is known about the breeding habits of *Tepuihyla*, and it is only recently that the tadpole of one species (*T. edelcae*) has been described (Myers & Donnelly 2008). All *Tepuihyla* species for which reproductive ecology is known breed in marsh and relatively shallow rocky pools in upland and highland savannahs (on white sand or sandstone) where several males congregate and call partially immersed in the water or from very low vegetation close to the water (PJRK, personal observation). Amplexus is axillary and eggs are laid as gelatinous masses (PJRK, personal observation). Tadpoles can tolerate acidic water (pH values *c.* 4). They are opportunistic feeders and have been seen feeding on dead animals including congeners (PJRK, personal observation). Virtually nothing is known about the reproductive ecology of *T. exophthalma* and *T. warreni*, which in contrast to other *Tepuihyla* species are primarily forest dwellers (MacCulloch & Lathrop 2005). Kok & Kalamandeen (2008) hypothesized that *T. exophthalma* (as *O. exophthalmus*) could be a phytotelm breeder, but this remains uncorroborated.

Discussion

Amazonian and Guiana Shield diversity

The Amazonian and Guianan regions (pan-Amazonia) form a continuous area integrating a mosaic of habitats that harbour a significant portion of the global amphibian fauna. About 1039 (16%) of the approximately 6370 amphibian species worldwide listed by the IUCN (2012) [AmphibiaWeb (2012) lists 7083 named species, but see Frost (2013)] are pan-Amazonian, with records of more than 100 species occurring in a few square kilometres (e.g. Lynch 2005). This already outstanding species diversity of amphibians, however, appears to be greatly underestimated because of a number of factors. First, large areas still remain unexplored from a taxonomic perspective. Second, most of the historical species hypotheses are based on brief and cursory phenotypic descriptions often lacking vouchered types (either lost, destroyed or never designated), specific type localities or both; this implies that new species descriptions are hampered by lack of sufficient information. An example is the toad *Rhinella margaritifera* (Laurenti, 1768) with 'Brasilia' (= Brazil) as its type locality, but in fact a complex of numerous species occurs within and outside Brazil (Fouquet *et al.* 2007b). Third, many species can be considered morphologically cryptic because they lack clear diagnostic characters; therefore, information on behaviour, reproductive biology, ecology and genetics is needed to discover species (e.g. Padial & De la Riva 2009). And fourth, although our understanding of the biogeography and origin of the Amazonian fauna is growing rapidly (Fouquet *et al.* 2012; see also summaries by Antonelli *et al.* 2010; Lovejoy *et al.* 2010; Wesselingh *et al.* 2010), we are still far from having a general understanding of the history and mechanisms responsible for species diversity across groups of organisms and areas (Hoorn *et al.* 2010), which in turn suggests that important hotspots of diversity still remain undetected under the apparently uniform forest cover.

These factors, combined with the realization that pan-Amazonia is biogeographically much more complex than previously thought, led some researchers to wonder if there are amphibian species in the Amazon region with wide distributions (i.e. distributions occupying most of the region) (e.g. Wynn & Heyer 2001). Since then, a few studies have specifically addressed this question for amphibians using two different strategies: (i) focussing on the diversity of a relatively small (compared to the total area of the pan-Amazon region), but well-sampled location, such as parts of French Guiana (Fouquet *et al.* 2007a) and the Chiquitania of Bolivia (Jansen *et al.* 2011), or (ii) studying the diversity of only a few species in genera such as *Adelophryne* and *Phyzelaphryne* (Fouquet *et al.* 2012), *Amereega* (Brown & Twomey 2009), *Engystomops* (Ron *et al.* 2006), *Hyalinobatrachium* (Castroviejo-Fisher *et al.* 2011), *Leptodactylus* (e.g. Heyer 2005; Heyer & de Sá 2011), *Oreobates* (Padial *et al.* 2012), *Pristimantis* (Elmer *et al.* 2007; Padial & De la Riva, 2009) and *Ranitomeya* (Brown *et al.* 2011).

The present contribution, with information from 328 specimens and 218 localities encompassing eight countries, constitutes one of the most extensive sampling efforts of pan-Amazonian amphibian groups. Our results reveal an intriguing pattern where four widespread nominal species (*O. buckleyi*, *O. leprieurii*, *O. planiceps* and *O. taurinus*) are indeed composites of species, with lower than previously reported genetic variability, but with wide distributions in some lineages. This pattern is similar to that found in certain groups of dendrobatids such as species of *Ranitomeya* and *Amereega* with low genetic distances (1–2%) among species, some of them having very restricted distributions (e.g. *R. summersi*), while others occur throughout thousands of kilometres (e.g. *R. amazonica*) (Brown *et al.* 2011).

Our results support an increase in the species diversity of *Osteocephalus* from 24 recognized species (taking into account three species removed from *Osteocephalus* and two synonymized here) to 27 (12.5% more species), if we only include confirmed lineages, or 33 (37.5%) more species, if we include all candidate species. A high level of overlooked diversity is not exclusive of *Osteocephalus*, but has been found in several amphibian groups in the region (Fouquet *et al.* 2007a,b, 2012; Jansen *et al.* 2011; Funk *et al.* 2012) and in other tropical areas such as Madagascar (Vieites *et al.* 2009; Vences *et al.* 2010), Central America (Crawford *et al.* 2010) or South-East Asia (Stuart *et al.* 2006). Recent studies of other vertebrates confirm this pattern, for example, for birds (Milá *et al.* 2012), mammals (Pavan *et al.* 2012) or reptiles (Bergmann and Russell 2007).

Candidate species within *Osteocephalus* are not randomly distributed across the species groups. The *O. taurinus* Group alone contributes five candidate species and accounts for 56% of the previously unrecognized species diversity within the genus. The *O. leprieurii* (two candidate species), *O. buckleyi* (1) and *O. planiceps* (1) Groups contribute more candidate species, while we did not detect any additional diversity in the *O. alboguttatus* Group.

To facilitate comparison, we recognize four geographic areas of unequal size within the pan-Amazon region. Each of these geographic regions harbours important areas of endemism that have been previously recognized in other studies (e.g. da Silva *et al.* 2005). We divide the pan-Amazon longitudinally into a northern and a southern area, along the main course of the Amazon River. Most of the north-eastern region coincides with the Guianan region (Hoogmoed 1979), divided from the north-western area by the Río Orinoco, the Casiquiare Canal and the Rio Negro. The southern area includes the basins of the rivers Tapajos, Tocantins, Xingu, Madeira and Juruá and is divided by the Rio Madeira, which separates the lowlands of Bolivia and the south-western part of Brazilian Amazonia from the basins of those rivers originating on the Brazilian Shield. The western area has a strong Andean influence, while the eastern area is more influenced by the uplands and highlands of the Precambrian shields of Guiana and Brazil.

Most of the diversity of the 33 species (24 nominal and 9 candidate species considered here) of *Osteocephalus* is concentrated in the western parts of the Amazon (24 spp.). The *O. buckleyi* Group is much more diverse (10 spp.) in the west than in other regions (one in the NE and two in the SE), especially in species that inhabit elevations above 500 m a.s.l. in the Andean foothills (seven spp.). This diversification is coincidental with the origin of a derived mode of reproduction (breeding in mountain streams; see Reproductive diversity). The presence of at least five species with mostly allopatric distributions (some overlap at the species' contact zones) suggests vicariance as the origin of speciation. One species in the SW (*O. helenae*) also occurs in the NE and spans a huge area in contrast to the other species in the group.

The *O. leprieurii* Group has one representative in each of the four sections, while all three species in the *O. alboguttatus* Group occur in the NW. The *O. planiceps* Group (six spp.) has a western distribution (five spp.) with one apparently isolated species (*O. planiceps* Ca1) at Pico de Neblina, an outcrop of the Guiana Shield, in the north-eastern sector.

The *O. taurinus* Group (seven spp.) is predominantly northern (six spp.), with five species associated with the Guianan region. Two species occur in the SE and SW sectors, among them *O. taurinus* s. str. that occurs in all four sectors. It is the most widely distributed species in the genus. Our results show that the eastern exemplars of *O. taurinus* s. str., from the gallery forests of the Cerrado, represent those of the most recent divergence in the clade, indicating a recent occupation of the Amazonian periphery.

Our findings of species richness in *Osteocephalus* corroborate the results found in other Amazonian frog groups (Fouquet *et al.* 2007a,b; Jansen *et al.* 2011; Funk *et al.* 2012) that the frog biodiversity in Amazonia is severely underestimated. While Funk *et al.* (2012) locally compared two species of *Engystomops* and two species of *Hypsiboas* with wide pan-Amazonian distributions and found an increase of species to 150–250% and 200–350%, respectively, of the current number, our results show a lower increase of 113–138% for the entire genus, suggesting either that *Osteocephalus* was a well-studied (and well-known) group or that its evolutionary history was different from that of *Engystomops* and *Hypsiboas*. Both assumptions appear to be incorrect. The difference is simply caused by our inclusion of species with smaller ranges. Thus, if we considered only the three widespread nominal species *O. buckleyi*, *O. leprieurii* and *O. taurinus* and added the candidate species, the number of species would increase from three to six CCS (200%), or to 9 CCS + UCS (300%). Considering that 1000 + species of amphibians are currently known to occur in pan-Amazonia, and combining our results with those of other studies, we consider that the diversity of Amazonia is highly underestimated. Broad-scale taxonomic analyses as the one presented here are much needed for other groups of organisms to understand the evolution, distribution patterns and biogeography of Amazonia, as well as to guide effective conservation measures.

Morphological and genetic data in Osteocephalus

There are few nominal species of *Osteocephalus* exhibiting fixed qualitative or quantitative characters to separate them from all other species in the genus (e.g. iris colouration in *O. subtilis*). Usually a set of characters is needed to diagnose currently accepted nominal species. The issue becomes more acute with increasing character variability within a supposed species. Most of the morphological characters found in 20 of the 24 nominal species of *Osteocephalus* vary intraspecifically by 7–21% (KHJ, unpublished data). The lowest variation is found in species with small ranges that are also represented in low numbers in collections. The three species with the largest ranges, and also the highest numbers of specimens examined, *O. buckleyi*, *O. leprieurii* and *O. taurinus sensu lato*, respectively, also have the highest percentages of variation (57–71%). These preliminary data substantiate our molecular findings with respect to candidate species. Similarly, the molecular data support the view that a great deal of the morphological variation might be due to the presence of cryptic species. Using morphological data, several (candidate) species can be further subdivided, most of them representing clades also recognizable in the trees (Figs 1–4). Likewise, among populations of *O. taurinus* s. str., there are clades that are extremely different. While breeding specimens from near the type locality measure 82 mm SVL in males and 104 mm in females, breeding specimens from some 250 km SW of that site measure 50 and 57 mm, respectively, only slightly more than half the size of the former specimens. This indicates that our molecular view of candidate species is conservative when compared with one focussed on morphology. Unfortunately, few bioacoustical or other data on isolating mechanisms between populations are available to allow a more integrative approach to decipher species diversity in the genus.

Reproductive diversity and evolution of reproductive biology in Osteocephalus

Exploitation of different breeding sites may be one reason for the successful colonization and sympatric occurrence of *Osteocephalus* species in pan-Amazonia. Our phylogenetic hypothesis indicates that pond breeding and egg clutches laid as a surface film is the plesiomorphic reproductive mode in *Osteocephalus* (Appendix S10). Both adaptations are advantageous in facilitating rapid development in warm water with low oxygen contents (e.g. Wells 2007). This mode is common in hylids, including many Lophiohylini (Faivovich *et al.* 2005). Males call while floating in water with lateral vocal sacs inflated, but they can also call while out of water. Jungfer & Hödl (2002) speculated that a tuberculate dorsum in large choruses of males, like those of *O. leprieurii* (Lescure & Marty 2000) or *O. taurinus* (Bokermann 1964), may facilitate recognizing that the wrong sex has been clasped. This reproductive mode occurs in all known (candidate) species of the *O. taurinus* Group except *O. oophagus*. The latter is not only the smallest species in the group, it also breeds in narrow phytotelmata. An amplecting pair deposits small clutches of about 250 eggs altogether in short intervals of about 5 days, and males lack dorsal tubercles and have a subgular vocal sac. All these character states have been proposed to be adaptations to breeding in phytotelmata (Jungfer & Weygoldt 1999). Small size allows for using very small bodies of water such as bromeliad leaf axils. Small sites can only hold few eggs, but if fewer eggs are laid, they can be produced at shorter intervals. Phytotelmata are not available *ad libitum*, so remembering and re-using them might be an advantage. Females of *O. oophagus* avoid sites where tadpoles of other females are already present. Parents return to phytotelmata where they have oviposited and provide their larvae with nutritive eggs. Larvae are dependent on them and will starve if the female does not return at least five times to feed them. Like other oophagus tadpoles in several anuran families, they have a reduced number of labial tooth rows (Silverstone 1975; Jungfer 1985; Ueda 1986; Lannoo *et al.* 1987; Duellman 2001). A small subgular vocal sac may be advantageous over large paired sacs in the confines of a narrow phytotelm, which in many cases acts as a resonator itself (KHJ, personal observation).

The shift from pond breeding sites to phytotelmata could be advantageous not only for tadpoles avoiding interspecific competition and numerous predators. The shift in call strategy away from large choruses to spaced individuals scattered in the forest may be seen as an adaptation to avoid predators of adults, too. The cost of a prolonged breeding season appears to be lower than that of explosive breeding.

Members of the *O. leprieurii* Group are pond breeders depositing eggs in surface films. Males of *O. leprieurii* have coloured keratinized nuptial pads, spiny backs and keratinized structures on other parts of the body (Jungfer & Hödl 2002). Explosive breeding in large choruses after heavy rains is the rule in this group, and male nuptial excrescences are advantageous not only on the hands. Dorsal spines in male–male interactions may be analogous to release calls. Furthermore, males become conspicuously yellow during breeding, possibly to warn other males or to attract females. This phenomenon is also known in other explosive breeders in the genera *Scinax*, for example *S. elaeochroa* (Duellman 2001), *Dendropsophus*, for example *D. microps* (Kwet 2001), *D. minutus* (Marty & Gaucher 1999), *Trachycephalus mesophaeus* (MTR, personal observation) or *Triprion petasatus* (KHJ, personal observation). Very often males in the *O. leprieurii* Group call while floating in water. Vocal sacs are lateral, as it occurs in several frog groups that call while floating on the water surface, such as *Pelophylax* or *Pseudis*.

The sister taxon of the *O. leprieurii* Group is the *O. buckleyi* Group, the only clade of stream-breeding *Osteocephalus*. Stream breeding is a synapomorphy of this group. Although they breed in moving, oxygen-rich water, most species have retained the ancestral character of a surface film of eggs. However, eggs of this group that become submerged develop normally (KHJ, personal observation), unlike those of lentic breeding *O. oophagus* (Jungfer & Weygoldt 1999), *O. taurinus* (KHJ, personal observation) or other hylids (e.g. Kluge 1981) in the field. Vocal sacs in the group do not appear to be different from the lateral ones of the *O. leprieurii* Group, although at least half of the (candidate) species usually call from vegetation along streams (KHJ, personal observation). The dorsal skin of breeding males ranges from dense keratinized spicules (*O. mimeticus*, *O. verruciger*) to simple, non-keratinized tubercles (*O. cabrerai*). It is not known whether skin texture is correlated with size of choruses or distribution of choruses along streams. Breeding period at least in *O. buckleyi* s. str. is lengthy (Jungfer & Weygoldt 1999).

Members of the *O. planiceps* Group breed in phytotelmata (breeding site unknown in *O. fuscifacies*) and all of them have similar smooth backs (except *O. planiceps*) and single, subgular vocal sacs in males (barely distensible and lacking vocal slits in *O. castaneicola*), similar to *O. oophagus* of the *O. taurinus* Group. Coloured keratinized spines on the nuptial excrescences of the thumb are reduced in most species.

Osteocephalus planiceps is exceptional for the group in being large, having extensive dark brown keratinized nuptial pads and tuberculate backs in males (although some sexually active males are smooth-backed). Tadpoles, at least of *O. castaneicola* and O. *deridens*, eat conspecific eggs and *O. deridens* larvae have a reduced number of labial tooth rows (Jungfer *et al.* 2000; Moravec *et al.* 2009). As already stated by Moravec *et al.* (2009), our consensus tree suggests that phytotelm breeding and tadpoles feeding on conspecific eggs have evolved independently in *Osteocephalus* at least twice and members of both groups independently evolved similar character states associated with this reproductive mode (subgular vocal sacs, smooth dorsa in males, reduction of tooth rows in tadpoles). They also exhibit nuptial excrescences that are reduced (less keratinization) or absent in males of three species, suggesting a lack of male-male competition by clasping. In one of those, *O. leoniae*, we observed females returning without males to tadpoles and feeding them on unfertilized nutritive eggs (KHJ, unpublished data). This reproductive mode has so far only been found in a few groups of frogs breeding in phytotelmata (reviewed by Lehtinen *et al.* 2004) or subterranean burrows (Gibson & Buley 2004).

Amplexus is a relatively conservative character in anurans. In most neobatrachians and all hylids, the amplexus is axillary (Duellman & Trueb 1986). In some *Osteocephalus*, there is a previously unknown position, in which the male clasps the female exclusively around the female's throat, here termed gular amplexus (Appendix S11). It was observed in two closely related species, *O. deridens* and *O. leoniae*, both in the *O. planiceps* Group, and can be expected in *O. fuscifacies* as well. In both species, this type of amplexus is employed exclusively and we observed it 100 + times, while we have never seen it in other species of *Osteocephalus*. These were always found in axillary amplexus. Gular amplexus may also be considered an adaptation to phytotelm breeding, because in both species, females carry amplecting males around, indicating that it is they who select the breeding site, although a male calling from a phytotelm may 'propose' one. This latter behaviour is similar to that of *O. oophagus* (Jungfer & Weygoldt 1999). Being held in the gular region may allow a female to climb more freely. Other females in the genus approach males at or near the breeding site on the ground (except the large *O. planiceps*).

Almost nothing is known about the reproduction of the members of the *O. alboguttatus* Group. However, the smooth backs and subgular vocal sacs of males might indicate that these frogs are phytotelm breeders as well.

Summarizing the evolutionary history of reproduction, it can be stated that explosive or opportunistic pond breeding is the ancestral state in *Osteocephalus*, as exhibited in the *O. taurinus* Group and retained in the *O. leprieurii* Group. Phytotelm breeding (partly combined with parental care for tadpoles) evolved in the *O. planiceps* Group and independently in *O. oophagus* (*O. taurinus* Group), and stream breeding in the *O. buckleyi* Group.

Authors' contributions

JF and KHJ designed the work. MTR, KHJ, JMP, SCF, VKV, JCC, CPTG, PHV, PJRK, SR, JM, CFBH, RDM, GGU, DBM, RE, IDR, APL and JCS contributed materials. JF, JMP and SCF analysed molecular data. JF, MLL, BvMB, PI, CFBH and WCW produced sequences. KHJ, JF, SCF and JMP wrote the paper. PHV prepared maps. All authors read, contributed and approved the final version of the manuscript.

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Appendix 1: Synonyms among species of *Osteocephalus* and *Tepuihyla*

Mainly because of differences in juvenile and adult colourations, several species of *Osteocephalus* have been described more than once and/or placed into different genera (Jungfer 2010). We are aware of more such cases. While synonymizing junior taxon names and establishing old neglected ones is beyond the scope of this work, we nonetheless deem it unwise to use names that will soon be considered outdated. On the other hand, it is hard to follow synonymizations when no sufficient reasons are given. The following account – though still not complete – gives a brief overview of the changes proposed here and why. In the case of old taxon names (such as '*Hyla helenae*'), a more detailed study is in progress.

Likewise, several species of *Tepuihyla* have been described from isolated mountain tops using few specimens for which intraspecific variation was poorly known, but which were treated as distinct species basically because of their isolated distributions. More populations and specimens together with molecular data indicate that several of these taxa have to be treated as junior synonyms.

Osteocephalus helenae (Ruthven, 1919) nov. comb. This species was considered as *incertae sedis* by Faivovich *et al.* (2005). However, its holotype (UMMZ 52681) from Dunoon, Guyana, is a recently metamorphosed juvenile with the typical colouration of most of the members of the *O. buckleyi* Species Group (dark spots on light ground and light colour of distal part of upper arm and elbow, and also of knee and tarsus, compare Appendix S1). Based on molecular data of tissue taken from frogs earlier considered to be *O. buckleyi* (Kok & Kalamandeen 2008) or *O. cabrerai* (Gorzula & Señaris '1998' [1999]; Lescure & Marty 2000) from north-eastern South America, we consider it a valid species of *Osteocephalus*. This well-supported clade is widely distributed from north-eastern South America south-westward to southern Peru and Bolivia.

Osteocephalus germani Ron, Venegas, Toral, Read, Ortiz & Manzano, 2012. The type locality of *O. germani* is near Pongo de Mainique, La Convención, Cusco, Peru. Our specimen MHNC7004 from Río Nusiniscato, Cusco, Peru, agrees well morphologically with the holotype and genetically with a paratopotype of *O. germani* included in our tree. They form a large, well-supported clade that also includes specimens from Guyana considered to be *O. helenae*. For this reason, we consider *O. germani* a junior synonym of *O. helenae*. That *O. germani* and specimens from north-eastern South America were likely to be conspecific was also stated by Ron *et al.* (2012).

Osteocephalus vilmae Ron, Venegas, Toral, Read, Ortiz & Manzano, 2012. Within *O. buckleyi* as presented here, there are two clades with low genetic distances (uncorrected *p* distance 1.6%). They were also found by Ron *et al.* (2012). One of these is considered a distinct species, *O. vilmae*, by them. We have seen specimens of the '*vilmae*' clade from Yasuní, Ecuador and the Iquitos region in Loreto, Peru, one of each locality is contained in our tree. For the following reasons, we do not follow their arguments, but consider *O. vilmae* a junior synonym of *O. buckleyi*: Morphologically, we were unable to distinguish '*O. vilmae*' from other *O. buckleyi*. In the original description, '*O. vilmae*' is distinguished from *O. buckleyi* by having scattered and weakly keratinized dorsal tubercles (abundant and keratinized in *O. buckleyi*). We have seen both character states in both males of the '*vilmae*' and the '*buckleyi*' clade. Especially keratinization is strongly dependent on reproductive activity and not a reliable character when breeding and non-breeding frogs are compared. '*Osteocephalus vilmae*' is furthermore characterized by its larger SVL in males (50.74 mm ± 3.17 SD; *n* = 6; maximum 55.77 mm), while *O. buckleyi* are stated to have an SVL of 41.12 mm ± 2.49 SD. Five of our males genetically belonging to the '*vilmae*' clade or collected with them are 42.2–50.6 mm in SVL (46.0 ± 3.80) and six more males, like the holotype of '*O. vilmae*' from Loreto, Peru, make the range 38.2–50.6 mm (43.3 ± 3.78) SVL, close to the mean given for *O. buckleyi* by Ron *et al.* (2012). Remarkably, our only female in the '*vilmae*' clade, a mature specimen of 54.9 mm, is smaller than the largest male measured by Ron *et al.* (2012), which is exceptional in *Osteocephalus*. In almost all species, females are considerably larger than males.

Ron *et al.* (2012) found a more extensive and conspicuous areolate area on the flanks in '*O. vilmae*', reaching from the axilla to the groin (to anterior half of flank in *O. buckleyi*). In our specimens, the areolate area covers the first ¼ to ¾ of the flanks in '*O. vilmae*' and ⅓ to ½ in other specimens of *O. buckleyi* from Ecuador, leaving too much overlap to distinguish between both.

At least in captivity, specimens ascribed morphologically and genetically to *O. buckleyi* and '*O. vilmae*' (*sensu* Ron *et al.* 2012) were able to interbreed. The offspring was able to reproduce successfully as well. One such 'cross-breed' (Trier 028_2742) was included in our tree and grouped with the '*vilmae*' clade. Ron *et al.* (2012) state that both 'species' were sympatric at one site and assume reproductive barriers between them. These apparently do not exist.

It has been demonstrated only recently that Guianan upland and highland species, including populations from different tepui summits, are less divergent genetically than previously thought, suggesting that they have evolved in isolation only for a relatively short time (Kok *et al.* 2012; Salerno *et al.* 2012). Although some morphological differences between *T. exophthalma* and *O. phasmatus* (= *T. phasmata*), namely in SVL, tympanum size and amount of webbing, have been reported (Smith & Noonan 2001; MacCulloch & Lathrop 2005; Kok & Kalamandeen 2008), examination of a larger sampling shows that these characters are variable and are not strongly diagnostic. This, coupled with the low interspecific genetic divergence (ranging from 0.0% to 0.2%), strongly suggests that a single species with a wider morphological and geographic range is involved. Therefore, we consider *T. phasmata* a junior synonym of *T. exophthalma*.

Likewise, Kok *et al.* (2012) demonstrated the low genetic divergence between *T. rodriguezi*, *T. galani* and *T. talbergae* even in the rapidly evolving mitochondrial ND1 gene. Descriptions of *T. galani* and *T. talbergae* were based on only four and two specimens, respectively. Examination of additional specimens of these two species, including some from the type localities, showed that the morphological characters used to diagnose *T. galani* and *T. talbergae* are more variable than was stated in the original descriptions. Furthermore, these diagnostic characters exhibit considerable overlap among *T. galani*, *T. talbergae* and *T. rodriguezi* (PJRK, unpublished data). We therefore consider *T. galani* and *T. talbergae* to be junior synonyms of *T. rodriguezi*.

Salerno *et al.* (2012), on the basis of personal communication with César Barrio-Amorós, argued that *T. celsae*, a species purportedly from Falcón, Venezuela (outside the Guiana Highlands and pan-Amazonia), most likely is a junior synonym of *T. luteolabris* based on a specimen with incorrect locality data. We follow this argumentation after inspection of type material of *T. celsae* by one of us (JCS).

Supporting Information

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

Appendix S1. Recently metamorphosed juveniles of *Osteocephalus* and of juveniles previously assigned to that genus. The constant pattern in the colouration of juveniles of *Osteocephalus* (*sensu* this work) is proposed as a morphological synapomorphy for the genus. *Osteocephalus taurinus* Species Group: (a) *O. taurinus* (Reserva Ducke, Manaus, Amazonas, Brazil); (b) *O. oophagus* (Reserva Ducke, Manaus, Amazonas, Brazil). *Osteocephalus leprieurii* Species Group: (c) *O. leprieurii* (Arataï, French Guiana). *Osteocephalus planiceps* Species Group: (d) *O. castaneicola* (San Antonio, Pando, Bolivia); (e) *O. deridens* (Iquitos, Loreto, Peru); (f) *O. leoniae* (Tarapoto, San Martín, Peru); (g) *O. planiceps* (Jatun Sacha, Napo, Ecuador). *Osteocephalus buckleyi* Species Group: (h) *O. buckleyi* (Jatun Sacha, Napo, Ecuador); (i) *O. carri* (Picachos, Huila, Colombia); (j) *O. mimeticus* (Tarapoto, San Martín, Peru); (k) *O. mutabor* (Cordillera Galeras, Napo, Ecuador); (l) *O. verruciger* (Reventador, Sucumbíos, Ecuador). *Dryaderces pearsoni* (m) (Rurrenabaque, Beni, Bolivia). *Itapotihyla langsdorffii* (n) (Rio de Janeiro, RJ, Brazil). *Tepuihyla rimarum* (o) (Ptari-tepui, Bolívar, Venezuela) (not to scale).

Appendix S2. Localities, voucher information and GenBank accession numbers for DNA sequences used (in bold: sequences produced for this study). Abbreviations used for vouchers (unless downloaded from GenBank): AMNH: American Museum of Natural History, New York, USA. APL: Albertina P. Lima, Laboratório de Vertebrados da Ecologia - INPA collection, Manaus, Brazil. AJC: Andrew J. Crawford field numbers. CBF: Colección Boliviana de Fauna, La Paz, Bolivia. CFBH Collection Célio F. B. Haddad, Departamento de Zoologia, I.B., UNESP, Rio Claro, SP, Brazil. CPI: Coastal Plains Institute and Land Conservancy (Field numbers of D. Bruce Means), Tallahassee, FL, USA. EPN: Escuela Politecnica Nacional, Quito, Ecuador. GGU: Giussepe Gagliardi-Urrutia field numbers at UNAP (Universidad Nacional de la Amazonía Peruana, Iquitos, Peru). IRSNB: Royal Belgian Institute of Natural Sciences, Brussels, Belgium. JMP: José M. Padial field numbers. KHJ-F: Karl-Heinz Jungfer field numbers, to be divided between MUSM and MTD. MACN: Museo Argentino de Ciencias Naturales "Angel Gallardo"—CONICET, Buenos Aires, Argentina. MAR: Marco Rada field numbers. MHNC Museo de Historia Natural, Universidad Nacional de San Antonio Abad del Cusco, Cusco, Peru. MHNLS: Museo de Historia Natural La Salle, Caracas, Venezuela. MNCN Museo Nacional de Ciencias Naturales, Madrid, Spain. MUSM: Museo de Historia Natural de la Universidad de San Marcos, Lima, Peru. MSH: Marinus S. Hoogmoed field numbers. MTD: Senckenberg Naturhistorische Sammlungen, Dresden, Germany. MTR: Miguel T. Rodrigues field numbers. MZUSP: Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil. NMP: National Museum, Zoology, Praha, Czech Republic. PHV: Paula Hanna Valdujo field numbers (to be accessioned in MZUSP). PK: Philippe Kok field numbers deposited at IRSNB. ROM: Royal Ontario Museum, Toronto, Canada. SMNS: Staatliches Museum für Naturkunde, Stuttgart, Germany. SMS: Sergio Marques de Souza field numbers (to be accessioned in MZUSP). TG: Taran Grant field numbers. TNHC: Texas Natural History Collections, Austin, USA. UA: Universidad de los Andes, Bogotá, Colombia. Vogt: Richard Vogt field numbers. Locality coordinates are given for specimens newly accessed to GenBank.

Appendix S3. Models of nucleotide substitution for the partitions used in the maximum likelihood phylogenetic analyses.

Appendix S4. (a) Inter- and intraspecific uncorrected genetic distances (diagonal and below the diagonal respectively) within members of the *Osteocephalus taurinus* species group (sample size in parentheses) inferred from 551 aligned characters of a fragment of the mitochondrial 16S gene. *ss* = *sensu stricto*. (b) Inter- and intraspecific uncorrected genetic distances (diagonal and below the diagonal respectively) within members of the *Osteocephalus planiceps* species group (sample size in parentheses) inferred from 551 aligned characters of a fragment of the mitochondrial 16S gene. (c) Inter- and intraspecific uncorrected genetic distances (diagonal and below the diagonal respectively) within members of the *Osteocephalus leprieurii* species group (sample size in parentheses) inferred from 551 aligned characters of a fragment of the mitochondrial 16S gene. ss = *sensu stricto*. (d) Inter- and intraspecific uncorrected genetic distances (diagonal and below the diagonal respectively) within members of the *Osteocephalus buckleyi* species group (sample size in parentheses) inferred from 551 aligned characters of a fragment of the mitochondrial 16S gene. ss = *sensu stricto*.

Appendix S5. Phylogenetic relationships of *Dryaderces*, *Osteocephalus*, *Tepuihyla*, and outgroups inferred from a maximum likelihood analysis, executed in the program GARLI 2.0, of a partitioned matrix of a static alignment (generated with a multiple sequence alignment in Clustal-W). Partitions and their respective models of sequence evolution are detailed in Appendix S3. Not all loci are available for all terminals.

Appendix S6. (1–6) Phylogenetic relationships of *Dryaderces*, *Osteocephalus*, *Tepuihyla*, and outgroups inferred from maximum parsimony analysis under a static alignment (generated with a multiple sequence alignment in Clustal-W) in the program T.N.T., Willi Hennig Society Edition. This topology reflects one of the 4797 most parsimonious trees (length 13 254 steps), with black nodes on dots indicating collapsed clades in strict consensus tree; not all loci are available for all terminals. Tips are labelled with the initial and tentative field identifications. See Appendix S2 and/or Figs 1–4 for current classification.

Appendix S7. List of some of the molecular transformations common to all most parsimonious trees of the static parsimony analysis, supporting the monophyly of each of the three genera discussed in the text. Positions correspond to the alignment stored in Dryad Repository DOI: http://dx.doi.org/10.5061/dryad.j04vf.

Appendix S8. (1) Map of localities of sampled exemplars of *Dryaderces* gen. n. in central and southern Amazonia. (2) Map of localities of sampled exemplars of the *Osteocephalus alboguttatus* Species Group. (3a) Map of localities of sampled exemplars of the *Osteocephalus buckleyi* Species Group (part). (3b). Map of localities of sampled exemplars of the *O. buckleyi* Species Group (part). (4) Map of localities of sampled exemplars of the *Osteocephalus leprieurii* Species Group. (5) Map of localities of sampled exemplars of the *Osteocephalus planiceps* Species Group. (6) Map of localities of sampled exemplars of the *Osteocephalus taurinus* Species Group. (7) Map of localities of sampled exemplars of *Tepuihyla* on the Guiana Shield.

Appendix S9. Fully inflated vocal sacs in *Osteocephalus*: (a) paired, lateral with subgular expansion (*O. leprieurii*, pond breeder); (b) and (c) paired, lateral with subgular expansion (*O. verruciger*, stream breeder); (d) and (e) paired, lateral with subgular expansion (*O. buckleyi*, stream breeder); (f) single, subgular (*O. oophagus*, phytotelm breeder).

Appendix S10. Egg clutches of *Osteocephalus*: (a) surface film (*O. taurinus*, pond breeder); (b) clutch attached to a bromeliad leaf axil at surface level (*O. oophagus*, phytotelm breeder); (c) surface film of a phytotelm breeder at a spacious site (*O. planiceps*) forming during egg-laying.

Appendix S11. Types of amplexus in *Osteocephalus*: (a) axillary (*O. yasuni*, pond breeder); (b) axillary (*O. verruciger*, stream breeder); (c) gular (*O. leoniae*, phytotelm breeder); (d) gular (*O. deridens*, phytotelm breeder); (e) axillary *O. oophagus*, phytotelm breeder.