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RELAÇÕES FILOGENÉTICAS EM PERERECAS MARSUPIAIS (ANURA: HEMIPHRACTIDAE): IMPACTO DA EVIDÊNCIA TOTAL, CRITÉRIOS DE OTIMIZAÇÃO, MÉTODOS DE ALINHAMENTO E TRATAMENTO DE INDELS

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Orientador: Dr. Santiago Castroviejo-Fisher

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Sumário

Agradecimentos

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Resumo

O efeito do critério de otimização tem sido amplamente discutido na sistemática filogenética, especialmente em relação ao desempenho dos métodos paramétricos e não paramétricos. Porém, outros fatores analíticos menos discutidos na literatura (p. ex. alinhamento, pessagem de caracteres, codificação de indels, seleção de modelos), podem ter efeitos tão importantes como o critério de otimização nas análises de inferência filogenética. Neste trabalho, foram utilizadas seis estratégias analíticas, combinando diferentes critérios de otimização (Parcimônia vs. Máxima Verosimilhança), métodos de alinhamento (*tree-aligment* ou otimização direta vs. alinhamento por similaridade), e três métodos de codificação de indels, para avaliar o efeito de cada uma destas variáveis na inferência das relações filogenéticas de Hemiphractidae a partir de uma matriz de evidência total. A matriz analisada foi construída principalmente por dados gerados nos mais recentes e extensos trabalhos filogeneticos de Hemiphractidae, e 219 sequências de DNA geradas neste estudo correspondentes a 10 genes mitocôndrias e nucleares de 30 especies de pererecas marsupiais. O conjunto de dados final incluiu, 143 terminais de Hemiphractidae, 127 terminais do grupo externo, sequências de DNA de 20 genes mitocondriais e nucleares, e 51 caracteres fenotípicos. A comparação dos resultados das seis estratégias analíticas, mostrou que tanto o método de alinhamento como o método de codificação de indels, podem gerar diferenças da mesma magnitude que o critério de otimização. Portanto, os resultados deste trabalho contribuem com evidencia empírica sobre o importante rol das homologias inferidas a través do alinhamento, e da informação dos indels na inferência de relações filogenéticas.

Palavras chave: alinhamento por similaridade, homologia, indels, máxima verosimilhança otimização direta, parcimônia.

Phylogenetic relationships of egg-brooding frogs (Anura: Hemiphractidae), impact of total evidence, optimality criteria, alignment methods, and indel information

Abstract

Alternative and often discordant phylogenetic hyphoteses are published simultaneously or successively for many groups. Therefore, studies that investigate the potential effect of relevant analytical factors become a useful approach needed to choose among analytical options and detect the causes of observed differences. Six analytical strategies were used, combining different optimality criteria (parsimony vs. maximum likelihood), alignment methods (tree- vs. similarity-alignment), and three indel coding schemes to study their effect on the inferred phylogenetic relationships based on a total-evidence dataset of Hemiphractidae. The comparison of the results of the six analyses demonstrated that: (i) alignment and indel coding methods can both influence phylogenetic relationships in the same degree as the optimality criterion; (ii) previous differences observed among studies were not caused by differences in character or taxon sampling, but by differences in analytical factors; (iii) current implementations of maximum likelihood support topologies without evidence due to undersampling limitations; and (iv) the two current supraspecific taxonomies used for *Gastrotheca* are incompatible with my results.

Key words: direct optimization, homology, indels, maximum likelihood, parsimony, similarity-alignment.

Apresentação

Este trabalho teve como objetivo principal avaliar o impacto do uso de diferentes critérios de otimização (parcimônia vs. máxima verossimilhança), métodos de alinhamento (*tree-alignment* vs. similaridade) e de diferentes tratamentos dos eventos de inserção e deleção (*indels*), na inferência das relações filogenéticas de Hemiphractidae, a partir da análise de um conjunto de dados de evidência total.

A dissertação consiste em um artigo, escrito em inglês, intitulado "*Phylogenetic relationships of egg-brooding frogs (Anura: Hemiphractidae): impact of total evidence, optimality criteria, alignment methods, and indel information*". O artigo foi escrito no formato da revista Cladistics.

A Introdução informa sobre o contexto atual da sistemática filogenética em relação as três variáveis analíticas estudadas no trabalho. Apresentam-se, brevemente, aspetos teóricos e práticos importantes do alinhamento e codificação de *indels*. A seguir, a apresentação de Hemiphractidae destaca a importância desta família no estudo da evolução do desenvolvimento em anfíbios, pela diversidade e singularidade da biologia reprodutiva das pererecas marsupiais, e também a constante controvérsia em torno de suas relações filogenéticas. Tais características fizeram de Hemiphractidae, um grupo de estudo ideal para o presente trabalho.

A seção de Materiais e Métodos inclui uma descrição detalhada dos dados analisados, considerações teóricas relevantes para a interpretação dos resultados, a descrição das seis análises filogenéticas utilizadas no estudo, das análises utilizadas para a comparação dos resultados de cada uma das análises (e.g. custos recíprocos e clados compartilhados), e da análise para a deteção de terminais inestáveis (*wildcards*).

A seção de Resultados inclui a descrição qualitativa das árvores filogenéticas mais ótimas obtidas através das análises. A subseção Comparação de Árvores descreve os resultados quantitativos das análises de comparação de árvores filogenéticas. A subseção Wildcard taxa inclui os resultados da análise para a deteção de terminais inestáveis (*wildcards*) a partir do conjunto de árvores óptimas para cada uma das análises de parcimônia. Finalmente, são apresentadas quatro subseções com a descrição detalhada das diferenças causadas pelas três variáveis analíticas estudadas, através da comparação das árvores filogenéticas das seis análises (*e.g.* posição de Hemiphractidae em Nobleobatrachia, monofilia e relações entre taxa supraespecíficos)

A Discussão está dividida em quatro subseções que correspondem a considerações em relação aos principais estudos filogenéticos da família, discussão dos efeitos dos três fatores analíticos estudados, e uma proposta taxonômica para *Gastrotheca*. Na seção de Conclusões, enfatiza-se a importância dos fatores analíticos estudados no trabalho, especialmente o método de alinhamento e o tratamento dos *indels*, na inferência de relações filogenéticas. Finalmente, o artigo inclui as seções de Agradecimentos, Referências Bibliográficas, Apêndices, e Figuras e Tabelas suplementares.

Autoría

O presente trabalho será usado como a base de um manuscrito em conjunto com os seguintes colaboradores: Santiago Castroviejo-Fisher, Ignacio De la Riva, Carles Vilà, Pablo J. Venegas e Fernando J. M. Rojas-Runjaic.

CAPÍTULO 1

Phylogenetic relationships of egg-brooding frogs (Anura: Hemiphractidae), impact of total evidence, optimality criteria, alignment strategy, and indel information

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Abstract

Alternative and often discordant phylogenetic hyphoteses are published simultaneously or successively for many groups. Therefore, studies that investigate the potential effect of relevant analytical factors become a useful approach needed to choose among analytical options and detect the causes of observed differences. Six analytical strategies were used, combining different optimality criteria (parsimony vs. maximum likelihood), alignment methods (tree- vs. similarity-alignment), and three indel coding schemes to study their effect on the inferred phylogenetic relationships based on a total-evidence dataset of Hemiphractidae. The comparison of the results of the six analyses demonstrated that: (i) alignment and indel coding methods can both influence phylogenetic relationships in the same degree as the optimality criterion; (ii) previous differences observed among studies were not caused by differences in character or taxon sampling, but by differences in analytical factors; (iii) current implementations of maximum likelihood support topologies without evidence due to undersampling limitations; and (iv) the two current supraspecific taxonomies used for *Gastrotheca* are incompatible with my results.

Introduction

Systematics experienced a renaissance in the last three decades invigorated by technological and theoretical advances related to the generation and analysis of geneticdata (e.g., Felsenstein, 1981; Drummond *et al*., 2006; Wheeler *et al*., 2006; Zwickl, 2006; Goloboff *et al*., 2008; Rannala and Yang, 2008; Lemmon and Lemmon, 2013; Goodwin *et al*., 2016). This wealth of data and analytical factors creates a diversity of alternative results that deserve detailed comparisons, to both evaluate the effect on the results of analytical factors (e.g., optimality criteria, intensity of tree searches) and determine the most explanatory phylogenetic hypotheses given the data. Adding to the difficulty created by the variety of results that stem from using different characters and analyses is the hierarchical nature of phylogenetic hypotheses, which leads to marked differences in taxon sampling among studies depending on which taxon is considered the ingroup.

The cyclic nature of phylogenetics and the necessity of repeatability, replicability, and maximization of explanation leads to "total evidence" analysis (Kluge 1989, 1997, 1998; Eernisse and Kluge, 1993)—other synonymous terms are "supermatrix", "simultaneous-analysis", "combined-analysis" or "concatenated-analysis" (de Queiroz and Gatesy, 2007). Total evidence has two components, (i) simultaneous analysis of all relevant available data for the problem at hand, and (ii) its epistemological strength resides in the maximization of explanatory power (Kluge, 1989; Kluge and Wolf, 1993; Kluge, 1998). Thus, revisionary approaches that combine legacy empirical data derived from independent studies of partially overlapping datasets are crucial to summarize our state of understanding of the phylogenetic relationships among any group of organisms (Driskell *et al*., 2004; Gatesy *et al*., 2004; Frost *et al*., 2006; Pyron and Wiens, 2011; Padial *et al*., 2014; Castroviejo-Fisher *et al*., 2015; Goicoechea *et al*., 2016; Peloso *et al*., 2016). Nonetheless, combining characters and terminals from independent studies often result in datasets with large amounts of missing data (often >

60 %; Simmons and Goloboff, 2014) and only tackles the problem of differences among results caused by variations in taxon and character sampling.

Missing data can neither support nor reject relationships and analytical methods should reflect this absence of information. In other words, evidence that does not exist should not affect the choice of optimal tree (s). However, current implementations—regardless of optimality criterion—may lead to biased results (Lemmon *et al*., 2009; Siddall, 2010; Simmons, 2012a, b; Simmons and Goloboff, 2013, 2014; Simmons and Norton, 2013; Goloboff, 2014; Simmons and Randle, 2014) unless specific measures are taken. Due to computational limitations, most of these measures (e.g. saving a representative set of equally optimal trees and implementing: perturbation-based methods, sectorial searches, simulated annealing, and genetic algorithms) are currently unavailable for parametric approaches to phylogenetics (Simmons and Goloboff, 2013, 2014).

Although a phylogenetic total evidence analysis allows evaluation of differences among previous competing hypotheses due to character sampling and taxon sampling, it is mute about the effect that other analytical factors may have played in selecting incompatible optimal topologies. Arguably, most attention has been directed to differences caused by choice of optimality criterion, on both simulated and empirical studies (e.g., Felsenstein, 1978; Huelsenbeck and Hillis, 1993; Huelsenbeck, 1995, 1997; Yang, 1996a; Siddall and Kluge, 1997, 1999; Siddall, 1998; Wiens and Servedio, 1998; Siddall and Whiting, 1999; Huelsenbeck *et al*., 2001; Pol and Siddall, 2001; Swofford *et al*., 2001; Leaché and Reeder, 2002; Huelsenbeck and Lander, 2003; Kolaczkowski and Thorton, 2004; Yu *et al*., 2008; Puttick *et al*., 2017). However, a variety of other variables could be equally influential on the inferred phylogenetic hypothesis. For example, alignment of sequence data, indel coding, character weighting, model selection, heuristic tree-search strategies, and representation of results such as optimal trees versus a variety of consensus trees (Chippindale and Wiens, 1994; Simon *et al*., 1994; Yang *et al*., 1994;

Milinkovitch *et al*., 1996; Yang, 1996b; Morrison and Ellis, 1997; Simmons and Ochoterena, 2000; Simmons *et al*., 2001; Brandley *et al*., 2005; Ogden and Rosenberg, 2006; Zwickl, 2006; Brown and Lemmon, 2007; Kumar and Filipski, 2007; McGuire *et al*., 2007; Simmons *et al*., 2007; Li *et al*., 2008; Wong *et al*., 2008; Ward *et al*., 2010; Sanderson *et al*., 2011; Goloboff, 2014). Although these and other analytical variables deserve careful investigation, herein, I will focus on optimality criterion, alignment method, and indel coding—the last two are briefly introduced below.

A critical variable to phylogenetic analyses are the alignments as representations of homology schemes (Wheeler, 1996, 2001, 2007). The highly simplified nature of DNA sequences and the non-transitivity of the correspondences between its elements makes homology determination very complex (Wheeler, 2012). The universe of potential alignments that exists for large datasets could be even greater than the number of potential trees (Wheeler, 2012), and different alignment methods lead to different alignments, hence different phylogenetic trees (Wheeler, 1996; Morrison and Ellis, 1997; Whiting *et al*., 2006; Lindgren and Daly, 2007; Wong *et al*., 2008; Wheeler, 2012; Padial *et al*., 2014; Goicoechea *et al*., 2016; Peloso *et al*., 2016). The Tree-Alignment Problem (TAP) (Sankoff, 1975) considers the inference of homologies from DNA sequences as a problem inseparable from the phylogenetic tree inference, and the overall cost of the alignment is minimized on a phylogenetic tree (Wheeler and Giribet, 2009; Varón and Wheeler, 2012). Given its compound complexity, no polynomial time solutions exist, so heuristic approaches to the TAP must be implemented. Multiple sequence alignments (MSA) and tree searches are traditionally performed in two steps. First, fixed homologies are established, by inserting gaps, for DNA sequences of different length. These character correspondences are based on similarity by optimizing an objective function (e.g. "sum-of-pairs", "weighted sum-of-pairs", or "consensus" functions) (Wheeler, 1996, 2012; Notredame, 2002; Edgar and Batzoglou, 2006). The second step consists of tree searches, evaluating all trees under the set of homologies previously defined, using an optimality criterion different from the one implemented in the MSA step and often treating gaps as nucleotides of unknown identity. Direct Optimization (DO) (Wheeler, 1996, 2012) is a TAP heuristic that evaluates simultaneously and under the same optimality criterion, both base-to-base correspondences and trees, finding the homologies that minimize tree cost (Wheeler *et al*., 2006). As a result, each tree has a set of specific homologies (Wheeler, 2007, 2012) that can be visualized as a matrix through an implied alignment (Wheeler, 2003a).

Alignments of DNA sequences of unequal length include gaps representing insertion or deletion events (Wheeler, 1996; Giribet and Wheeler, 1999). The information carried by indel events is known to be useful in phylogenetic analyses (e.g. Klopfstein *et al*., 2013; Luan *et al*., 2013; Ren *et al*. 2019). However, indels are not commonly incorporated into phylogenetic analysis because it is considered that existing methods or models to incorporate indel information into analyses do not represent its complexity or due to computational difficulties (McGuire *et al*. 2001; Yang, 2014). Indel information can be included into phylogenetic analysis by using a TAP algorithm or by coding indels after the alignment step. Some parsimony programs consider gaps as a fifth state, where contiguous gaps are treated as an independent single indel event (e.g., Swofford, 2002; Wheeler *et al*., 2006; Goloboff *et al*., 2008). For statistical approaches treatment of indels include: treating indels as a fifth state (McGuire *et al*., 2001) by extending models as F84 (Felsenstein and Churchill, 1996); full statistical alignment, joint estimation of alignment and phylogenetic tree, incorporating a model for indels (Fleissner *et al*., 2005; Lunter *et al*., 2005; Redelings and Suchard, 2005; Suchard and Redelings, 2006; Novák *et al*., 2008).

The many available coding methods can be discriminated by their complexity; however, they are all similar in considering contiguous gaps as non-independent. The Single Indel Coding method (SIC) of Simmons and Ochoterena (2000) gaps with the same start and end positions as presence-absence characters. Other methods code single gaps as a fifth state, but contiguous gaps as presence/absence characters (Barriel, 1994) or consider only the first gap position, coding adjacent gaps as missing data (Bena *et al.*, 1998). The more complex methods code overlapping indels as multistate characters (Baum *et al.*, 1994; Freudenstein and Chase, 2001; Gonzalez, 1996; Simmons and Ochoterena, 2000; Müller, 2006).

While total evidence approaches can be used to evaluate the effect of different character and taxon sampling, only through sensitivity analysis can one address issues related to analytical variables (Giribet and Wheeler, 2007). Contrived data have the advantage of allowing the researcher access to the objective truth before the analysis, enabling clear-cut evaluations of the performance of different discovery operations and their assumptions. However, an unresolvable question is how much can one extrapolate from simulations, necessarily simplistic, into the real world. Thus, studying empirical data, even with its associated uncertainty, still plays a major role on our understanding of analytical methods in phylogenetics. Focusing on the empirical case of egg-brooding frogs, a group with an old pedigree of controversy in amphibian systematics, I evaluate the effect that optimality criterion, alignment strategy, and indel coding have on phylogenetic inference obtained from a total-evidence dataset.

Egg-brooding frogs (Hemiphractidae) are non-aquatic Neotropical anurans, with 117 described species, grouped in six genera: *Cryptobatrachus*, *Flectonotus*, *Fritziana*, *Gastrotheca*, *Hemiphractus*, and *Stefania* (Frost, 2019). Their distribution includes a wide variety of habitats, from Neotropical lowland or montane rainforest to humid high-elevation Andean grasslands and the tepuis of the Guiana Shield (Castroviejo-Fisher *et al.*, 2015; Duellman, 2015). The reproductive biology of hemiphractids is not only unique but diverse. For instance, their common name derives from the fact that females carry their eggs on their backs, either inside a pouch or exposed on the dorsum (Del Pino, 1980). A variety of developmental modes are present among the members of this family, from direct development in species of

Cryptobatrachus, *Hemiphractus,* and *Stefania*, to development with a free-living tadpole phase in species of *Flectonotus* and *Fritziana*, and some species presenting direct development and other aquatic larvae in *Gastrotheca* (Duellman, 2015). Given the diversity of developmental modes within Hemiphractidae, its internal phylogenetic relationships and position within Nobleobatrachia are key to the study of the evolution of development in amphibians.

Despite their relevance, egg-brooding frogs' systematics are controversial. For most of the twentieth century and part of the twenty-first century, hemiphractids were considered as Hemiphractinae within Hylidae (Noble 1927, 1931; Duellman and Hoogmoed, 1984; Silva, 1998; Mendelson *et al*., 2000; Burton, 2004). Novel hypothesis of the phylogenetic position of Hemiphractidae were proposed by phylogenetic studies using DNA sequences (e.g., Darst and Canatella, 2004; Faivovich *et al*., 2005; Wiens *et al*., 2005; Frost *et al*., 2006; Wiens *et al*., 2006; Wiens *et al*., 2007; Guayasamin *et al*., 2008; Heinicke *et al*., 2009; Pyron and Wiens, 2011; Blackburn and Duellman, 2013; Padial *et al*., 2014). However, different taxon and character sampling, as well as analytical approaches led to different results regarding the monophyly of the family and the relationships among genera (Wiens, 2007; Frost *et al*., 2008; Guayasamin *et al*., 2008). In 2015, two independent studies revisited the evolutionary relationships of egg-brooding frogs (Castroviejo-Fisher *et al*., 2015; Duellman, 2015). Both studies included a broad, although different, sampling of hemiphractids (more than 70 % of the species); but a markedly different outgroup sampling. While Duellman (2015) only included three nobleobatrachian outgroup terminals, Castroviejo-Fisher *et al*. (2015) included 127 outgroup terminals with representatives of all nobleobatrachian families recognized at the time. The analytical approaches of both studies were also broadly different. The phylogeny presented by Castroviejo-Fisher *et al*. (2015) was based on a total-evidence parsimony analysis under tree-alignment of DNA sequences (up to 20 markers, including mitochondrial and nuclear genes) combined with 51 phenotypic characters. The phylogeny presented by Duellman (2015) was based on maximum likelihood and Bayesian analyses of a similarity-alignment of DNA sequences of two mitochondrial (16S and ND1) and two nuclear (RAG-1 and POMC) markers. Both studies agreed on the monophyly of Hemiphractidae and its six genera (although Duellman, 2015 only include one terminal of *Cryptobatrachus*), but differed most noticeably on the relationships among genera and among infrageneric taxa of *Gastrotheca* (Fig. 1). Although Duellman (2015) presented an additional phylogeny of combined molecular and osteological data, information on which characters were included and how they were coded was completely missing so that repeatability and replicability were not possible. More recently, Kok *et al*. (2017) publish a detailed study on the biogeography of *Stefania* including a more comprehensive taxon sampling of the genus than either Castroviejo-Fisher et al. (2015) or Duellman (2015). Also, Walker *et al*. (2018a) published a comprehensive study about the genetic diversity of *Fritziana*.

Given the importance of hemiphractid phylogenetic relationships, the recent publication of two revisionary studies with conflicting results based on different datasets and analytical assumptions, and the intrinsic characteristics of the available data (i.e. moderate levels of missing data and species diversity as to not obfuscate tree-searches, but complex enough to mimic the analytical complexity of supermatrices), I consider this group an excellent example to discuss some of the most relevant analytical assumptions currently used in phylogenetic studies.

The objective of this study was to identify and discuss the importance of the effect, on the inferred phylogenetic relationships of Hemiphractidae of different alignment methods, optimality criteria, and coding of indels, as well as compare the results of increasing taxa and characters with regard to the most recent and comprehensive studies (Castroviejo-Fisher *et al*., 2015; Duellman, 2015; Kok *et al*., 2017; Walker *et al*., 2018a). A single dataset of all available and newly generated evidence for Hemiphractidae and relevant outgroup taxa was analyzed through six strategies that combined all the analytical factors of interest.

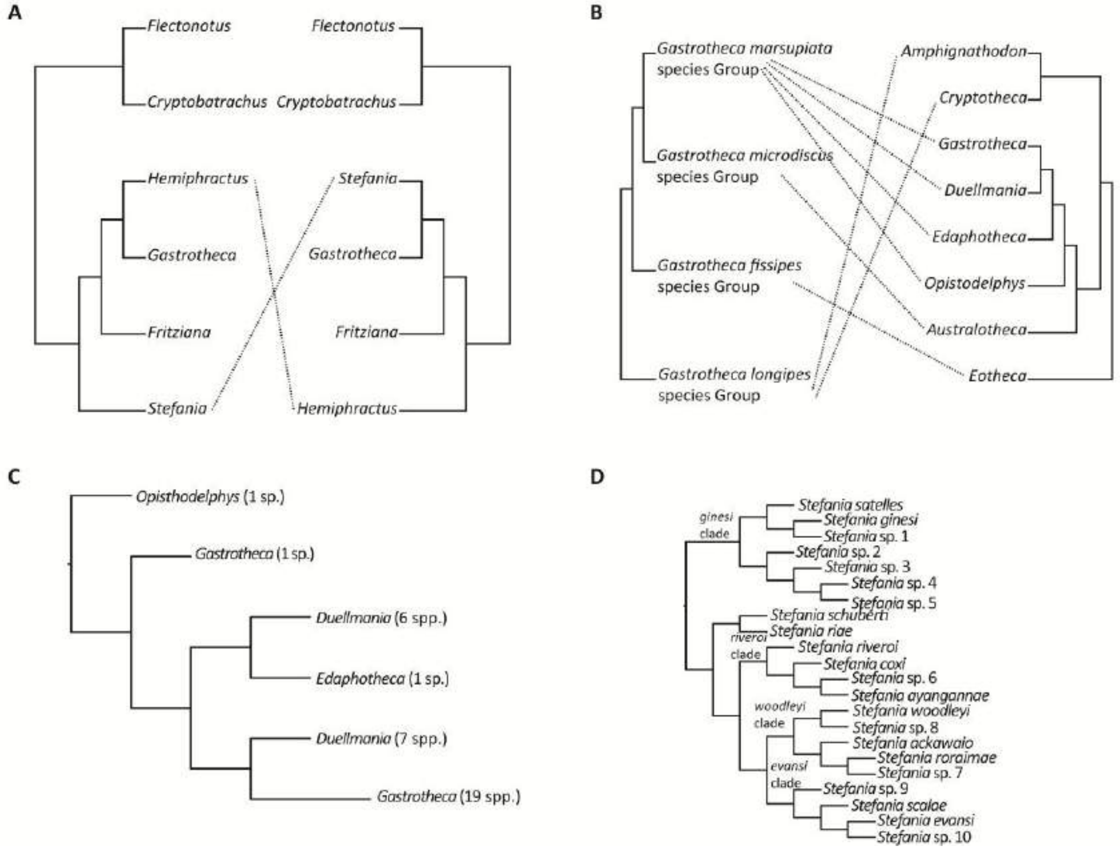

Figure 1. **(A)** Differences in relationships among genera of Hemiphractidae between Castroviejo-Fisher *et al*. (2015), right, Duellman (2015), right; **(B)** Relative position of species groups (Castroviejo-Fisher *et al*. 2015) and subgenera (Duellman, 2015) within *Gastrotheca*; **(C)** Non-monophyly of *Gastrotheca* subgenera according to the results of Castroviejo-Fisher *et al.* (2015); (D) Simplified topology showing the phylogenetic relationships among *Stefania* clade proposed by Kok *et al*. (2017).

Materials and Methods

Taxon sampling

The dataset of Castroviejo-Fisher *et al*. (2015) was used as an initial scaffold. I selected this study because to date it constitutes the most complete dataset when both terminals and characters are considered simultaneously. Because the objective was to study the phylogenetic relationships among hemiphractids, the outgroup of Castroviejo-Fisher *et al*. (2015) was not modified, it includes 121 terminals representing 20 nobleobatrachian families, and six non-nobleobatrachian terminals (Appendix I). The ingroup was modified to include one terminal per species of as many species as possible. Molecular data used in the analysis including sequences downloaded from GenBank (NCBI), prior to April 30, 2018, and newly generated sequences are listed in Appendices I and II.

The name or identification of several terminals was updated to reflect taxonomic proposals since 2015, and also re-identification of specimens (Table 1). When data from different individuals of the same species resulted in sister relationships in exploratory phylogenetic analysis (*i.e.*, similarity-alignment and equal weights parsimony, results not shown), the corresponding uncorrected proportional genetic distances (p-distances) between shared 16S ribosomal RNA (rRNA) gene fragments (between 414–1157 bp) were calculated. In order to reduce the amount of missing entries per terminal, when genetic distances were < 1 % sequences from different specimens were assigned to a single species, which occurred in 38 ingroup terminals (Appendix I). The only exception was the terminal *Gastrotheca ovifera*, which combines DNA data from KU 185758 and MHNLS 20979, a decision based on morphological identification. On the other hand, for fifteen species more than one terminal was included (Appendix I). The terminal *Gastrotheca riobambae* UIMNH 94580 was not included in the dataset because it corresponds to *G. pseustes*, as pointed out by Castroviejo-Fisher *et al*. (2015) and Carvajal-Endara *et al*. (2019), given the genetic p-distances between 399 bp of the marker 16S rRNA (0.5 %) and its collection locality (Azuay, Ecuador) were *G. pseustes* is known to occur.

The total evidence dataset was composed by 143 terminals of egg-brooding frogs, including nominal and undescribed species. The outgroup was represented by 121 nobleobatrachian and six non-nobleobatrachian terminals of Castroviejo-Fisher *et al*. (2015). *Heleophryne purcelli* was used to root the trees.

Molecular dataset

Sequences of 20 genes were included: tRNAPhe, 12S rRNA, tRNAVal, two non-overlapping fragments of the 16S rRNA, tRNALeu, NADH dehydrogenase subunit 1 (ND1), cytochrome oxidase I (COI), cytochrome b (cytb), 28S rRNA, proto-oncogene cellular myelocytomatosis exons 2 (C-MYC 2) and 3 (C-MYC 3), chemokine receptor 4 (CXCR4), histone (H3a), proopiomelanocortin A (POMC), two non-overlapping fragments of the recombination activating gene 1 (RAG1), rhodopsin exon 1 (Rho), seven in absentia homolog 1 (SIAH), solute carrier family 8 member 1 (SLC8A1), solute carrier family 8 member 3 (SLC8A3), and tyrosinase (Tyr). A total of 219 new sequences were generated, representing ten genes (two fragments of RAG1) from 58 specimens of 30 species of egg-brooding frogs (Appendix II). In addition, 194 sequences, representing 13 genes from 52 hemiphractid terminals were downloaded from GenBank (Appendix I). A new cytochrome b sequence was downloaded for *Acris crepitans*. Genomic DNA was extracted from ethanol-preserved tissues, using the Wizard® Genomic DNA Purification Kit from PROMEGA. Amplification, sequencing, and editing protocols followed those of Guayasamin *et al*. (2008) and Castroviejo-Fisher *et al*. (2015).

Phenotypic data

The phenotypic dataset of Castroviejo-Fisher *et al*. (2015), which is an updated version of Mendelson *et al*. (2000), was complemented with data for the following terminals: *Fritziana* cf. *fissilis* 1 MNRJ 62845, *Fritziana* cf. *fissilis* 2 CFBH 28886, *F. goeldii* Go III CFBH 10910, *F. ohausi* Oh III CFBH 7611, *F*. sp. CS1 I CFBH 24810, *F*. sp. CS1 II MZUFV 11721, *F*. sp. CS1 III CFBH 30747, *F*. sp CS 2 MCNAM 12341, *F. mitus* CFBH 8273, *Fritziana tonimi* MNRJ 34921, *F. ulei* MNRJ 44622, *Gastrotheca aguaruna* KU 212026, *G. aratia* KU 212056, , *G. flamma* (845 + 846), *G. griswoldi* CORBIDI 16066, *G. oresbios* CORBIDI 11076, *G. rebeccae* CORBIDI 10821, *G.* sp. G CORBIDI 16614, *G. pulchra* (1058 + G3), *G. spectabilis* CORBIDI 11790, and *G*. sp. G CORBIDI 16614. Pertinent information was taken from the literature (Juancá and Nunes, 2008;

Duellman *et al*., 2014; Duellman, 2015; Duellman and Venegas, 2016; Folly *et al*., 2014; Walker *et al*., 2016) or from direct observations on specimens. Also, skull characters for *Hemiphractus fasciatus* ZSM_36/0, *H. elioti* MVUP1927, and *H. panamensis* (EVACC 061 + CHP 6670) were coded from high-resolution computed tomography reconstructions (Hill *et al*., 2018), available at MorphoSource. The data same phenotypic data, taken from *Cryptobatrachus furhmanni* KU 169378 according to Mendelson *et al*. (2000) and as modified by Castroviejo-Fisher *et al*. (2015), was assigned for *Cryptobatrachus fuhrmanni* JDL 14865 and *C. fuhrmanni* (KU 204891 + TNHC-GDC 451) terminals. These terminals have 4.4 % p-distances between 809 pb 16S ribosomal RNA (rRNA) fragments and procede supposedly from the same locality (Colombia: Santander: Municipio San Gil: 7 km by road SW San Gil). Since it was not possible to revise the specimens, it was considered better to assign to both the same phenotypic data.

The data of *Fritziana ohausi* KU 92226 (Teresópolis, Rio de Janeiro) was assigned to the terminal *F. ohausi* Oh I CFBH 16287 (Parque Nacional da Serra dos Orgãos, Teresópolis, Rio de Janeiro) because terminals from the other two *F. ohausi* lineages come from localities in São Paulo. The phenotypic data of *F. goeldii* KU 84721 was assigned to the terminals *F. goeldii* GoI (MNRJ 44592 + CFBH 30938) and *F. goeldii* GoII MNRJ 53758, both from Rio de Janeiro, whilst terminal *F. goeldii* GoIII CFBH 10910 comes from São Paulo. Data of *Gastrotheca stictopleura* was assigned to the terminal nearest to the type locality, *G. stictopleura* CORBIDI 14563. The phenotypic data of *Gastrotheca testudinea* KU 163275 (río Piene, Tutumbaro, Ayacucho, Peru) was assigned to the terminal *G. testudinea* CORBIDI 8009 (CC. NN. Aendoshiari, Cusco, Peru) because both localities lie on southern Peru, approximately 500 km apart. Data from the re-indentified terminals *Stefania ginesi* LM 1056 and *S. satelles* VUB 3755 was transferred to the terminals *S. ginesi* IRSNB 16736 and *S. satelles* IRSNB 16728.

Table 1. Updated identification of ingroup and outgroup samples used in previous studies.

Previous	This study	Taxonomic authority
Ingroup		
Fritziana goeldii MNRJ 34921 in Castroviejo-Fisher *et al.* (2015)	*Fritiziana tonimi*	Walker *et al.* (2016)
Fritziana fissilis MNRJ 62845 Castroviejo-Fisher *et al.* (2015)	*Fritziana* cf. *fissilis* 1	Walker *et al.* (2018a)
Fritziana aff. f*issilis* sp. 3 MZUSP 133700 in Castroviejo-Fisher *et al.* (2015)	*Fritziana* sp. CS 1 IV	Walker *et al.* (2018a)
Fritziana aff. *fissilis* sp. 1 CFBH 5726 in Castroviejo-Fisher *et al.* (2015)	*Fritziana mitus*	Walker *et al.* (2018b)
Fritziana aff. *fissilis* sp. 1 13546 in Castroviejo-Fisher *et al.* (2015)	*Fritziana mitus*	Walker *et al.* (2018b)
Fritziana sp. MNRJ 44592 in Castroviejo-Fisher *et al.* (2015)	*Fritziana goeldii* Go I	Walker *et al.* (2018a)
Fritziana aff. *fissilis* sp. 2 MNRJ 44622 in Castroviejo-Fisher *et al.* (2015)	*Fritziana ulei*	Walker *et al.* (2018a)
Gastrotheca abdita (?) in Duellman (2015)	*Gastrotheca* sp. J	This study
Gastrotheca excubitor KU 173171 in Castroviejo-Fisher *et al.* (2015)	*Gastrotheca* sp.	Carvajal-Endara *et al.* (2019)
Gastrotheca lateonota QCAZ 45113 in Castroviejo-Fisher *et al.* (2015) and Duellman (2015)	*Gastrotheca pseustes* 1	Carvajal-Endara *et al.* (2019)
Gastrotheca pseustes QCAZ 42862 in Castroviejo-Fisher *et al.* (2015)	*Gastrotheca pseustes* 2	Carvajal-Endara *et al.* (2019)
Gastrotheca pseustes TNHC 62492 in Castroviejo-Fisher *et al.* (2015)	*Gastrotheca pseustes* 2	Carvajal-Endara *et al.* (2019)
Gastrotheca pulchra MTR 16228 in Castroviejo-Fisher *et al.* (2015)	*Gastrotheca* sp. L	This study
Gastrotheca sp. A QCAZ 22635 in Castroviejo-Fisher *et al.* (2015) and Gastrotheca pseustes in Duellman (2015)	*Gastrotheca pseustes* 2	Carvajal-Endara *et al.* (2019)
Gastrotheca sp. B QCAZ 21105 in Castroviejo-Fisher *et al.* (2015) and *Gastrotheca* sp. D in Duellman (2015)	*Gastrotheca yacuri*	Carvajal-Endara *et al.* (2019)
Gastrotheca sp. C QCAZ 47299 in Castroviejo-Fisher *et al.* (2015) and Duellman (2015)	*Gastrotheca turnerorum*	Carvajal-Endara *et al.* (2019)
Gastrotheca sp. D QCAZ 42725 in Castroviejo-Fisher *et al.* (2015)	*Gastrotheca lojana*	Duellman (2015)
Gastrotheca sp. E QCAZ 21213 in Castroviejo-Fisher *et al.* (2015) and *Gastrotheca* sp. B in Duellman (2015)	*Gastrotheca elicioi*	Carvajal-Endara *et al.* (2019)
Hemiphractus fasciatus MVUP 1927 in Castroviejo-Fisher *et al.* (2015)	*Hemiphractus elioti*	Hill *et al.* (2018)
Hemiphractus fasciatus sp. 1 CHP 6397 in Castroviejo-Fisher *et al.* (2015)	*Hemiphractus kaylockae*	Hill *et al.* (2018)

Table 1. Continuation.

Previous	This study	Taxonomic authority
Hemiphractus fasciatus sp. 1 EVACC 207 in Castroviejo-Fisher *et al*. (2015)	*H. kaylockae*	Hill *et al*. (2018)
Hemiphractus fasciatus sp. 1 EVACC 065 in Castroviejo-Fisher *et al*. (2015)	*H. kaylockae*	Hill *et al*. (2018)
Hemiphractus fasciatus sp. 2 *EVACC 061* in Castroviejo-Fisher *et al*. (2015)	*Hemiphractus panamensis*	Hill *et al*. (2018)
Hemiphractus fasciatus sp. 2 *CHP 6670* in Castroviejo-Fisher *et al*. (2015)	*H. panamensis*	Hill *et al*. (2018)
Stefania aff. *evansi* VU B3555 in Castroviejo-Fisher *et al*. (2015)	*S*. sp. 9 VUB 3555	Kok *et al.* (2017)
Stefania roraimae ROM 42843 in Castroviejo-Fisher *et al*. (2015)	*S. ackawaio* VUB 3546	Kok *et al.* (2017)
Stefania satelles VUB 3755 in Castroviejo-Fisher *et al*. (2015)	*S*. sp. 3 VUB 3755	Kok *et al.* (2017)
Stefania sp. VUB 3266 in Castroviejo-Fisher *et al*. (2015)	*S*. sp. 6 VUB 3266	Kok *et al.* (2017)
Stefania ginesi LM 1056 in Castroviejo-Fisher *et al*.(2015); Duellman (2015)	*S*. sp. 1 LM 1056	Kok *et al.* (2017)
Stefania evansi IRSNB 14586 in Castroviejo-Fisher *et al*. (2015)	*Stefania* sp. 10	Kok *et al.* (2017)
Stefania evansi ROM 39450 in Castroviejo-Fisher *et al*. (2015)	*Stefania* sp. 10	Kok *et al.* (2017)
Outgroup		
Amietophrynus brauni in Castroviejo-Fisher *et al*. (2015)	*Sclerophrys brauni*	Ohler and Dubois (2016)
Hyla arenicolor in Castroviejo-Fisher *et al*. (2015)	*Dryophytes arenicolor*	Duellman *et al*. (2016)
Hyla cinerea in Castroviejo-Fisher *et al*. (2015)	*Dryophytes cinereus*	Duellman *et al*. (2016)

Table 1. Continuation.

Previous	This study	Taxonomic authority
Hypsiboas boans in Castroviejo-Fisher *et al*. (2015)	*Boana boans*	Dubois (2017)
Hypsiboas lanciformis in Castroviejo-Fisher *et al*. (2015)	*Boana lanciformis*	Dubois (2017)
Litoria caerulea in Castroviejo-Fisher *et al*. (2015)	*Ranoidea caerulea*	Dubois and Frétey (2016)
Litoria infrafrenata in Castroviejo-Fisher *et al*. (2015)	*Nyctimystes infrafrenatus*	Duellman *et al*. (2016)

Modifications on Character 46 (state of development of young at hatching) from Mendelson *et al*. (2000), by Castroviejo-Fisher *et al.* (2015), included: the fusion of States 1 and 2 of Mendelson *et al.* (2000), specific developmental stages were included on the description of the character, and the feeding information was removed from the description (i.e., endotrophic vs. exotrophic) because it constitutes a different character (Sereno, 2007). Thus, Character 46 was coded as follows:

0—hatch as an early 'embryonic' tadpole, < 30 Gosner state (1960)

1—hatch as a well-developed tadpole, ≥ state 30 of Gosner (1960)

2—hatch as a froglet, ≥ state 46 of Gosner (1960)

In summary, the final phenotypic dataset includes 243 terminals, of which 116 correspond to hemiphractids, and 51 characters. However, characters are well represented for 34 terminals—mostly those of Mendelson *et al*. (2000)—while most other terminals are coded just for four behavioral and developmental characters (i.e., characters 41, 42, 46, and 47 of Mendelson *et al*., 2000). The terminals *Cryptobatrachus* sp., *Hemiphractus fasciatus*, and *Hemipractus johnsoni* are only represented by phenotypic data.

Theoretical considerations

Regardless of optimality criterion, a scientific hypothesis is interpreted as supported if it is not refuted by the critical evidence. A phylogenetic hypothesis is not refuted when it corresponds to the optimal solution according to a justified optimality criterion (parsimony or ML in this study) or to other, equally optimal hypotheses (i.e., evidence is ambiguous, such as when multiple most-parsimonious cladograms are obtained). Frequency of clades based on resampling measures (i.e., jackknife and bootstrap) is interpreted as a proxy of the relative amount of favorable and contradictory evidence for each group present in the optimal topology when > 50 % (Goloboff *et al*. 2003; Ramírez 2005; Kopuchian and Ramírez, 2010). These indices are specific to each dataset and analytical assumptions; they should not be extrapolated between different datasets and analyses, used to judge the validity of a method or hypothesis, or to predict the stability of a clade. In other words, at their best they only convey information on the relative amount of supporting and contradicting evidence for each optimal clade obtained for a specific dataset under specific analytical conditions.

Tree-alignment+ Parsimony analysis (TAP)

Sequences of tRNAPhe, tRNAVal, tRNALeu, COI, cytb, C-MYC 2, C-MYC 3, CXCR4, H3a, ND1, POMC, the two RAG(1) fragments, Rho, SIAH, SLC8A1, SLC8A3, and Tyr were aligned independently in Aliview 1.17.1 (Larsson, 2014), using Muscle (Edgar, 2004) with default parameters. Sequences of 12S, the two 16S fragments, and 28S were aligned in MAFFT v7 online version (Kuraku *et al*., 2013; Katoh *et al*., 2017) using the FFT-NS-i strategy, all parameters were left at default values. The resulting multiple sequence alignments were visualized and edited in Aliview. Alignments of each DNA marker, were modified to correct a clear artifact created by the alignment program in which, sometimes, the end and/or the beginning of shorter fragments within a gene were placed at the corresponding end or beginning of the whole alignment. Those fragments were placed at the beginning or end of the corresponding sequence. Subsequently, the aligned matrix of each marker was divided into putatively homologous blocks where length variation among DNA sequences is only due to insertions and/or deletions of nucleotides, a requisite for tree-alignment analysis in POY (Wheeler *et al*., 2006). For non-coding genes, fragment partitions were located in conserved regions (no gaps and few or no nucleotide substitutions) according to the multiple sequence alignments. For coding genes, fragment partitions were stablished considering groups of codon triplets. Finally, for each fragment, gaps implied by the multiple sequence alignment were removed. The DNA sequence fragments, along with the phenotypic matrix, were analyzed in POY 5.1.1 (Varón *et al*., 2010; Wheeler *et al*., 2015). Forty-eight phenotypic characters were coded as additive, according to Mendelson *et al*. (2000). Tree-alignment was performed under parsimony with equal weights for all transformations using direct optimization. Tree searches were conducted using the command "search"—which implements an algorithm based on random addition sequence Wagner builds, subtree pruning and regrafting (SPR), and tree bisection and reconnection (TBR) branch swapping (see Goloboff, 1996, 1999), parsimony ratcheting (Nixon, 1999), and tree fusing (Goloboff, 1999)—running consecutive rounds of searches within a specified run-time, storing the shortest trees of each independent run and performing a final round of tree fusing on the pooled trees. A round of 24 hours of search was followed by 168 hours of search, using as starting topologies the trees from the previous round, with an extra round of fuse at the end. The resulting trees were submitted to a round of search of 72 hours, with an extra round of fuse at the end. The best topology was used as starting point for a 96 hours search with extra round of fuse and swap at the end. There was no improvement in the tree cost, so the resulting tree was submitted to a round of swap using iterative pass optimization (Wheeler, 2003b) in POY 4.1.2 (Varón *et al*., 2010). Finally, the optimal implied alignment from iterative pass optimization was converted to a data matrix (Wheeler, 2003a) and submitted to driven searches in TNT 1.5 (Goloboff *et al*., 2008; Goloboff and Catalano, 2016). All the New Technologies algorithms (Sectorial Search, Ratchet, Drift, Tree Fusing) in their default mode were implemented. Search was set for all taxa, at level 70, with the minimum length tree to be found set to 100 times and random seed = 1.

Jackknife frequencies (JK) were calculated in TNT using the implied alignment for 1000 pseudoreplicate searches under a Traditional Search analysis with 50 replicates and 50 trees saved per replication, gaps treated as fifth state, and removal probability 0.36 (~e^{-1}) to render bootstrap and JK values comparable (Farris *et al.*, 1996).

POY analyses were run on a cluster housed at the *Laboratório de Alto Desempenho* (LAD) – PUCRS. The cluster Amazonia consists on two HP Proliant BL620C G7 servers, each with two 2.0 GHz processors Intel Xeon E7-2850. Jointly, both processors have 40 cores and 80 threads. The servers are interconnected by four 10 Gigabit-Ethernet nets.

Similarity-alignment + Parsimony analysis, gaps as a fifth state (SAP$_{5th}$)

For all similarity-alignment analyses, blocks of each marker generated for the TAP analysis, but including gaps implied by the multiple sequence alignment, were concatenated into a single matrix using SequenceMatrix 1.7.8 (Vaidya *et al.*, 2011). The DNA sequence matrix was concatenated with the phenotypic matrix using POY 5.1.1, the resulting Nexus file was exported in .tnt format from Mesquite 3.31 (Maddison and Maddison, 2017). Tree searches were run in TNT 1.5, all transformations were equally weighted, gaps treated as a fifth state, and phenotypic characters were coded as additive or non-additive following Mendelson *et al*. (2000). Searches for the shortest trees and JK were conducted in TNT as explained in the previous section.

Similarity-alignment + Parsimony analysis, gaps as missing data (SAPm)

For this analysis, the same total evidence matrix as in the SAP_{5th} analysis was used. Tree searches and calculation of JK frequencies were executed in TNT 1.5 as for the implied alignment of TAP. TNT was set to consider gaps as missing data.

Similarity-alignment + Parsimony analysis, gaps as binary characters (SAPg)

The molecular matrix analyzed in SAP_{5th} was used to code indels as the longest possible fragments. Indels were incorporated in the dataset as a block of binary characters according to the Simple Indel Coding method (SIC) of Simmons and Ochoterena (2000) as implemented in SeqState 1.4.1 (Müller, 2005, 2006). All datasets (i.e., DNA sequences, indels coded as binary characters, and phenotypic characters) were concatenated using POY 5.1.1. The resulting Nexus file was exported as a .tnt file using Mesquite 3.31 and analyzed in TNT 1.5 as explained above. Gap characters ("-") of the molecular matrix were recoded as missing data (“?”).

Similarity-alignment + Maximum Likelihood analysis, gaps as binary characters (SALg)

The program PartitionFinder v 1.1.1 (Lanfear *et al*., 2012) was used to select the most optimal partition scheme and substitution models for the molecular dataset, under the corrected Akaike information criterion (AICc). Branchlengths were stimated as linked. A tree topology generated in TNT (Traditional Search of 1000 replicates, swapping algorithm TBR, and saving 10 trees per replication) was supplied fot the analysis. Three partition schemes were evaluated: all data combined; two partitions scheme, mtDNA versus nuDNA; and a 20-partition scheme (each locus independently). To incorporate indel information the SIC method was used, as in SAPg analysis. The aligned DNA sequences, with gap characters ("-") recoded as missing data (“?”), indels as binary characters block, and additive and non-additive phenotypic characters were included in a single Nexus file.

Tree searches were executed using GARLI 2.01 (Zwickl, 2006). A total of 500 independent searches were conducted using as starting point a random topology, 100 000 generations without topology improvement required for termination (genthreshfortopoterm), treerejectionthreshold at 50, and the limsprrange parameter was set at 15. Finally, I selected the best tree among the 500 independent searches by comparing the likelihood scores.

A total of 250 independent bootstrap pseudoreplicates were calculated under less rigorous parameters than tree searches (genthreshfortopoterm: 10 000; limsprrange: 6) to reduce execution time. The 250 pseudoreplicates were compiled in the R package Ape 4.1 (Paradis *et al*., 2004) and the bootstrap frequencies were assigned to the corresponding clades of the optimal tree using SumTrees 4.3.0 (Sukumaran and Holder, 2010a) of the DendroPy 4.3.0 package (Sukumaran and Holder, 2010b).

Tree searches were run using the Amazonia cluster housed at the Laboratório de Alto Desempenho (LAD) – PUCRS. Bootstrap pseudoreplicates were run using the Amazonia cluster and the CIPRES Science Gateway (Miller *et al*., 2010).

Similarity-alignment + Maximum likelihood analysis, gaps as missing data (SALm)

Partitions and model selection, tree searches, selection of optimal trees, and calculation of bootstrap frequencies were performed as explained in the SALg analysis. Gap characters ("-"), of the DNA sequences alignment remained unaltered, as GARLI automatically treats gaps as missing data.

Comparisons and the effect of analytical factors

The six analyses were designed to single out, heuristics aside, the effect of each variable of interest through alternative comparison of the results (Fig. 2). The comparison of the results of TAP and SAP_{5th} analyses allowed identifying the direct effects of using different alignment methods. The effect of using different optimality criteria can be more easily identified by comparing differences between the results of SAPm vs. SALm and SAPg vs. SALg. Finally, the effect of different treatment of indels becomes clear-cut when comparing the results among SAP_{5th}, SAPg, and SAPm on the one hand and among SALm and SALg on the other. All other comparisons were cautiously done connecting analyses (Fig. 2) and keeping in mind the potential additive effects that more than one varying analytical parameter could imply. To compare TAP with SALm and SALg, the following five scenarios were evaluated with the help of the corresponding SAP result: i) clades shared by TAP and SALg/m, but not SAPg/m, show that the optimality criterion (maximum likelihood) caused congruence between SALg/m and TAP results, by overcoming the role of the alignment and/or the indel coding that caused the differences between TAP and SAPg/m; ii) clades shared by TAP and SAPg/m, but not by SAL, indicate that the optimality criterion is the single cause of the differences; iii) clades exclusive of each of the three analytical strategies cannot be used to discern between the effect of optimality criteria, alignment method, and indel treatments; iv) clades shared by SALg/m and SAPg/m, but not TAP, are examples of differences caused by the alignment method and/or indel coding; and v) clades shared by the three analyses are evidence of relationships not sensitive to any of the analytical factors under study.

Differences among topologies were quantified using the program ybara_sa.py of YBYRÁ (Machado, 2015), which implements an algorithm to calculate local and global distances between trees based on the Robinson-Foulds metric (Robinson and Foulds, 1981). Robinson-Foulds local distances are calculated by 1 – (S/U), where S is the number of shared clades or splits and U is the number of unique clades or splits in the compared trees. Local distances were calculated considering the number of shared clades among trees. The prevalence of clades per tree file and sensitivity plots were also generated through a sensitivity analysis, using ybara_sa.py of YBYRÁ (Machado, 2015). Additionally, the reciprocal cost of optimal topologies of each analytical strategy was calculated under the conditions of all the other implemented strategies. For all parsimony analyses, the reciprocal cost of both one optimal tree and the strict consensus were calculated. Costs in POY were calculated under IPO.

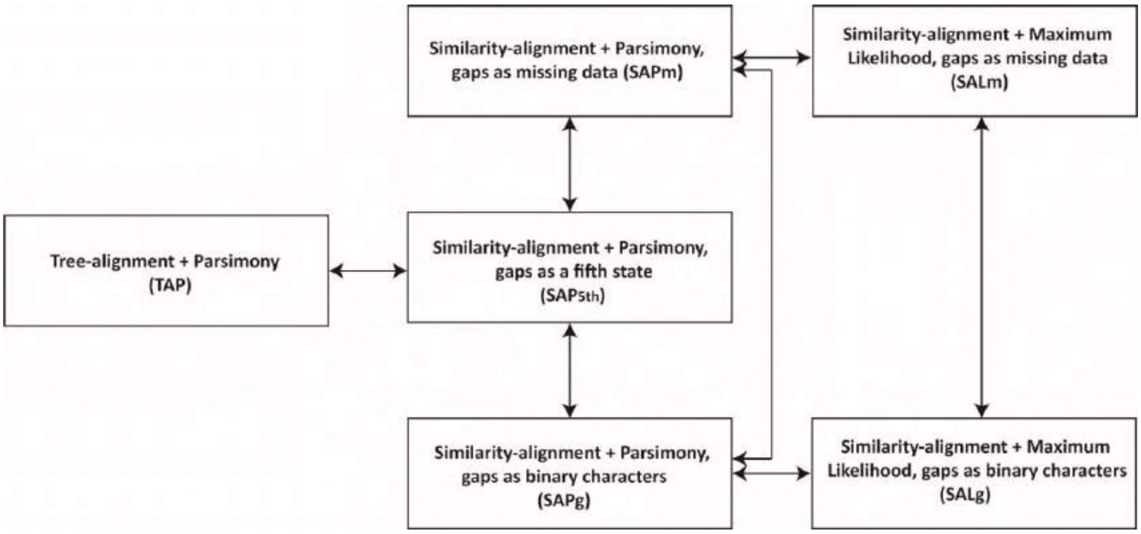

Figure 2. The six main phylogenetic analyses performed in this study to evaluate the effect of optimality criterion, alignment and indel coding. Arrows indicate direct pathways between analyses where just a single analytical factor differs (heuristics aside).

Wildcard taxa

Analyses to search for potential wildcard taxa for each set of resulting trees of parsimony analyses were performed using YBYRÁ. First, consensus and optimal tree files were pruned one terminal at a time using ybyra_sa.py. Then, pruned tree files were submitted to MSdist to calculate how each terminal affects the average matching split distances (MSD) among trees. Terminals resulting in the lowest MSD were identified as more likely to cause decrease of resolution and considered potential wildcards.

Results

At the outgroup level, half of the analyses, except SAP5th, SALg and SALm, recovered a monophyletic Athesphatanura and a non-monophyletic Brachycephaloidea (Fig. 3, Supplementary Figures 1–6). In the strict consensus of SAP5th, the non-monophyly of

Athesphatanura was determined by the position of *Pseudopaludicola falcipes* as sister to all other nobleobatrachians, and the non-sister relationships of four main athesphatanuran clades (Supplementary Figure 2). In the SALg optimal tree, Dendrobatoidea (Aromobatidae + Dendrobatidae) resulted sister to all other athesphatanurans and Hemiphractidae (Supplementary Figure 5). In the SALm optimal tree, Dendrobatoidea resulted sister to all other nobleobatrachians (Supplementary Figure 6). The position of *Ceuthomantis smaragdinus* as sister to all nobleobatrachians determined the non-monophyly of Brachycephaloidea in TAP, SAPg, and SAPm analyses. Two analyses, SALm and SAP5th, recovered Hemiphractidae as sister to Brachycephaloidea, the other four analyses recovered Atesphathanura, or all athesphatanurans excluding Dendrobatoidea according to SALg optimal tree, as sister to Hemiphractidae (Fig. 3, Supplementary Figures 1–6).

At the ingroup level, all analyses recovered the monophyly of Hemiphractidae. All analyses agreed on the monophyly of *Fritziana*, *Gastrotheca*, *Hemiphractus*, and *Stefania*. However, *Cryptobatrachus* and *Flectonotus* resulted in a single clade with unresolved relationships according to TAP, SAPg, and SAPm analyses (Supplementary Figures 1, 3, and 4). Cryptobatrachinae and Hemiphractinae were recovered only by the SALm and SALg analyses. All analyses, although with differences on the relationships among terminals, recovered all population lineages proposed by Walker *et al*. (2018a) for *Fritziana*, and all supraspecific clades according to Kok *et al*. (2017) within *Stefania*.

Due to the large number of terminals, detailed comparisons among the relative position of each terminal in all analyses would be excruciating and of lesser interest for the general reader. Therefore, the following sections are focused on the differences with respect to the relationships among hemiphractid genera, the monophyly of previously recognized supraspecific taxa (i.e., subgenera, species groups) in Castroviejo-Fisher *et al*., (2015) and Duellman (2015), the relationships among and within named clades (Kok *et al*., 2017) and populations lineages (Walker *et al.*, 2018a) proposed for hemiphractid genera, as well as a brief description of the outgroup relationships at the family level. A summary of the main phylogenetic relationships among supraspecific groups is presented in Fig. 4. Appendices IX–XXIII include detailed visual comparisons of Hemiphractidae among the results of all analyses.

Table 2. Summary of the six analyzed matrices detailing the total number of aligned characters, the number of missing data and gap cells. Total number of terminals = 270.

Matrix	Aligned characters	Missing Data Cells	Gap Cells
TAP*	17 852	2 705 026	715 223
SAP5th	14 315	2 378 915	141 124
SAPg	15 736	2 718 617	1 421
SAPm	14 315	2 520 039	–
SALg	15 736	2 718 617	1 421
SALm	14 315	2 520 039	–

*Implied Alignment.

Tree-alignment + Parsimony analysis (TAP)

Tree searches in POY yielded one most parsimonious tree of 85 162 steps. A final round of swap, under Iterative Pass Optimization (IPO), reduced the cost to 84 885 steps. The optimal implied alignment contains 17 801 molecular transformation series combined with 51 phenotypic characters, for a static dataset of 17 852 transformation series with 2 705 026 cells coded as missing data (56 %) and 715 223 cells with gaps (15 %) (Table 2). Driven tree searches of the static dataset in TNT found 393 most parsimonious trees of 84 885 steps. The strict consensus of these trees contained 256 nodes out of 269 of a fully resolved tree (Fig. 3, Supplementary Figure 1).

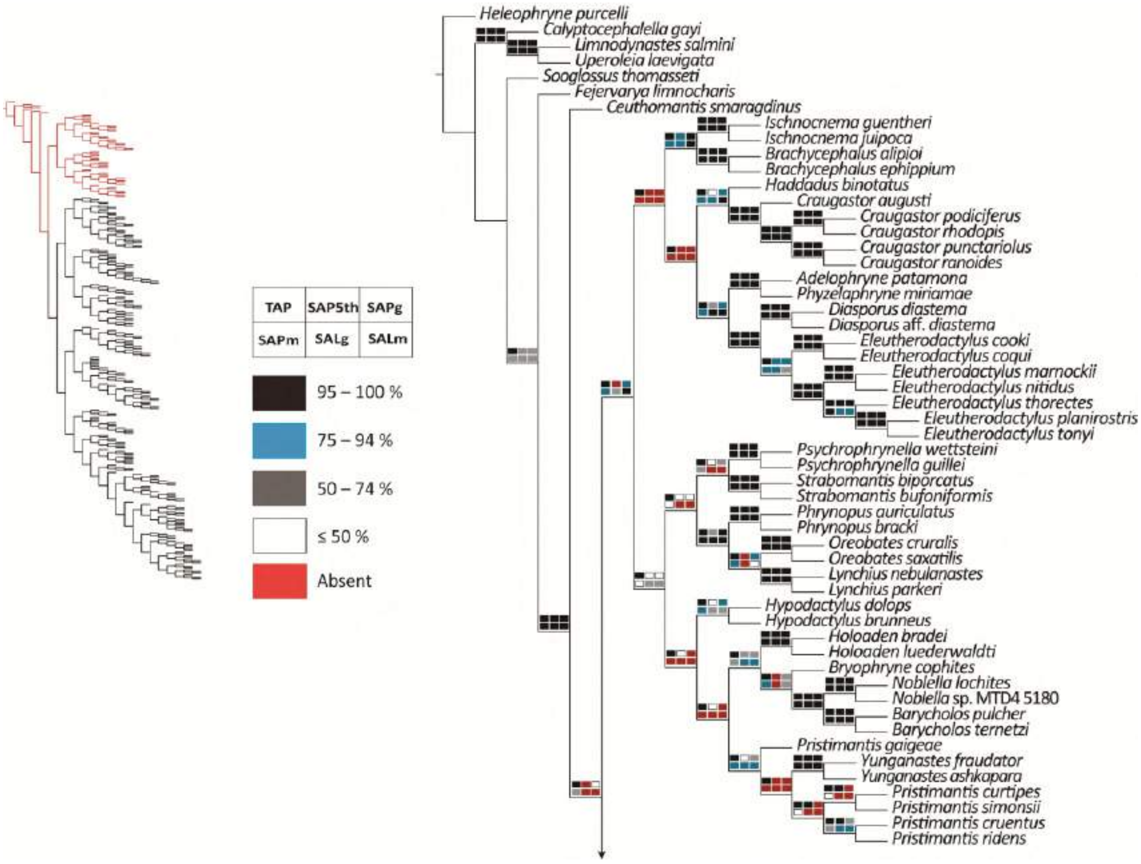

Figure 3. Phylogenetic relationships of egg-brooding frogs and outgroups inferred from a tree-alignment + parsimony analysis of phenotypic characters and DNA sequences. This topology reflects the strict consensus of the 393 most parsimonious trees (tree costs = 84 885 steps). Colored boxes over branches indicate monophyly (all colors but red) and frequency of clades in jackknife or bootstrap resampling measures in parsimony and maximum likelihood analyses, respectively, as inferred from each of the six analyses performed in this study: Tree-alignment + parsimony (TAP); Similarity-alignment + Parsimony, gaps as binary characters (SAPg); Similarity-alignment + Parsimony, gaps as missing data (SAPm); Similarity-alignment + Parsimony, gaps as a fifth state (SAP_{5th}); Similarity-alignment + Maximum Likelihood analysis, gaps as missing data (SALm); and Similarity-alignment + Maximum Likelihood, gaps as binary characters (SALg).

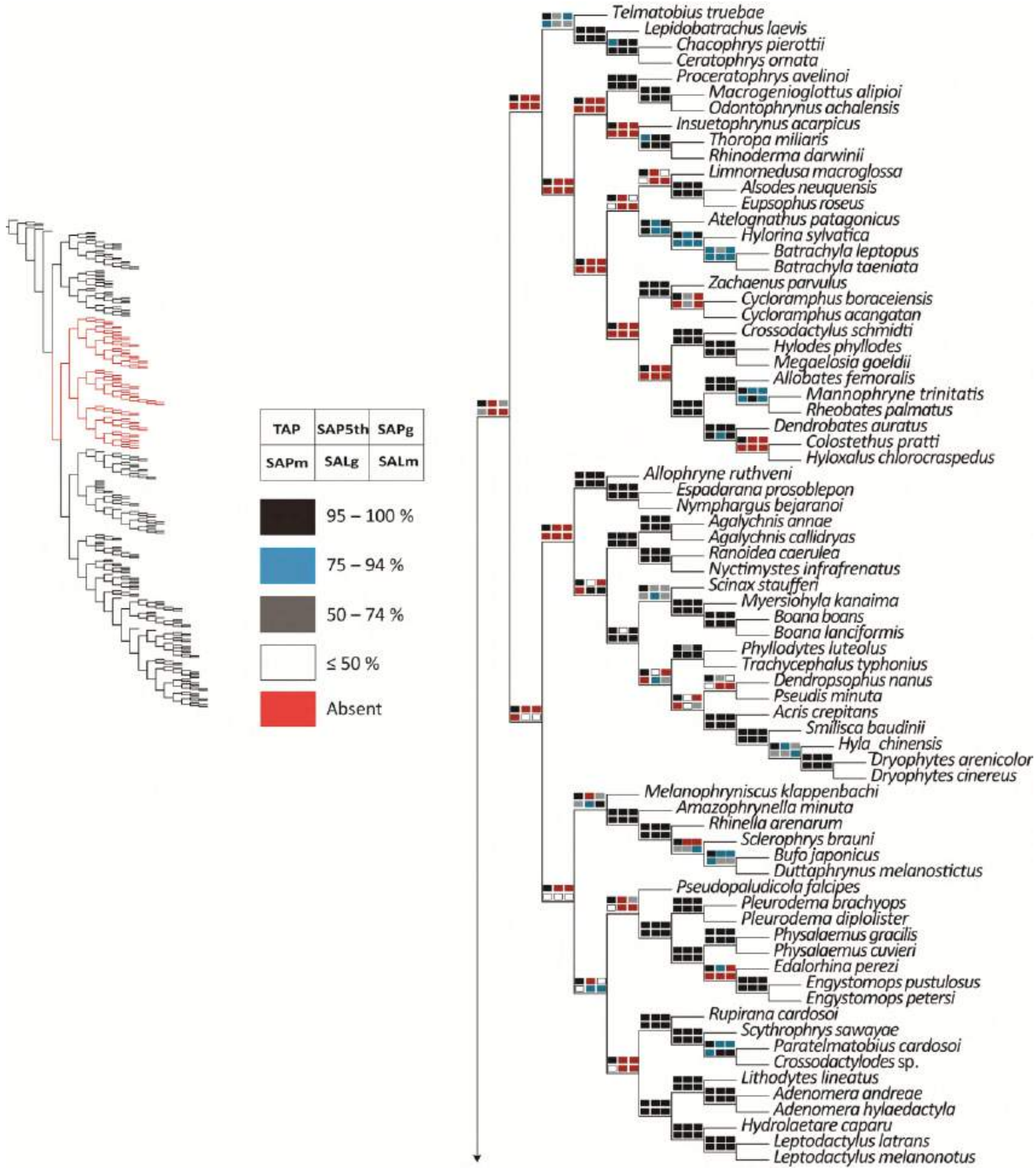

Figure 3. Continuation.

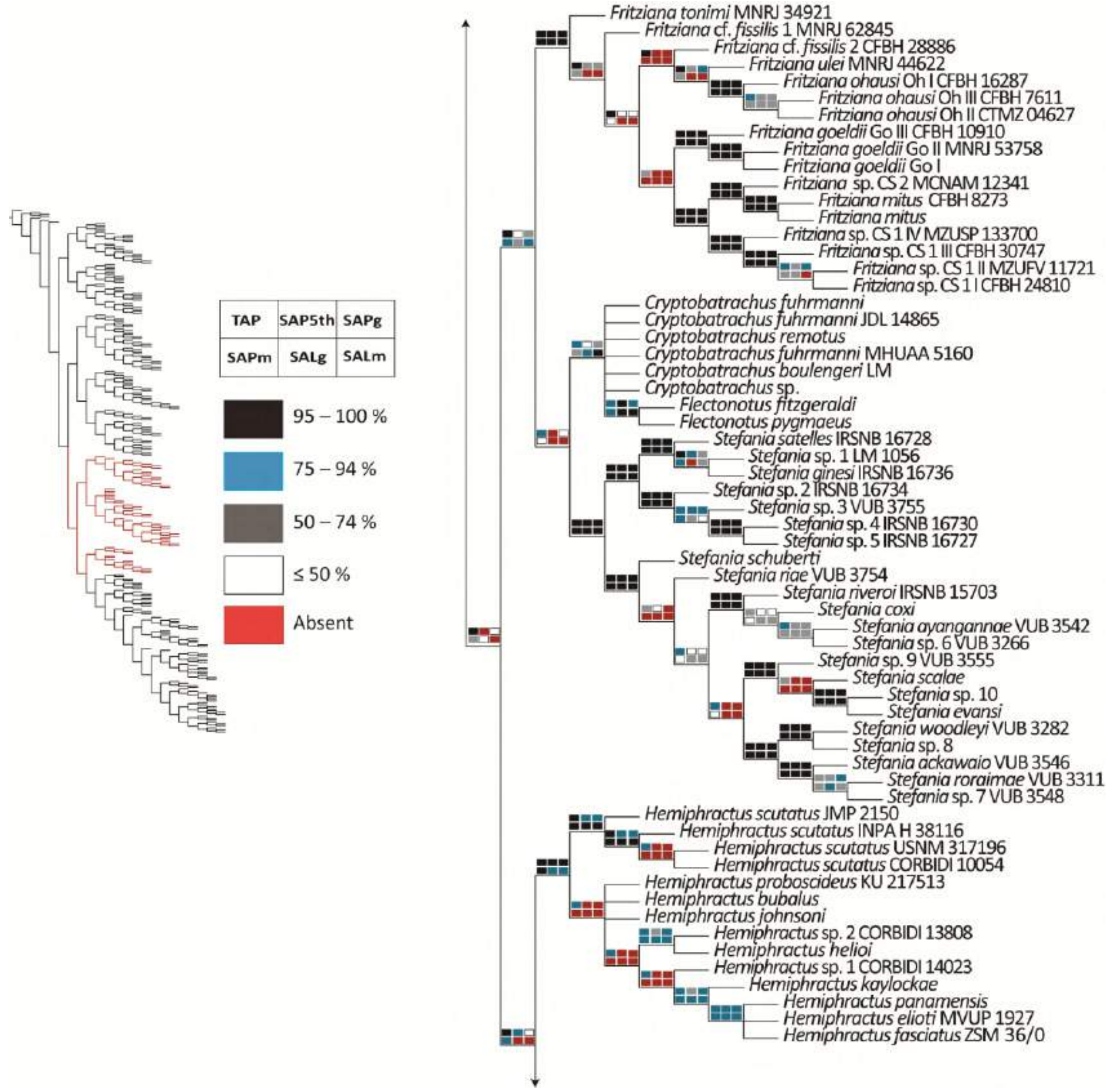

Figure 3. Continuation.

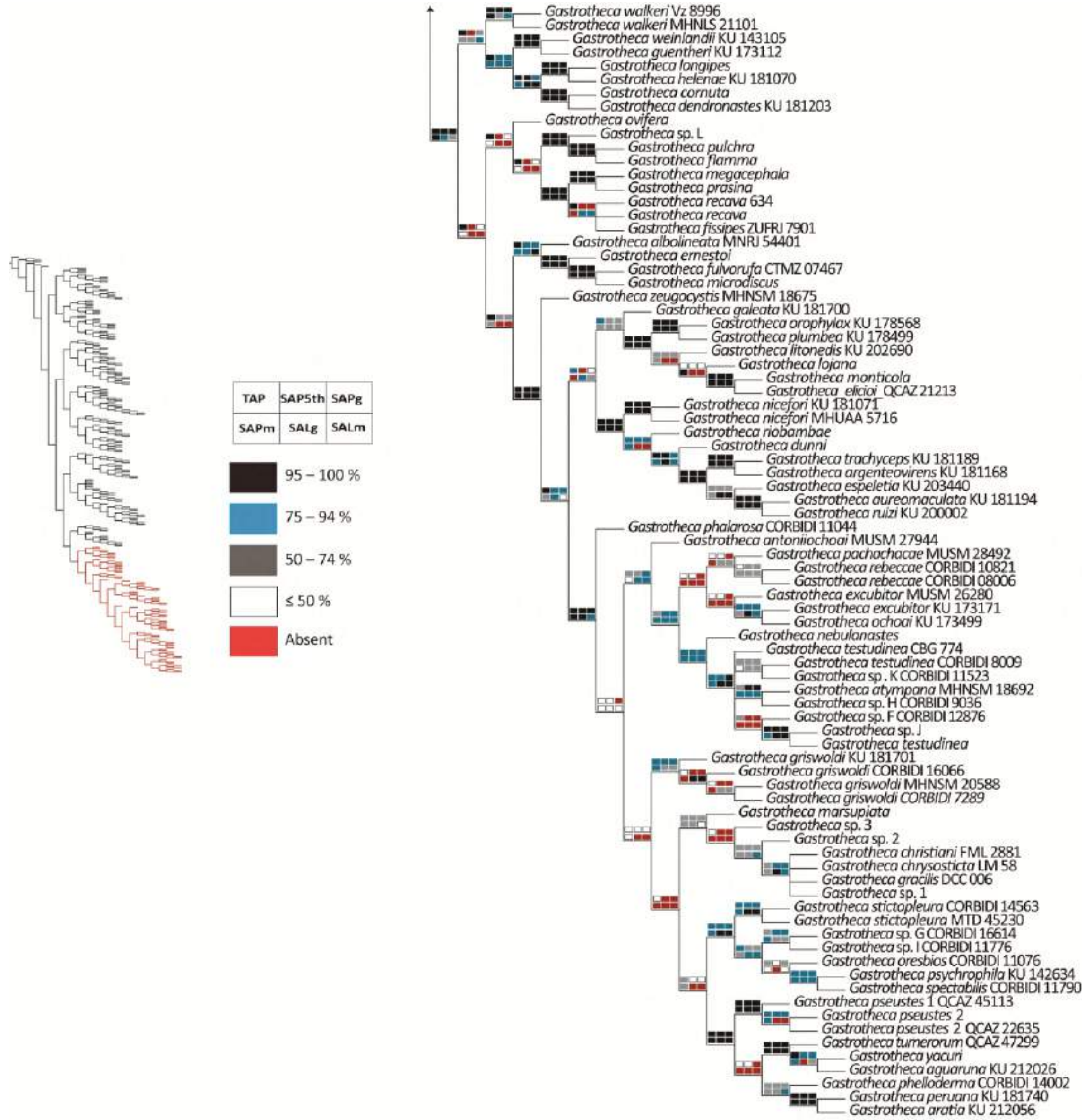

Figure 3. Continuation.

Within the outgroup Craugastoridae and Cycloramphidae resulted non-monophyletic. Within Hemiphractidae (JK = 99), *Cryptobatrachus* and *Flectonotus* resulted within a clade (JK = 94) with unresolved relationships among all *Cryptobatrachus* and the two *Flectonotus* sister terminals. The clade (*Fritziana* ((*Cryptobatrachus* + *Flectonotus*) (*Stefania*))) (JK = 94) resulted sister to (*Gastrotheca* + *Hemiphractus*) (JK = 100). Within *Fritziana* (JK = 100), *F. tonimi* resulted sister to all other terminals among which there are two clades. A clade grouping *F. ulei*, *F.* cf. *fissilis* 2 and the *F. ohausi* lineages resulted sister to a clade including *F. goeldii* lineages, *F. mitus*, and *F.* sp. CS 1 and *F.* sp. CS 2; *F.* cf. *fissilis* 1 was not recovered as part of any of these clades. Relationships recovered among *Stefania* (JK =100) clades were the same as in Kok *et al*. (2017), only the sister relationship between *S. riae* and *S. schuberti* was not recovered. Within *Hemiphractus* (JK =100), a clade including all *H. scutatus* resulted sister to all other terminals. The relationships of *H. bubalus*, *H. johnsoni* and *H. proboscideus* remained unresolved, as well as those among *H. elioti*, *H. fasciatus* and *H. panamensis*. Within *Gastrotheca* (JK =100), the *G. longipes* species Group (*Cryptotheca* and *Amphignathodon* of Duellman *et al*., 2015) resulted sister to all remaining terminals. The *G. fissipes* species Group (*Eotheca* of Duellman *et al*., 2015) included *G.* (*Opisthodelphys*) *ovifera*, from the *G. marsupiata* species Group, as sister to all its terminals. The *G. microdiscus* species Group (*Australotheca* of Duellman *et al*., 2015) resulted sister to the terminals of the non-monophyletic *G. marsupiata* species Group. Within the latter clade, a non-monophyletic *Gastrotheca* (Duellman *et al*., 2015) was recovered as *G. zeugocystis* resulted sister to all other terminals. *Duellmania* (Duellman *et al*., 2015) was recovered as non-monophyletic by including *G. galeata* (*Edaphotheca* of Duellman *et al*., 2015) as sister to six *Duellmania* terminals.

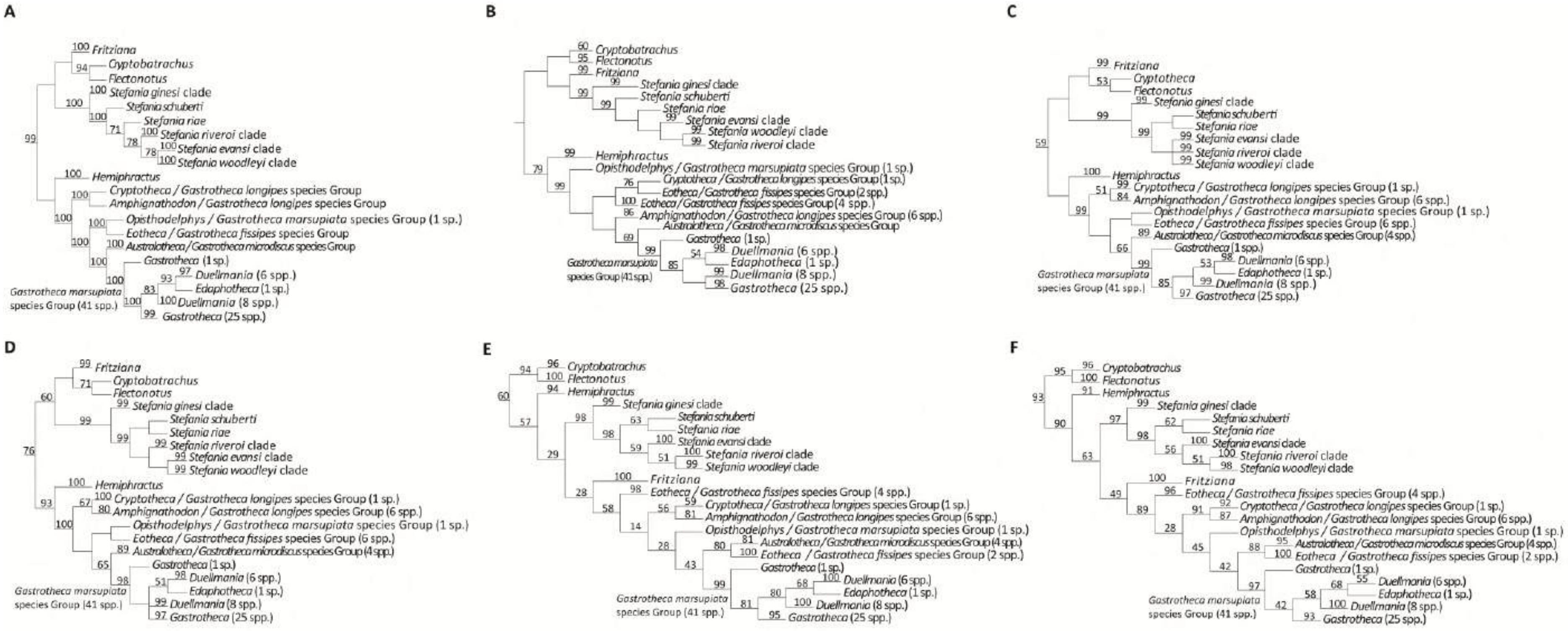

Figure 4. Summarized topologies of (A) Tree alignment + Parsimony (TAP), (B) Similarity-alignment + Parsimony, gaps as a fifth state (SAP$_{5th}$), (C) Similarity-alignment + Parsimony, gaps as binary characters (SAPg) strict consensus trees, (D) Similarity-alignment + Parsimony, gaps as missing data (SAPm), and (E) Similarity-alignment + Maximum Likelihood, gaps as binary characters (SALg) and (F) Similarity-alignment + Maximum Likelihood, gaps as missing data (SALm) optimal trees, showing the main phylogenetic relationships among genera and the supraspecifc groupings proposed by Castroviejo-Fisher *et al*. (2015) and Duellman (2015) for *Gastrotheca*, and by Kok *et al*. (2017) for *Stefania*. Numbers above branches are jackknife or bootstrap proportions in parsimony and maximum likelihood analyses, respectively.

Similarity-alignment + Parsimony analysis, gaps as a fifth state (SAP$_{5th}$)

The final dataset included 14 315 characters, 2 378 915 cells coded as missing data (62 %) and 141 124 cells with gaps (4 %) (Table 2). A total of 309 trees of 90 684 steps (5 799 more steps than the TAP) were retained. The strict consensus had 255 nodes out of 269 of a fully resolved tree (Supplementary Figure 2).

At the outgtoup level, Craugastoridae, Bufonidae and Leptodactylidae were recovered as non-monophyletic (Appendix IV). Within the ingroup, the clade ((*Cryptobatrachus* + *Flectonotus*)(*Fritziana* + *Stefania*)) resulted sister to (*Gastrotheca* + *Hemiphractus*). Relationships among *Cryptobatrachus* (JK = 60) terminals were not resolved. Within *Fritziana* (JK = 99), *F. tonimi* resulted sister to all other terminals. The clade of *F. ulei* and the *F. ohausi* lineages resulted sister to a clade including *F*. cf. *fissilis* 2, *F. goeldii* lineages, *F. mitus*, and *F*. sp. CS 1 and *F*. sp. CS 2. *F*. cf. *fissilis* 1 was not recovered as part of any of these clades. Relationships among *Stefania* (JK = 99) clades were the same as in Kok *et al*. (2017), only the sister relationships between *S. riae* and *S. schuberti* was not recovered, and relationships within the *evansi* clade were not completely resolved. Within *Hemiphractus* (JK = 99), H. *helioi* and *H*. sp. 2 are sister to all remaining terminals. The relationships among three *H. scutatus* terminals and among *H. elioti, H. fasciatus and H. panamensis* were not resolved. Regarding relationships within *Gastrotheca* (JK = 100), *G.* (*Opisthodelphys*) *ovifera* (from the *G. marsupiata* species group) resulted sister to all other *Gastrotheca* terminals. *G*. (*Cryptotheca*) *walkeri* terminals, from the *G*. *longipes* species Group, resulted within the *G. fissipes* species Group (*Eotheca*). A clade with the remaining members of the *G*. *longipes* species Group (*Amphignathodon*) resulted sister to a clade composed by the *G*. *microdiscus* species Group (*Australotheca*) as sister to the non-monophyletic *G. marsupiata* species group. The monophyly of *Gastrotheca* and *Duellmania* was not recovered. *G*. (*Gastrotheca*) *zeugocystis* resulted sister to *Duellmania* and the remaining *Gastrotheca* terminals. *Duellmania* terminals resulted in two non-sister clades, *G*. (*Edaphotheca*) *galeata* resulted within one of these clades.

Similarity-alignment + Parsimony analysis, gaps as binary characters (SAPg)

The final dataset included 15 736 characters, of which 1 421 characters correspond to the presence/absence matrix of indels (Table 2). A total of 2 718 617 cells (64 %) correspond to missing data, 339 702 more than the SAP5th matrix and 198 578 more than the SAPm matrix (Table 2). The increase in the number of missing cells is due to recodification of gap characters of the DNA sequence alignment as missing data and missing entries in the presence/absence matrix of indels. The analysis yielded 1 402 trees of 87 670 steps (2 785 more steps than TAP, 3 014 less than SAP_{5th}, and 3 872 more than SAPm). The strict consensus had 225 nodes out of a maximum of 269 in a fully bifurcating tree (Supplementary Figure 3).

At the outgroup level, Cycloramphidae, Craugastoridae, Hylidae, and Rhinodermatidae were recovered as non-monophyletic. Also, relationships among athesphatanuran clades remained unresolved. Within Hemiphractidae (JK = 59), the clade (*Fritziana*((*Cryptobatrachus* + *Flectonotus*)*Stefania*)) resulted sister to (*Gastrotheca* + *Hemiphractus*). Within *Fritziana* (JK = 99) relationships were completely resolved, *F. tonimi* and *F.* cf. *fissilis* 1 were not recovered within any of the resulting clades. *Cryptobatrachus* and *Flectonotus* resulted within a clade with unresolved relationships among *Cryptobatrachus* terminals and the position of *Flectonotus* terminals not resolved. Relationships recovered among *Stefania* (JK =99) clades were the same as in Kok *et al*. (2017), however relationships within the *evansi* clade and the position of the *riveroi* clade were not resolved. Within *Hemiphractus* (JK =100), a clade including *H. scutatus* terminals and *H*. sp. 1 resulted sister to all other terminals. The relationships of *H. bubalus*, *H. johnsoni* and *H. proboscideus* remained unresolved, as well as the relationships among *H. elioti*, *H. fasciatus* and *H. panamensis*. Within

Gastrotheca (JK = 99), the *G*. *longipes* species Group (*Amphignathodon* and *Cryptotheca*) resulted the sister clade to the remaining terminals. *G*. (*Opisthodelphys*) *ovifera*, from the *G*. *marsupiata* species Group, was recovered within a clade as sister to the *G*. *fissipes* species Group (*Eotheca*). The *G*. *microdiscus* species Group (*Australotheca*) resulted sister to the remaining terminals from the *G*. *marsupiata* species Group. Within the *G*. *marsupiata* species Group*, Gastrotheca* was recovered as non-monophyletic, as *G*. (*Gastrotheca*) *zeugocystis* resulted sister to all other terminals. Also *G*. (*Edaphotheca*) *galeata* was recovered within *Duellmania*.

Similarity-alignment + Parsimony analysis, gaps as missing data (SAPm)

The final dataset included 14 315 characters, with 2 520 039 cells coded as missing data—an increase of 6 % when compared to *SAP*$_{5th}$, caused by reading indels as unknown nucleotides (Table 2). The analysis yielded 1 132 trees of 83 798 steps (1 087 fewer steps than TAP, and 6 886 fewer steps than SAP$_{5th}$). The strict consensus had 247 nodes out of a maximum of 269 of a fully resolved tree (Supplementary Figure 4).

Two outgroup families, Craugastoridae and Hylidae, resulted non-monophyletic. Within the ingroup, the clade (*Fritziana*((*Cryptobatrachus* + *Flectonotus*)*Stefania*)) (JK = 76) is sister to (*Gastrotheca* + *Hemiphractus*) (JK = 93). Relationships within *Fritziana* (JK = 99) were completely resolved, *F*. *tonimi* and *F*. cf. *fissilis* 1 were not recovered within the clades composed by the remaining terminals. *Cryptobatrachus* and *Flectonotus* were recovered within a clade with unresolved relationships among *Cryptobatrachus* terminals and the position of *Flectonotus* terminals not resolved. Relationships recovered among *Stefania* (JK =99) clades were the same as in Kok *et al*. (2017), only relationships within the *evansi* clade resulted different. Within *Hemiphractus* (JK =100), *H*. sp. 1 resulted sister to all other terminals. The relationships of *H. bubalus*, *H. johnsoni* and *H. proboscideus* remained unresolved, as well as the relationships among *H. elioti*, *H. fasciatus* and *H. panamensis*. Within *Gastrotheca* (JK = 100), the *G*. *longipes* species Group (*Amphignathodon* and *Cryptotheca*) resulted sister to all remaining terminals. *G*. (*Opisthodelphys*) *ovifera*, from the *G*. *marsupiata* species Group, was recovered as sister to the terminals from the *G*. *fissipes* species Group (*Eotheca*). The *G*. *microdiscus* species Group (*Australotheca*) resulted sister to the remaining terminals from the *G*. *marsupiata* species Group. *Duellmania* and *Gastrotheca*, within the *G*. *marsupiata* Group, resulted non-monophyletic. *G*. (*Gastrotheca*) *zeugocystis* resulted sister to all other terminals, *Duellmania* terminals were recovered in two non-sister clades. *G*. (*Edaphotheca*) *galeata* resulted sister to six *Duellmania* terminals.

Similarity-alignment + Maximum Likelihood analysis, gaps as binary characters (SALg)

The final dataset included 15 736 characters; there were 2 718 617 cells coded as missing data (64 %), the same amount as in the SAPg matrix (Table 2). The PartitionFinder analysis found that the 20-partitions scheme (i.e. considering each loci independently) was the best partition strategy among the three alternatives evaluated, and the models selected for each locus are listed in Table 3. A Standard Variable model was assigned to the indel character block and the non-additive phenotypic characters' block. A Standard Variable Ordered model was assigned to the additive phenotypic characters' block. The best maximum log likelihood score obtained by Garli was - 368 553.75. The optimal tree topology had 269 nodes (Supplementary Figure 5).

Table 3. Partition schemes evaluated in PartitionFinder and resulting scores for the corrected Akaike Information Criterion (AICc).

Partition Scheme	lnL	Parameters	Sites	Subsets	cAIC
All	-355 328.76	541	14 264	1	711 782.26
mtDNA/nuDNA	-351 704.49	552	14 264	2	704 557.50
20 partitions	-349 097.98	728	14 264	20	699 730.38

Within the outgroup, Alsodidae and Craugastoridae were recovered as non-monophyletic. Within Hemiphractidae (BS =60), Cryptobatrachinae (BS = 94) and Hemiphractinae (BS = 57) clades were recovered. Within Hemiphractinae the clade (*Hemiphractus*(*Stefania*(*Fritziana* + *Gastrotheca*))) was recovered. *Cryptobatrachus remotus* resulted as sister to all other *Cryptobatrachus* terminals, and *C. fuhrmanni* MHUAA 5160 was not recovered as sister to the other two *C. fuhrmanni* terminals. Within *Hemiphractus* (BS = 94), the clade (*H. helioi* + *H.* sp. 2) resulted sister to the remaining *Hemiphractus* terminals. Within *Stefania* (BS = 98), relationships among the *evansi*, *riveroi*, and *woodleyi* clades differed from those recovered by Kok *et al*. (2017), as well as relationships within the *ginesi and woodleyi* clades. *Fritziana* (BS = 100) terminals resulted in two main clades. *F. goeldii* lineages and *F.* cf. *fissilis* 2 resulted sister to a clade including *F. ulei*, *F. ohausi* lineages, *F.* cf. *fissilis* 1, *F. tonimi*, *F.* sp. CS2, *F. mitus*, and *F.* sp. CS 1. Within *Gastrotheca* (BS = 58), five terminals of the *G. fissipes* Group (*Eotheca*) were recovered as sister to all remaining terminals. The *G. longipes* species Group (*Amphignathodon* and *Cryptotheca*) resulted monophyletic. *G.* (*Opisthodelphys*) *ovifera*, from the *G. marsupiata* species Group, was recovered as sister to a clade including the remaining terminals from the *G. fissipes* (*Eotheca*), and the *G. microdiscus* (*Australotheca*) and *G. marsupiata* species Groups. The clade (*G.* sp. L(*G. flamma* + *G. pulchra*)) was recovered as sister to the *G. microdiscus* (*Australotheca*) species Group. The subgenus *Gastrotheca* was recovered as non-monophyletic, as *G*. (*Gastrotheca*) *zeugocystis* resulted sister to a clade containing *Gastrotheca* and *Duellmania* terminals. *G*. (*Edaphotheca*) *galeata* was recovered within *Duellmania*.

Similarity-alignment + Maximum Likelihood analysis, gaps as missing data (SALm)

The final dataset included 14 315 characters, with 2 520 039 cells coded as missing data (65 %), the same amount of characters and missing data as the SAPm matrix (Table 2). This matrix had 198 578 less cells coded as missing data than SALg matrix. The 20-partition scheme (i.e. considering each loci independently) and the same models of evolution per loci as in the SALg analysis were used. A Standard Variable model was assigned to the non-additive phenotypic characters' block and a Standard Variable Ordered model to the additive phenotypic characters' block. The best maximum log likelihood score obtained by Garli was - 348 389.07. The optimal tree topology had 269 nodes (Supplementary Figure 6).

Within the outgroup, the monophyly of Alsodidae and Craugastoridae was not recovered. Within Cryptobatrachinae (BS = 95) and Hemiphractinae (BS = 90), the same internal relationships of SALg were recovered. *Hemiphractus* sp. 1 resulted sister to all other *Hemiphractus* (BS = 91) terminals. The relationships within *Fritziana* (BS = 100) and *Stefania* (BS = 97) were the same of the SALg analysis, except for relationships among terminals from the *F*. sp. CS1 clade. Within *Gastrotheca* (BS = 89), the analysis recovered the same overall topology of SALg analysis, only with differences on the relationships among clades corresponding to the *Gastrotheca* subgenus.

Comparison of trees

The costs of optimal trees under the conditions of all alternative analytical strategies did not result in better costs than the best cost of each analytical strategy (Table 4). Among parsimony analyses, the best costs corresponded to the SAPm and TAP analyses, the SAPm analysis obtained shorter trees than TAP, a result explained by the exclusion of indels from the SAPm analysis. Similarly, the SALm analysis resulted in a better likelihood score than SALg.

Under TAP conditions, the optimal tree with the closest tree length to TAP was SAPm (373 steps longer), followed by SAPg (377 steps longer), SAP_{5th} (454 steps longer), SALm (572 steps longer), and SALg (596 steps longer). The optimal tree with the closest cost to SALg was SALm, followed by the parsimony optimal trees of SAP_{5th}, TAP, SAPg, and SAPm. The optimal tree with the closest cost to SALm (excluding SALg) was SAP_{5th} followed by TAP, SAPm, and SAPg optimal trees.

The total number of clades for each final topology per analysis and the number of shared clades among them are presented in Table 5. The optimal trees of the SALm and SALg analyses had the highest number of clades (269), followed by the strict consensuses of TAP (256), SAP_{5th} (255), SAPm (247), and SAPg (225). The topologies that share the greatest number of clades were SALm and SALg (256), despite the different treatment of indels. The topologies that share the least number of clades, the consensus tree of SAPg and and SALg optimal tree (188), differ in optimality criteria. Among the parsimony analyses, the topologies that share the greatest number of clades were the consensus trees of SAPm and SAPg (222), both differ in the treatment of indels. The topologies that share the least number of clades, from analyses with different treatment of indels, were the consensus trees of SAP_{5th} and SAPg (197). The parsimony analysis tree sharing more clades with SALg was the consensus tree of SAP_{5th} (199), and the one sharing the least number of clades was the consensus tree of SAPg (188). The parsimony consensus trees sharing more clades with SALm are SAP_{5th} and SAPm (198), and the one sharing the least number of clades was the consensus tree of SAPg (191).

The Robinson-Foulds (RF) local distance values were overall congruent with the number of clades shared between trees of the six analyses, the higher the number of shared clades, the smaller the RF local distance values (Table 4). However, trees sharing the least number of clades, less than 200, resulted in RF local distance values affected by the number of unique clades on compared trees. Thus, distance corresponding to the pair of trees with the lowest number of shared clades did not correspond to the highest RF local distance value.

Regarding resampling indices (Table 6), the Jackknife values for the Hemiphractidae clade were high for parsimony matrices, only the Jackknife analysis of the SAP_{5th} matrix did not recovered the Hemiphractidae clade. The Jackknife values corresponding to *Fritziana*, *Gastrotheca*, *Hemiphractus* and *Stefania* were equally high for all parsimony analyses. The BS frequencies for the SAL matrices resulted generally high (Table 6), although the BS analysis of the SALm matrix reported the overall highest values for the main clades within the Hemiphractidae.

The effect of alignment method: Tree-alignment vs. Similarity-alignment

The results of TAP and SAP_{5th} analyses were compared directly because, heuristics aside, these two analyses only differ in the alignment method. For the TAP analysis, the implied alignment of DNA sequences along with the phenotypic characters constituted a total of 4 820 040 characters, of which 15 % correspond to gaps. The SAP_{5th} total-evidence matrix included 3 865 050 characters, of which 4 % correspond to gaps. The strict consensuses of TAP and SAP_{5th} analyses resulted almost equally resolved (256 vs. 255 clades; Table 4), and shared 80 % of its clades.

Table 4. Costs of all optimal trees (in bold) and reciprocal cost of single optimal trees inferred by the six different analyses, this later value is followed by percentage of decrease in optimal cost. The reciprocal cost for strict consensus of parsimony analyses is in parenthesis. Column defines optimization strategy. For parsimony analyses the cost of optimal trees were calculated using the first of all optimal trees of each analysis.

	TAP	SAP$_{5th}$	SAPm	SAPg	SALg	SALm
TAP	**84 885**	90 768 / 0.09 % (91 221)	83 851 / 0.06 % (84 233)	87 724 / 0.06 % (88 132)	369 449.19 / 0.24 % (369 045.05)	349 434.25 / 0.3 % (348 837.62)
SAP$_{5th}$	85 281 / 0.47 % (85 339)	**90 684**	83 897 / 0.12 % (84 111)	87 769 / 0.11 % (87 997)	369 443.03 / 0.24 % (369 142.38)	349 231.27 / 0.24 % (348 963.24)
SAPm	85 258 / 0.44 % (85 521)	90 756 / 0.08 % (91 312)	**83 798**	87 673 / 0.03 % (88 188)	369 729.38 / 0.32 % (368 964.45)	349 436.53 / 0.3 % (348 766.44)
SAPg	85 262 / 0.44 % (85 814)	90 750 / 0.07 % (92 476)	83 801 / 0.004 % (85 404)	**87 670**	369 656.08 / 0.3 % (368 924.56)	349 581.02 / 0.34 % (348 742.01)
SALg	85 481 / 0.7 %	90 979	84 132	87 988	**368 553.75**	348 416.14
SALm	85 457 / 0.67 %	90 995	84 099	87 979	368 585.44	**348 389.07**

Table 5. Number of clades (in parenthesis) and clades shared (above the diagonal) by parsimony strict consensus trees and optimal maximum likelihood trees. Robinson-Foulds local distance values are shown below the diagonal. Total number of terminals = 270.

	TAP (256)	SAP_{5th} (255)	SAPm (247)	SAPg (225)	SALg (269)	SALm (269)
TAP		206	215	209	192	194
SAP_{5TH}	32.5		203	197	199	198
SAPm	25.4	32.1		222	193	198
SAPg	23.2	30.4	11.3		188	191
SALg	42.3	38.8	40.3	38.6		256
SALm	41.4	39.3	37.7	37.0	9.2	

At the outgroup level, Athesphatanura resulted non-monophyletic only in SAP_{5th} and a non-monophyletic Brachycephaloidea was recovered only by TAP. The SAP_{5th} and TAP analyses recovered a different group as sister to Hemiphractidae, Brachycephaloidea and Athesphatanura, respectively. Both analyses recovered unresolved clades only at the ingroup level (Supplementary Figures 1 and 2), within *Cryptobatrachus*, *Gastrotheca* and *Hemiphractus*, and within *Stefania* only in SAP_{5th}.

Both TAP and SAP_{5th} analyses recovered the (*Gastrotheca* + *Hemiphractus*) clade as sister to a clade including the other four genera. However, only SAP_{5th} recovered a sister relationship between *Fritziana* and *Stefania*, while in TAP results *Stefania* resulted sister to the *Cryptobatrachus* and *Flectonotus* clade. Although both analyses resulted in a polytomy among *Cryptobatrachus* terminals, only in the strict consensus of TAP the *Flectonotus* clade resulted in an unresolved position, within the *Cryptobatrachus* clade. Within *Fritziana*, the strict consensus trees differed only in the position of *F*. cf. *fissilis* 2. The TAP analysis recovered the *evansi* and *woodleyi* clades as sister, whereas the SAP_{5th} recovered a sister relationship between the *riveroi* and *woodleyi* clades. Within *Hemiphractus* and *Gastrotheca*, the TAP and SAP_{5th} analyses recovered very different relationships. Within *Hemiphractus*, in TAP results, the *H*. *scutatus* clade resulted as sister to all other terminals, whereas in SAP_{5th} results, *H*. *helioi* and *H*. sp. 2 resulted sister to the remaining terminals. Although both recovered a polytomy among *H*. *elioti*, *H*. *fasciatus*, and *H*. *panamensis*, only in the TAP strict consensus tree the relationships of *H*. *bubalus*, *H*. *johnsoni*, and *H*. *proboscideus* remained unresolved. Also, only the SAP_{5th} strict consensus resulted in a polytomy among the *H*. *scutatus* terminals. Regarding relationships within *Gastrotheca*, the position of *G*. *ovifera*, and the non-monophyly of the *G*. *longipes* (*Amphignathodon*) species Group were among the main differences of SAP_{5th} compared to TAP results. Supplementary Figure 7 includes visual detailed comparisons between TAP and SAP_{5th} results.

The effect of optimality criteria: Parsimony vs. Maximum Likelihood

This comparison was made by contrasting the results of SAPg vs. SALg and of SAPm vs. SALm because, heuristics aside, these two pairs of analyses only differed in optimality criterion.

Table 6. Percentage of resampling indices, Bootstrap (BS) and Jackknife (JK), for the main clades considered in this study. An asterisk denotes Jackknife = 0–50 %, while an em dash indicates non-monophyly.

	TAP (JK)	**SAP$_{5th}$** (JK)	**SAPm** (JK)	**SAPg** (JK)	**SALg** (BS)	**SALm** (BS)
Athesphatanura	99	–	57	64	–	–
Brachycephaloidea	–	56	–	–	65	92
Hemiphractidae	99	*	76	59	60	93
Athesphatanura + Hemiphractidae	99	–	52	53	–	–
Brachycephaloidea + Hemiphractidae	–	*	–	–	–	62
Cryptobatrachinae Frost *et al.* (2006)	–	–	–	–	94	95
Hemiphractinae Peters (1862)	–	–	–	–	57	90
Cryptobatrachus	–	60	–	–	96	96
Flectonotus	–	95	–	–	100	100
Fritziana	100	99	99	99	100	100
Gastrotheca	100	100	100	99	58	89
Hemiphractus	100	99	100	100	94	91
Stefania	100	99	99	99	98	97
Gastrotheca species groups (Castroviejo-Fisher *et al.*, 2015)						
Gastrotheca fissipes species group	–	–	–	–	–	–
Gastrotheca longipes species group	100	–	67	51	56	91
Gastrotheca marsupiata species group	–	–	–	–	–	–
Gastrotheca microdiscus species group	100	91	89	89	–	–
Gastrotheca subgenera (Duellman, 2015)						
Amphignathodon	100	–	67	51	81	87
Australotheca	100	91	89	89	–	–
Cryptotheca	100	–	100	99	59	92
Duellmania	–	–	–	–	–	–
Edaphotheca	–	–	–	–	–	–
Eotheca	–	–	–	–	–	–
Gastrotheca	–	–	–	–	–	–
Opisthodelphys	–	*	–	–	*	*
Stefania clades (Kok *et al.*, 2017)						
evansi clade	100	99	99	99	100	100
ginesi clade	100	99	99	99	99	99
riveroi clade	100	99	99	99	100	100
woodleyi clade	100	99	99	99	99	98

The SAPg strict consensus of 1 402 optimal trees was obviously a less-resolved optimal solution than SALg optimal tree (225 vs 269 clades; Table 4). Similarly, the SAPm strict consensus of 1 132 trees resulted in 247 clades, and the SALm optimal solution in 269 clades. The results of SALg (Supplementary Figure 5) and SAPg (Supplementary Figure 3) analyses shared 188 clades, 84 % of the 225 clades present in the SAPg strict consensus. The strict consensus of the SAPm analysis shared 80 % of its clades with the SALm optimal tree.

Within the outgroup, SAPg and SAPm, opposite to SAL analyses, recovered the monophyly of Athesphatanura and the non-monophyly of Brachycephaloidea. Also, in SAPg and SAPm analyses Athesphatanura resulted as sister to Hemiphractidae, whereas SALg recovered all athesphatanurans, except Dendrobatoidea, as sister to hemiphractids, and SALm recovered Brachycephaloidea as the sister taxon of the ingroup. At the ingroup level, the most striking differences of SAL analyses results, compared to SAPg and SAPm results, were the presence of Cryptobatrachinae and Hemiphractinae clades and the sister relationship of *Gastrotheca* and *Fritziana* (*Gastrotheca* and *Hemiphractus* in SAPg and SAPm results). Within *Fritziana*, SAPg and SAPm analyses recovered different relationships for the (*F*. cf. *fissilis* 2 + *F*. *goeldii* lineages) clade, *F*. *tonimi*, *F*. *ulei*, and *F*. cf. *fissilis* 1 and, than SAL analyses. The position of *Gastrotheca ovifera* and the polyphyly of the *G*. *fissipes* species Group (*Eotheca*) were the main differences within *Gastrotheca,* between the two SAL analyses and the SAPg and SAPm analyses.

Within *Hemiphractus*, SAPg and SALg analyses recovered different relationships for *H*. sp. 1, and the (*H*. *helioi* + *H*. sp. 2) and the *H*. *scutatus* clades. The SAPm and SALm results only had in common *H*. sp. 1 as sister to all other terminals. There were few differences between SAPg and SALg within *Stefania*, including the position of *S*. *ginesi* within the *ginesi* clade. The SAPm analysis recovered a sister relationship between the *evansi* and *woodleyi* clades, whereas the SALm analysis recovered the *riveroi* clade as sister to the *woodleyi* clade.

Detailed visual comparisons between SAPg and SALg, and of SAPm and SALm are presented in Supplementary Figures 17 and 20, respectively.

The effect of indel coding: Gaps as unknown nucleotides, a fifth state or as binary characters

Within parsimony, the three indel coding strategies were easily investigated by comparing the results of SAP_{5th} (Supplementary Figure 2), SAPg (Supplementary Figure 3), and SAPm (Supplementary Figure 4). Within a likelihood context, two coding options (i.e. indels as unknown nucleotides and as binary characters) were compared through the results of SALg (Supplementary Figure 5) and SALm (Supplementary Figure 6).

Among SAP strict consensus trees, the SAP5th analysis had the following unique results: i) the monophyly of Brachycephaloidea; ii) the non-monophyly of Athesthatanura; iii) the sister relationship between Brachycephaloidea and Hemiphractidae; iv) the sister relationship of *Fritziana* and *Stefania*. Also, *Cryptobatrachus* and *Flectonotus* clades resulted monophyletic in the SAP_{5th} strict consensus tree, opposite to other parsimony results. Results of SAPg and SAPm agreed on the non-monophyly of Brachycephaloidea, the monophyly of Athesphatanura and its sister relationship to Hemiphractidae, and the sister relationship of *Stefania* with the *Cryptobatrachus* and *Flectonotus* clade. All SAP analyses agreed on recovering *Hemiphractus* as sister to *Gastrotheca*.

The strict consensus of the SAP_{5th} analysis was more resolved than the SAPm strict consensus (255 vs. 247 clades; Table 5). These are the third pair of parsimony analyses sharing the most number of clades (203). The strict consensus of SAPm shared 82 % of its clades with the SAP_{5th} strict consensus. Differences between the results of SAP_{5th} and SAPm involved different relationships of few terminals and clades within *Stefania*, most important differences involved *Gastrotheca* and *Hemiphractus* relationships (Supplementary Figures 2, 4, and 13).

The SAPm strict consensus was more resolved than the SAPg strict consensus (247 vs. 225 clades; Table 5). These analyses shared the highest number of shared clades (222) among the results of parsimony analyses, the SAPg strict consensus tree shared all but three clades with the SAPm strict consensus tree. Both SAPg and SAPm recovered the same relationships among hemiphratid genera. Main differences between SAPg and SAPm involved relationships within *Gastrotheca* and *Stefania* (Supplementary Figures 3, 4, and 16).

The strict consensus of the SAP_{5th} analysis was better resolved than the SAPg strict consensus tree (255 vs. 225 clades, Table 5). These analyses shared the least number of clades (197) among the results of parsimony analyses. The SAPg strict consensus tree shared 88 % of its clades with the SAP_{5th} strict consensus. The results of SAPg and SAP5th had few differences within *Stefania*, with most important differences within *Gastrotheca* and *Hemiphractus* (Supplementary Figures 2, 3, and 12).

Both SALg and SALm optimal trees had 269 fully resolved clades. These trees shared 256 clades (95 %), the highest number of shared clades among all analyses. Within the outgroup, both analyses recovered the non-monophyly of Athesphatanura and the monophyly of Brachycephaloidea. However, different clades were recovered as sister to Hemiphractidae, the largest clade of athesphatanurans and Brachycephaloidea for SALg and SALm, respectively. The SALg and SALm analyses recovered the same relationships among hemiphractid genera. The most important differences between the SALg and SALm results involved relationships within *Hemiphractus*, followed by differences within the *Gastrotheca marsupiata* species Group (restricted to the *Gastrotheca* subgenus clade), and few differences within *Fritziana* and *Stefania* (Supplementary Figures 5, 6, and 21).

Identifying single and combined effects: comparisons between TAP and SAL analytical strategies

Given that the TAP and SAL strategies differed in all analytical factors, the TAP strict consensus was intended to be compared with each of the SAL optimal trees through the strict consensus trees of the SAP$_{5th}$ and SAPg or SAP$_{5th}$ and SAPm, as appropriate. However, the SAP$_{5th}$ strict consensus had multiple discordances with both SAPg and SAPm strict consensuses. This large amount of incompatibilities rendered very poorly resolved strict consensus trees between SAP$_{5th}$ and SAPg or SAP$_{5th}$ and SAPm (trees not shown) and made comparisons uninformative. A different strategy was devised to compare the TAP results with the respective SAL best topologies by defining five scenarios with the help of the corresponding SAP result: i) clades shared by TAP and SALg/m, but not SAPg/m, show that the optimality criterion (maximum likelihood) caused congruence between SALg/m and TAP results, by overcoming the role of the alignment and/or the indel coding that caused the differences between TAP and SAPg/m; ii) clades shared by TAP and SAPg/m, but not by SAL, indicate that the optimality criterion is the single cause of the differences; iii) clades exclusive of each of the three analytical strategies cannot be used to discern between the effect of optimality criteria, alignment method, and indel treatments; iv) clades shared by SALg/m and SAPg/m, but not TAP, are examples of differences caused by the alignment method and/or indel coding; and v) clades shared by the three analyses are evidence of relationships not sensitive to any of the analytical factors under study. Below, relevant examples of all five scenarios both related to comparisons of results of TAP with SALg and SALm are presented. Visual descriptions of detailed topological differences among the results of TAP, SALm, SALg, SAPm, and SAPg are presented in Supplementary Figures 8–11, 16–21.

The TAP strict consensus (256 clades) and SALg optimal tree (269 clades) shared 192 clades, while TAP and SALm (269) trees shared 194 clades (75 and 76 % of TAP strict consensus clades, respectively). The TAP and SAP5th strict consensus were the best resolved parsimony analyses (256 and 255 clades, respectively), while SAPg consensus, with 225 clades, was the least resolved tree. The TAP strict consensus shared 80 % of its clades with the SAP5th strict consensus, 83 % with the SAPm strict consensus, and 82 % with the SAPg strict consensus.

Common clades between the strict consensus of TAP and SALg were also present in the SAPg consensus tree. So, there were no instances of the optimality criterion (Maximum Likelihood) causing congruence between TAP and SALg. However, TAP and SALm shared a common result, absent in SAPm strict consensus, the clade (within the *Gastrotheca marsupiata* species Group) composed by *Duellmania* terminals and *Gastrotheca* (*Edaphotheca*) *galeata*. There were clades shared by TAP, SAPg, and SAPm, but not SAL analyses, indicating an effect of the optimality criteria. The monophyletic athesphatanuran clade as sister to Hemiphractidae, the intergeneric relationships within Hemiphractidae, the monophyly of the *G. microdiscus* species Group, and a single clade including *G. ovifera* as sister to the *G. fissipes* species Group terminals were common results among the strict consensus trees of TAP, SAPg, and SAPm not recovered by the SAL analyses. Other similar results between TAP, SAPg, and SAPm, not recovered by SAL analyses, include *Fritziana tonimi* as sister to the remaining *Fritziana* terminals, the sister relationship between *F. ulei* and *F. ohausi* lineages, relationships among species groups and/or subgenera within *Gastrotheca*. Most evident instances of differences among TAP, SAPg, SAPm and SAL analyses were found within *Hemiphractus*. The sister relationships of *Fritziana* cf. *fissilis* 2 and *F. ohausi* lineages, and between *Stefania schuberti* and *S. riae* were examples of differences caused by the alignment and/or the indel coding method, because these results were common between SAPg and SAPm and its corresponding SAL analysis, but not TAP. The position of *Hemiphactus* sp. 1 as sister to all other *Hemiphractus* terminals, shared by SALm and SAPm but absent in TAP, was also an example of the effect of the alignment and/or the indel coding method. The TAP, SAPg, SAPm and SAL analyses recovered the *Gastrotheca longipes* species Group (*Amphignathodon* and

Cryptotheca) clade, and the sister relationship between *Hemiphractus helioi* and *H*. sp. 2. Also, TAP, SAPg, SAPm and SAL analyses agreed on recovering a non-monophyletic *Gastrotheca marsupiata* species Group, and *G*. (*Edaphotheca*) *galeata* as sister to the same six *Duellmania* terminals.

Wildcard taxa

Search for wildcard taxa was performed for trees of all parsimony analyses. *Cryptobatrachus* sp. and *Gastrotheca* sp. 1 were found among the first ten terminals with the greater potential to cause resolution decrease in the strict consensus of all four parsimony analyses. For SAPg and SAPm, *Gastrotheca excubitor* MUSM 26280, a terminal poorly represented both in the molecular and phenotypic matrix, was found to be the terminal with the highest potential to have unstable behavior. For SAP5th and TAP, the terminals with the highest potential to have unstable behavior were two of the three only represented by phenotypic characters, *Cryptobatrachus* sp. and *Hemiphractus johnsoni*, respectively. A detailed list of taxa ordered by decreasing potential of wildcard behavior, for each analysis, is presented in Supplementary Tables 1–4.

Discussion

More taxa and more characters: Considerations concerning the studies of Castroviejo-Fisher et al. (2015) and Duellman (2015)

The TAP analysis applied the same analytical strategy as Castroviejo-Fisher *et al*. (2015). However, the dataset employed in the current TAP analysis included 46 % more hemiphractid terminals, 359 more DNA sequences for up to 13 genes (mitochondrial and nuclear), additional data for external morphology and behavioural characters of 21 *Fritziana* and *Gastrotheca* terminals, and for osteological characters of three *Hemiphractus* terminals. Observed differences can only be attributed to the increase in evidence.

The results of Castroviejo-Fisher *et al*. (2015) and the present analyses agreed on recovering Brachycephaloidea as non-monophyletic, and a monophyletic Athesphatanura as sister to Hemiphractidae. Differences within the outgroup will not be discussed, to focus only on the several important differences within the study group. Clades corresponding to Cryptobatrachinae and Hemiphractinae were not recovered by the TAP analysis. The only common result between TAP and Castroviejo-Fisher *et al*. (2015) results was the sister relationship of *Gastrotheca* and *Hemiphractus*.

The TAP analysis recovered the two *Flectonotus* sister terminals within a clade of unresolved relationships among *Cryptobatrachus* terminals. The *Cryptobatrachus* sp. terminal, represented only by phenotypic characters, resulted as sister to the two *Flectonotus* sister terminals, in 20 % of the TAP optimal trees; and was also found as the second most probable terminal to decrease resolution in the strict consensus of TAP results.

Differences between Castroviejo-Fisher *et al*. (2015) dataset and the one analyzed for this study were on the number of taxa and data, mainly DNA sequences, for *Fritziana* and *Stefania*. A relatively fewer amount of taxa and data was added for *Gastrotheca* and *Hemiphractus*. Regading *Fritziana* relationships, the only common result was the position of *Fritziana tonimi* as sister to all other *Fritziana* terminals. The addition of terminals and data changed most of the relationships within this genus, considering Castroviejo-Fisher *et al*. (2015) dataset was missing only terminals of *F*. cf. *fissilis* 2 and *F*. sp. CS2, as it included at least one terminal corresponding to the *F*. *goeldii*, *F*. *ohausi*, *F*. *mitus* and *F*. CS1 clades, when compared to the analyzed dataset. In contrast, the overall relationships among *Stefania* clades were stable, only the position of *Stefania riae* was different. Within *Gastrotheca*, relationships among species groups were the same. However, *G*. *ovifera* resulted sister to a clade of Atlantic

Forest *Gastrotheca* species, the *G. fissipes* species Group, and no longer closely related to the Andean species of the *G. marsupiata* species Group. Finally, within *Hemiphractus*, the most important change was the position of a clade of all *H. scutatus* terminals as sister to all other *Hemiphractus*, and a decrease in resolution regarding the relationships of six terminals.

The SALm analysis applied a similar analytical strategy of Duellman (2015). However, the data matrix analyzed in the present study had 86 % more terminals of egg-brooding frogs, 124 more outgroup terminals, up to 51 phenotypic characters for 243 terminals, and up to 9 580 bp from 16 nuclear and mitochondrial genes. Both studies agreed on recovering Hemiphractidae as sister of Brachycephaloidea and the monophyly of Cryptobatrachinae, Hemiphractinae, and all their genera. However, within Hemiphractinae, *Fritziana* was recovered as sister to *Gastrotheca*, instead of *Stefania* as in Duellman (2015). The limited number of terminals of Duellman (2015) dataset prevented comparison of the relationships within *Cryptobatrachus*, *Fritziana*, and *Hemiphractus*. Regarding *Gastrotheca,* only three subgenera proposed by Duellman (2015), *Amphignathodon*, *Cryptotheca*, and *Opistodelphys*, were recovered. *Gastrotheca* (*Opistodelphys*) *ovifera* was recovered as sister to a clade including *Australotheca*, *Edaphotheca*, some *Eotheca* terminals, *Duellmania* and *Gastrotheca*, instead of sister to Andean lineages (*Edaphotheca*, *Duellmania* and *Gastrotheca*) as in Duellman (2015).

Both, Castroviejo-Fisher *et al*. (2015) and Duellman (2015) results were sensitive to the addition of taxa and characters even when the increase of evidence was limited, considering the differences between the analyzed dataset and that of Castroviejo-Fisher *et al*. (2015). The convergence of TAP and SALm results has not increased despite identical taxa and character sampling—notwithstanding that there are still important differences and that indels are not considered characters in SALm. So, other analytical factors need to be explored as potential causes for the observed differences.

The impact of the optimality criteria

The comparison of SAPg vs. SALg, SAPm vs. SALm, and TAP vs. the two SAL strategies allowed the identification of qualitatively important differences attributable to the use of maximum likelihood or parsimony (e.g. the sister relationship of *Fritziana* and *Gastrotheca* vs. the sister relationship of *Hemiphractus* and *Gastrotheca*; the monophyly vs. the non-monophyly of Cryptobatrachinae and Hemiphractinae). A strong effect of the optimality criteria was evident, there are results exclusive of parsimony and maximum likelihood analyses, respectively. In fact, the two topologies sharing the least number of clades, the consensus tree of SAPg and SALg optimal tree, differ only in the optimality criterion. Also, many instances of common results between analyses differing in all studied factors except optimality criterion (i.e. the second scenario when analyzing single and combined effects of analytical factors) were identified. However, some results recovered by the SAP_{5th} analysis contrast with results shared by SAPg, SAPm, and TAP, some being exclusive to this analysis (e.g. *Fritziana* as sister to *Stefania*) and others shared with SAL results (e.g. a monophyletic Brachycephaloidea sister to Hemiphractidae; the monophyly of *Cryptobatrachus* and *Flectonotus*) indicating the effect other analytical factors over the effect of optimality criteria. Regarding the monophyly of *Cryptobatrachus* and *Flectonotus*, a small exploratory analysis was performed. Since *Cryptobatrachus* sp., the terminal recovered as sister to *Flectonotus* only in TAP, SAPg and SAPm analyses, was represented only by phenotypic characters, a matrix including all 51 phenotypic characters for 39 terminals, the best represented in the main matrix, was analyzed in TNT under Traditional Search (results not shown). Using one of the resulting optimal trees, the two sets of relationships obtained for parsimony analyses among *Cryptobatrachus*, *Flectonotus*, *Fritziana*, and *Stefania* were recreated in Mesquite. The position of *Cryptobatrachus* sp. as sister to the two *Flectonotus* terminals is as optimal as both clades being reciprocally monophyletic only when the intergeneric relationships corresponded to those recovered by TAP, SAPg and SAPm. When *Fritziana* and *Stefania* are sister clades, the position of *Cryptobatrachus* sp. as sister to the two *Flectonotus* terminals adds one step to the tree length.

A different issue, with more pragmatic implications, is the limitation of the maximum likelihood software, because of its implementation or computational difficulties, to report a single optimal tree for each log-likelihood value during tree search enmascarating lack of or ambiguous evidence in datasets. So those clades recovered as a polytomy by parsimony analyses but completely resolved by maximum likelihood analyses must be interpreted cautiously. As mentioned before, the completely resolved relationships among *Cryptobatrachus* terminals recovered by both SALm and SALg analyses (Appendices VII and VIII), as opposed to a complete polytomy in all parsimony strict consensuses (Appendices IV–VI; Figure 4), showed that available evidence is not enough to unambiguously resolved relationships within *Cryptobatrachus*. The terminal *Cryptobatrachus* sp. was identified among terminals most likely to behave as wildcard taxa for all parsimony results. It is also worth noting that between one to four *Cryptobatrachus* terminals were ranked among the ten terminals most likely to behave as wildcard taxa. The terminal *Cryptobatrachus* sp. is represented only by 48 out of 51 phenotypic characters, and the only other terminal within the genus with more than three phenotypic characters is *C. fuhrmanni* JDL 14865 (49/51 phenotypic characters). Furthermore, those shared three phenotypic characters are not variable among *Cryptobatrachus* species. In addition, among terminals with DNA data, no molecular marker is shared among all *Cryptobatrachus* terminals. Simply put, the available evidence is not adequate to resolve the relationships within that clade. Padial *et al*. (2014) provided a similar empirical example with *Eleutherodactylus* frogs. A related issue are the high support frequencies that can be obtained for properly unsupported clades for supermatrices with non-randomly distributed missing data (Simmons and Goloboff, 2013, 2014). For instance the BS frequencies of both SALg and SALm resulted as high as 96 % and 95 % (Table 6), respectively. These can particularly affect most used maximum likelihood software (i.e. GARLI) which are still limited by not performing through enough tree searching algorithms. However, ML software combining time efficient an more through tree search algorithms are available (i.e IQ-Tree Nguyen *et al*., 2014). Another issue affecting most used maximum likelihood software is not saving multiple trees per search replicate, which is primary related to the extremely long amount of time it would take for matrices with hundreds of terminals and thousands of characters.

The impact of alignment strategies

It was expected to find several similarities between SAP_{5th} and TAP results, despite the different nucleotide homology hypotheses of SAP_{5th} and TAP, inferred through a similarity-alignment and direct optimization, respectively. However, the results of these two strategies had numerous differences (Figure 4; Table 6), evidencing the impact of the alignment on the results of the phylogenetic analyses. Also, the TAP analysis and the two SAP strategies that did not incoporate as individual characters the indels inferred by the similarity-alignment or that dismissed such pattern of indels, SAPg and SAPm, recovered common clades not present in SAP5th consensus tree. This results show the strong effect of the pattern of indels on the SAP5th analyses, and also how parsimony overcame differences in nucleotide homologies and treatment of indels in the other two SAP analyses. The explanation is simple, while similarity-alignment searches for pairwise minimum phenetic distances among sequences, tree-alignment searches for the best topology-specific statements of homology as judged by an optimality criterion that allows transformations through time (e.g. parsimony and maximum likelihood). Also, through DO indels appear as transformations linking ancestral and descent nucleotide sequences, instead as patterns implied by MSA (Wheeler, 1996; Wheeler *et al*., 2006).

These results constitute empirical examples of the important impacts of the alignment strategy on phylogenetic analyses, once more demonstrating that this analytical factor is as important as the optimality criterion.

What shall we do with indels in phylogenetics?

Multiple sequence alignment creates gap characters representing insertion/deletion events, as these characters represent historical events should be included in phylogenetic analyses (Giribet and Wheeler, 1999; Simmon and Ochoterena, 2000). Three different treatments of indels allowed the evaluation of the effect of incorporating or excluding the information of indel/gap characters inferred through MSA.

With respect to SAP strategies, in terms of resolution, the SAP_{5th} strict consensus was the best resolved of all, while SAPm and SAPg analyses resulted in less resolved strict consensus trees (Table 5). The least resolved strict consensus tree of SAPg included the same unresolved clades as SAPm and some additional ones (Appendices V, VI). So, the diminished resolution of the SAPg strict consensus could be explained by a combination of coding all MSA-inferred indels as missing data combined with ambiguous signal in the 1 421 SIC indel characters. In addition, there are topological differences among the results of the three SAP analyses. The strict consensus of the SAP_{5th} strategy had several discordances with the strict consensus trees of SAPg and SAPm, the latter two analyses recovered more similar topologies (Appendices IV–VI; Figure 4; Table 6). As discussed in the previous section, parsimony analyses resulted in more similar topologies when dismissing the pattern of indels inferred through MSA or by coding gaps as separate characters. Also, although few, there are results exclusive of SAPm, involving relationships within *Gastrotheca*, *Hemiphractus* and *Stefania*, which can be attributed to an effect of dismissing the information of indel characters. Within Hemiphractidae, the support values of the three SAP strategies were very similar and overall high. These results indicate that the different treatments of indel information did not have any effect over the resampling measure.

Regarding the two SAL strategies, the effect of considering indels as missing data or as a matrix of presence/absence characters generated differences on the inferred phylogenetic relationships of both the ingroup and outgroup (Appendices VII, VIII; Figure 4; Table 6). The treatment of indels has an effect on BS values for the SAL analyses, although BS values for both matrices were high, the BS values for the SALm matrix were higher for some clades within Hemiphractidae. The comparison of the results of the two SAL analyses with different indel treatments contributes an example that demonstrates the effect of different treatments of indels in a parametric inference framework, which has been less explored (e.g. Egan and Crandall, 2008; Nagy *et al*., 2012; Luan *et al*., 2013; Boutte *et al*., 2019) and where indels are more frequently recoded as unknown nucleotides. These results highlight the importance to improve the computational features of methods that incorporate indel information into maximum likelihood and Bayesian phylogenetic software (Fleissner *et al*., 2005; Lunter *et al*., 2005; Redelings and Suchard, 2005; Suchard and Redelings, 2006; Novák *et al*., 2008; Westesson *et al*., 2012).

This study demonstrated that indel characters have quantitatively relevant phylogenetic information that can change the topology and resolution of phylogenetic hypothesis. Indeed, the fact that indels contain information relevant to phylogenetic analyses is nowadays widely known and accepted. However, lack of computational efficiency and biological realism of the currently available indel coding methods still prevent its common use to include MSA inferred indels on phylogenetic analyses. Although indel coding methods are still an alternative, currently available statistical alignment software (Fleissner *et al*., 2005; Lunter *et al*., 2005; Redelings and Suchard, 2005; Suchard and Redelings, 2006; Novák *et al*., 2008) coupled with the development of novel statistical alignment algorithms and improvements to existing software (Nute and Warnow, 2016; Levy Karin *et al*., 2019) offer the alternative to jointly stimate the alignment and phylogenetic tree. Indel information should be considered in phylogenetic analyses, given that there is no good reason to exclude a whole class of characters from any phylogenetic analysis. For two-step phylogenetic analysis, its inclusion is important in order to be consistent by analyzing all characters inferred through MSA.

Taxonomy of Hemiphractidae

The six implemented analytical strategies recovered the monophyly of Hemiphractidae, in accordance with the two previously most extensive phylogenies of egg-brooding frogs (Castroviejo-Fisher *et al*., 2015; Duellman, 2015), however novel phylogenetic relationships among genera were found. In addition, most parsimony analyses recovered a unresolved relationships among all *Cryptobatrachus* terminals within a clade including the two *Flectonotus* sister terminals. These relationships remain to be further tested due to ambivalence of current available evidence. The monophyly of *Fritziana* was recovered, in accordance with previous results (Duellman *et al*., 2011; Schmid *et al*., 2012; Blackburn and Duellman, 2013; Castroviejo-Fisher *et al*., 2015; Walker *et al*., 2016, 2018a). Walker *et al*. (2018a) analysis of DNA sequences from individuals of five of the six described species of *Fritziana*, corroborated the monophyly of the genus and resulted in the discovery of even more candidate species and species with marked population structure and high genetic diversity. According to the presented results, only relationships among species differed from Walker *et al*. (2018a); so no nomenclatural decision needs to be made because *Fritziana* does not include supraspecific taxa. With respect to *Stefania*, all analyses agreed on recovering the monophyly of the clades proposed by Kok *et al*. (2017), while the position of *S. riae* remained dependent on analytical assumptions. With respect to the relationships within *Hemiphractus*, although there is disagreement among most of the analyses, there are no further nomenclatural implications given that it does not include supraspecific taxa, notwithstanding the much needed description of all the putative new species.

Regarding the relationships within *Gastrotheca*, it is obvious that the current taxonomies in use are incompatible and dependent on analytical choices. Consequently, I choose to follow the strict consensus resulting from the TAP analysis because is the only analytical strategy that tested the nucleotide homologies dynamically, using indels as characters, applying a logically consistent optimality criterion between alignment and tree searches, while simultaneously optimizing the prealigned characters of the phenotypic matrix, on multiple alternative trees. This strategy maximizes the explanation of the observed differences, while simultaneously interpreting evidence more conservatively and throughly exploring tree space.

The subgenera proposed by Duellman (2015) were followed as an infrageneric rank to transmit the diversity within the genus *Gastrotheca*. Below, an updated and indented taxonomic summary of the genus with corresponding equivalences to the species groups of Castroviejo-Fisher *et al*. (2015) is provided.

Genus *Gastrotheca* as in Castroviejo-Fisher *et al*. (2015)

Subgenus *Amphignathodon* as in Duellman (2015)

Content: *Gastrotheca* (*Amphignathodon*) *andaquensis,* *G*. (*Amphignathodon*) *angustifrons*, *G*. (*Amphignathodon*) *antomia*, *G*. (*Amphignathodon*) *bufona*, *G*. (*Amphignathodon*) *cornuta*, *G*. (*Amphignathodon*) *dendronastes*, *G*. (*Amphignathodon*) *guentheri*, *G*. (*Amphignathodon*) *helenae*, and *G*. (*Amphignathodon*) *longipes.*

Remarks: Equivalent in content to the *Gastrotheca longipes* species group of Castroviejo-Fisher *et al*. (2015) except the two species of *Cryptotheca*.

Subgenus *Australotheca* as in Duellman (2015)

Content: *Gastrotheca* (*Australotheca*) *albolineata*, *G*. (*Australotheca*) *ernestoi*, *G*. (*Australotheca*) *fulvorufa*, and *G*. (*Australotheca*) *microdiscus*.

Remarks: Equivalent in content to the *Gastrotheca microdiscus* species group of Castroviejo-Fisher *et al*. (2015).

Subgenus *Cryptotheca* as in Duellman (2015)

Content: *Gastrotheca* (*Cryptotheca*) *walkeri*, *G*. (*Cryptotheca*) *williamsoni*.

Remarks: In the *Gastrotheca longipes* species group of Castroviejo-Fisher *et al*. (2015).

Subgenus *Eotheca* as in Duellman (2015)

Subgenus *Opisthodelphys sensu* Duellman (2015), new synonym

Content: *Gastrotheca* (*Eotheca*) *fissipes*, *G*. (*Eotheca*) *flamma*, *G*. (*Eotheca*) *megacephala*, *G*. (*Eotheca*) *ovifera*, *G*. (*Eotheca*) *prasina*, *G*. (*Eotheca*) *pulchra*, and *G*. (*Eotheca*) *recava*.

Remarks: Including the content of the *Gastrotheca fissipes* species group of Castroviejo-Fisher *et al*. (2015). My results confirm the inclusion of *G. flamma* in this taxon, which is also supported by SAPg and SAPm. I report a putative new species of this clade, the terminal *G*. sp. L. Also, *Gastrotheca ovifera*, a especies known to be restricted to the Cordillera de la Costa and the Sierra de Aroa in northern Venezuela was recovered for the fisrt time as sister to the species known to be restricted to the coastal regions of eastern Brazil.

Subgenus *Gastrotheca*

Subgenus *Duellmania sensu* Duellman (2015), new synonym

Subgenus *Edaphoteca sensu* Duellman (2015), new synonym

Content: *Gastrotheca* (*Gastrotheca*) *abdita*, *G*. (*Gastrotheca*) *aguaruna*, *G*. (*Gastrotheca*) *antoniiochoai*, *G*. (*Gastrotheca*) *aratia*, *G*. (*Gastrotheca*) *argenteovirens*, *G*. (*Gastrotheca*) *atympana*, *G*. (*Gastrotheca*) *aureomaculata*, *G*. (*Gastrotheca*) *caeruleomaculata*, G. (*Gastrotheca*) *carinaceps*, *G*. (*Gastrotheca*) *christiani*, *G*. (*Gastrotheca*) *chrysosticta*, *G*. (*Gastrotheca*) *cuencana*, *G*. (*Gastrotheca*) *dunni*, *G*. (*Gastrotheca*) *dysprosita*, *G*. (*Gastrotheca*) *elicioi*, *G*. (*Gastrotheca*) *espeletia*, *G*. (*Gastrotheca*) *excubitor*, *G*. (*Gastrotheca*) *galeata*, *G*. (*Gastrotheca*) *gracilis*, *G*. (*Gastrotheca*) *griswoldi*, *G*. (*Gastrotheca*) *lateonota*, *G*. (*Gastrotheca*) *lauzuricae*, *G*. (*Gastrotheca*) *litonedis*, *G*. (*Gastrotheca*) *lojana*, *G*. (*Gastrotheca*) *marsupiata*, *G*. (*Gastrotheca*) *monticola*, *G*. (*Gastrotheca*) *nebulanastes*, *G*. (*Gastrotheca*) *nicefori*, *G*. (*Gastrotheca*) *ochoai*, *G*. (*Gastrotheca*) *oresbios*, *G*. (*Gastrotheca*) *orophylax*, *G*. (*Gastrotheca*) *ossilaginis*, *G*. (*Gastrotheca*) *pacchamama*, *G*. (*Gastrotheca*) *pachachacae*, *G*. (*Gastrotheca*) *peruana*, *G*. (*Gastrotheca*) *phalarosa*, *G*. (*Gastrotheca*) *phelloderma*, *G*. (*Gastrotheca*) *piperata*, *G*. (*Gastrotheca*) *plumbea*, *G*. (*Gastrotheca*) *pseustes*, *G*. (*Gastrotheca*) *psychrophila*, *G*. (*Gastrotheca*) *rebeccae*, *G*. (*Gastrotheca*) *riobambae*, *G*. (*Gastrotheca*) *ruizi*, *G*. (*Gastrotheca*) spectabilis, *G*. (*Gastrotheca*) *splendens*, *G*. (*Gastrotheca*) *stictopleura*, *G*. (*Gastrotheca*) *testudinea*, *G*. (*Gastrotheca*) *trachyceps*, *G*. (*Gastrotheca*) *turnerorum*, *G*. (*Gastrotheca*) *yacuri*, and *G*. (*Gastrotheca*) *zeugocystis.*

Remarks: The subgenera *Gastrotheca*, *Duellmania*, *Edaphoteca* are rampantly non-monophyletic (although *Edaphoteca* is monotypic the preservation of this subgenus would requiere further nomenclatural changes to *Gastrotheca* and *Duellmania*) in all analyses.

Conclusions

For decades, the main concern of the phylogenetic systematics community has been the choice of an appropriate optimality criterion. Despite the importance of other analytical factors—such as the inference of homologies through alignments and the incorporation of indel information—have been recognized, the use of methods that offer alternatives to explore more thoroughly the universe homology schemes and allow for the incorporation of indel events to phylogenetic analyses is uncommon. The presented results, based on an empirical dataset, show clearly the impact of less discussed factors, the aligment method and the treatment of indels, in phylogenetic analyses. Different choices related to these factors can lead to important qualitative and quantitative differences among the results of phylogenetic analyses. The presented results stresses the fact that the choice of how to analyze data for phylogenetic inferences should be an *a priori* decision. Such a decision should consider properties as: realism; conservatism in the interpretation of the evidence; capacity to incorporate different kinds of evidence; ontological and epistemological congruence; operational flexibility; and computational speed. The other alternative is to be agnostic and take a pluralistic analytical approach. However, it would lead to an infinite sensitivity analysis where multiples analytical factors and the value of multiple parameters can be modified (e.g. indel opening cost, indel extension, substitution cost, transversion cost, transition cost, etc.).

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Appendix I. Terminals, voucher codes (for ingroup taxa), and GenBank accession numbers of DNA sequences used in this study. New sequences generated in this study are indicated by the number of base pairs for each marker in bold face. Acronyms follow Frost (2018) except for CBG (Centro de Biodiversidad y Genética, Cochabamba), CHP (Círculo Herpetológico de Panama), CORBIDI (Centro de Ornitología y Biodiversidad), CTMZ (tissue collection at MZUSP), DCC (David C. Cannatella field series), EVACC (El Valle Amphibian Conservation Center), GGU (Giussepe Gagliardi Urrutia field series), JDL (John D. Lynch field series), JLG (João Luis Gasparini field series), JMP (José M. Padial field series), LM (Linda Maxson field series), MNCN/ADN (tissue collection at MNCN), MJH (Martin J. Henzl field series), MTR (Miguel T. Rodrigues field series), MVUP (Museo de Vertebrados de la Universidad de Panamá), VUB (unknown, from Kok *et al*., 2012, 2017), Vz (unknown, from Wiens *et al*., 2007).

Taxon	**tRNAPhe**	**12S**	**tRNAVal**	**16S (1)**	**16S (2)**	**tRNALeu**	**ND1**	**COI**	**cytb**	**28S**	**C—MYC 2**	**C—MYC 3**	**CXCR4**	**H3a**	**POMC**	**RAG1 (1)**	**RAG1 (2)**	**Rho**	**SIAH**	**SLC8A1**	**SLC8A3**	**Tyr**
Acris crepitans	EF566970	EF566970	EF566970	EF566970	AY819491	AY819491	AY819491	—	EF988113	AY844194	AY819194	AY819268	EF107468	DQ284107	AY819109	EF107304 + AY844358		AY844533	AY844762	EF107244	EF107403	AY844019
Adelophryne patamona	—	EU186679	EU186679	EU186679	GQ345247	GQ345247	GQ345247	—	GQ345201	GQ345136	GQ345149	GQ345167	GQ345185	GQ345215	GQ345262	GQ345280 + GQ345296		GQ345302	GQ345312	GQ345233	GQ345331	EU186772
Adenomera andreae	—	HQ290944	HQ290944	HQ290944	HQ290944	HQ290944	HQ290944	KC520689	JQ321766	—	—	—	—	—	KC604061	KC604015 + KC604037		KC604094	—	HQ290704	—	KC520698
Adenomera hylaedactyla	—	KC603939	KC603939	KC603939	—	—	—	KC603995	KC603967	—	—	—	—	DQ284093	KC604063	KC604017 + KC604039		KC604095	—	—	—	JN691529
Agalychnis annae	—	GQ366221	GQ366221	GQ366221	GQ366291	GQ366291	GQ366291	—	GQ365913	—	EF174320	—	GQ365977	—	EF158394	EF174311		—	GQ366159	—	—	GQ366198
Agalychnis callidryas	—	DQ2834	DQ283	DQ28	FJ48	FJ48	FJ489334	FJ76	—	—	EF1	—	AB6	DQ2	EF1	AY32376		AY8	AY8	AB6	—	DQ2

Taxon	tRNAPhe	12S	tRNAVal	16S (1)	16S (2)	tRNALeu	ND1	COI	cytb	28S	C—MYC 2	C—MYC 3	CXCR4	H3a	POMC	RAG1 (1)	RAG1 (2)	Rho	SIAH	SLC8A1	SLC8A3	Tyr
		23	423	3423	9334	9334		6570			74321		12054	84401	58395	5		44537	44765	12055		83018
Allobates femoralis	—	AY364543	AY364543	AY364543	HQ290951	HQ290951	HQ290951	DQ502811	HQ290531	DQ283465	—	—	—	DQ502325	HQ290831	DQ503327		DQ503215	DQ503077	HQ290711	—	HQ290891
Allophryne ruthveni	—	AY843564	AY843564	AY843564	AY819458	AY819458	AY819458	—	AY843786	—	AY819162	AY819242	—	—	AY819077	EU663432		AY844538	AY844766	—	—	—
Alsodes neuquensis	—	AY843565	AY843565	AY843565	JX204017	JX204017	JX204017	JX203891	AY843787	AY844197	—	—	—	DQ284118	—	AY844362		AY844539	AY844767	—	—	—
Amazophrynella minuta	—	AY843582	AY843582	AY843582	AY819462	AY819462	AY819462	DQ502828	AY843804	—	AY819166	AY819246	DQ306496	DQ284096	AY819081	DQ158346 + DQ503337		AY844555	—	—	—	EF364362
Atelognathus patagonicus	—	AY843571	AY843571	AY843571	—	—	—	JX203909	AY843793	AY844203	—	—	—	—	—	AY844368		AY844545	AY844773	—	—	AY844027
Barycholos pulcher	—	EU186727	—	EU186709	—	—	—	—	—	—	—	—	—	—	—	—	EU186744	—	—	—	—	EU186765
Barycholos ternetzi	—	—	—	DQ283094	—	—	—	—	—	DQ283496	—	—	—	DQ284144	—	—		DQ283810	—	—	—	DQ282921
Batrachyla leptopus	—	AY843572	AY843572	AY843572	—	—	—	—	AY843794	AY844204	—	—	—	DQ284119	—	AY844369		AY844546	AY844774	—	—	AY844028
Batrachyla taeniata	—	AY578817	—	DQ864550	—	—	—	—	KC603962	—	—	—	—	—	KC604075	KC604007		—	—	—	—	—

Taxon	tRNAPhe	12S	tRNAVal	16S (1)	16S (2)	tRNALeu	ND1	COI	cytb	28S	C—MYC 2	C—MYC 3	CXCR4	H3a	POMC	RAG1 (1)	RAG1 (2)	Rho	SIAH	SLC8A1	SLC8A3	Tyr
Boana boans	—	AY843610	AY843610	AY843610		AY819496	AY819496	—	AY843835	AY844231	AY819199	381 (6 indels)	—	DQ284086	AY819114	—		AY844588	AY844809	—	—	AY844055
Boana lanciformis	—	AY843636	AY843636	AY843636		AY819543	AY819543	—	AY843870	AY844258	—	—	—	—	—	—		AY844619	AY844837	—	—	AY844081
Brachycephalus alipioi	—	HQ435676	—	HQ435690	—	—	—	—	HQ435703	—	—	—	—	—	—		HQ435718	—	—	—	—	HQ435732
Brachycephalus ephippium	—	DQ283091	DQ283091	DQ283091	GQ345243	GQ345243	GQ345243	—	HQ435706	DQ283494	GQ345145	GQ345162	GQ345180	GQ345212	GQ345256	GQ345275 + GQ345290	EU186761	DQ283808	DQ282673	GQ345228	GQ345326	DQ282919
Bryophryne cophites	—	EF493537	EF493537	EF493537	—	—	—	—	—	—	—	—	—	—	—	—	EF493423	—	—	—	—	EF493508
Bufo japonicus	AB303363	AB303363	AB303363	AB303363	AB303363	AB303363	AB303363	AB303363	AB303363	—	—	—	AB612061	—	JN653306	AB612057		—	—	AB612062	—	AB612059
Calyptocephalella gayi	—	DQ283439	DQ283439	DQ283439	AY819471	AY819471	AY819471	JX298374	JX298415	DQ283748	AY819175	—	EF107495	DQ284415	AY819090	AY583337		DQ284036	DQ282893	EF107275	EF107440	JX298244
Ceratophrys ornata	—		DQ347035	FJ882777	AY523774	AY523774	AY523774	—	L10983	—	—	—	AY364188	—	AY819091	AY364218		AY364399	—	AY523718	AY948886	DQ347168
Ceuthomantis smaragdinus	GQ345133	GQ345132	GQ345132	GQ345132	GQ345132	—	GQ345251	—	GQ345206	GQ345140	GQ345154	GQ345169	GQ345190	GQ345220	GQ345267	GQ345285		GQ345305	GQ345317	GQ345238	GQ345336	—
Chacophrys pierottii	—	DQ283328	DQ283328	DQ283328	—	—	—	—	—	—	—	—	—	—	—	—		—	—	—	—	—

Taxon	tRNAPhe	12S	tRNAVal	16S (1)	16S (2)	tRNALeu	ND1	COI	cytb	28S	C—MYC 2	C—MYC 3	CXCR4	H3a	POMC	RAG1 (1)	RAG1 (2)	Rho	SIAH	SLC8A1	SLC8A3	Tyr
Colostethus pratti	—	HQ290969	HQ290969	HQ290969	HQ290969	HQ290969	HQ290969	DQ502865	HQ290547	DQ503011	—	—	—	DQ502362	HQ290847	DQ503361		DQ503247	DQ503112	HQ290727	—	HQ290907
Craugastor augusti	—	AY326011	AY326011	AY326011	—	—	—	—	—	—	—	AY211289	—	DQ284291	—	—		DQ283935	DQ282786	—	—	DQ282963
Craugastor podiciferus	—	EF493360	EF493360	EF493360	EF493360	—	—	—	GQ345197	—	AY211319	GQ345164 + AY211319	GQ345182	—	GQ345258	GQ345277 + GQ345292	EF493450	—	—	GQ345230	GQ345328	EF493481
Craugastor punctariolus	—	DQ283168	DQ283168	DQ283168	—	—	—	FJ766667	—	DQ283558	—	—	—	DQ284206	—	—		DQ283862	—	—	—	—
Craugastor ranoides	—	DQ283105	—	DQ283106	—	—	—	—	DQ350246	DQ283505	AY211287	AY211287	—	DQ284154	—	—		DQ283820	—	—	—	DQ282928
Craugastor rhodopis	—	DQ283317	DQ283317	DQ283317	—	—	—	—	—	DQ283648	AY211315	AY211315	—	DQ284317	—	—		DQ283960	DQ282808	—	—	DQ282968
Crossodactylodes sp.	—	KC603957	KC603957	KC603958	—	—	—	KC603985	KC603963	—	—	—	—	—	KC604048	KC604011 + KC604033		KC604105	—	—	—	KC604077
Crossodactylus schmidti	—	AY843579	AY843579	AY843579	—	—	HQ290948	DQ502738	AY843801	AY844210	—	—	—	DQ284050	HQ290828	DQ503298		—	AY844780	HQ290708	—	AY844031
Cryptobatrachus boulengeri LM	—	—	—		AY819485	AY819485	AY819485	—	—	—	—	—	—	—	—	—		—	—	—	—	—
Cryptobatrachus	—	AY32605	AY326	AY326	—	—	—	—	—	—	—	—	—	—	—	—		—	—	—	—	—

Taxon	**tRNAPhe**	**12S**	**tRNAVal**	**16S (1)**	**16S (2)**	**tRNALeu**	**ND1**	**COI**	**cytb**	**28S**	**C—MYC 2**	**C—MYC 3**	**CXCR4**	**H3a**	**POMC**	**RAG1 (1)**	**RAG1 (2)**	**Rho**	**SIAH**	**SLC8A1**	**SLC8A3**	**Tyr**
fuhrmanni JDL 14865		0	050	050																		
Cryptobatrachus fuhrmanni (KU 204891 + TNHC-GDC 451)	—	JX564861	JX564861	JX564861	JX564861	JX564861	JX564861	JX564861	JX564861	—	—	—	—	—	—	KJ489582		—	—	—	—	—
Cryptobatrachus fuhrmanni MHUAA 5160	—	KR559912	KR270398	KR270398 + KR270414	—	—	—	—	—	—	—	—	—	—	KR270358	KR138418		—	—	—	—	—
Cryptobatrachus remotus MHNLS 18853 + MHNLS 17664	—	KR559914	KR559914	KR270400 + KR270416	KR138379	KR138379	KR138379	—	—	—	—	—	KR138404	—	KR270360	KR138419	KR138394	—	—	KR138381	KR270375	KR270387
Cycloramphus acangatan	—	HQ634162	—	FJ685683	—	—	—	—	FJ685663	—	—	—	—	—	—	HQ634170 + FJ685703		—	—	—	—	—
Cycloramphus boraceiensis	—	DQ283097	DQ283097	AY162397	—	—	—	DQ502856	DQ502588	DQ283498	—	—	—	DQ284147	—	DQ503357		DQ283813	DQ282675	—	—	DQ282924
Dendrobates auratus	—	DQ347026	DQ347026	AY364565	—	—	HQ290980	FJ766698	AY843803	AY844211	—	—	AY364184	DQ284072	HQ290857	EU325909		AY364395	AY844781	AY948823	AY948879	DQ347160
Dendropsophus nanus	—	AY549346	AY549346	AY549346	GQ366298	GQ366298	GQ366298	—	AY549399	AY844271	AY819208	AY819282	GQ365985	DQ284051	AY819123	AY844437		AY844634	AY844852	—	—	—
Diasporus aff. *diastema*	—	—	—	FJ784484	—	—	—	FJ766810	—	—	—	—	—	—	—	—		—	—	—	—	—
Diasporus diastema	—	EU186682	EU186682	EU186682	EU186682	—	—	—	GQ345200	GQ345135	GQ345148	—	GQ345184	GQ345214	GQ345261	GQ345279 + GQ34529	EU186752	—	GQ34531	GQ345232	GQ345330	EU186773

Taxon	tRNAPhe	12S	tRNAVal	16S (1)	16S (2)	tRNALeu	ND1	COI	cytb	28S	C—MYC 2	C—MYC 3	CXCR4	H3a	POMC	RAG1 (1)	RAG1 (2)	Rho	SIAH	SLC8A1	SLC8A3	Tyr
																5						
Dryophytes arenicolor	EF566960	EF566960	EF566960	EF566960		AY819494	AY819494	—	AY843824	—	AY819197	345 (6 indels)	AY364190	—	AY819112	AY364220 + AY844391		AY844577	AY844802	EF107241	EF107393	AY844048
Dryophytes cinereus	AY680271	AY680271	AY680271	AY680271		AY819498	AY819498	FJ226785	AY843846	AY844241	AY819201	279 (1 'N', 6 indels)	DQ306493	DQ284057	AY819116	AY323766		AY844597	AY844816	—	—	AY844063
Duttaphrynus melanostictus	—	AB331714	AB331714	FJ882791	FJ882791	NC 005794	NC 005794	NC 005794	—	DQ283658	—	—	DQ306508	DQ284324	DQ158317	EU712821		AF249097	DQ282815	AY948805	AY948851	—
Edalorhina perezi	—	AY843585	AY843585	AY843585	—	—	—	—	AY843807	DQ283474	—	—	—	DQ284095	—	—		AY844558	AY844784	—	—	—
Eleutherodactylus cooki	—	EF493539	EF493539	EF493539	EF493539	—	—	—	HQ831648	—	—	GQ345166	—	GQ345213	HQ831999	HQ831830 + GQ345294	EF493413	—	GQ345310	—	—	EF493455
Eleutherodactylus coqui	—	GQ345176	GQ345176	GQ345176	—	—	—	—	EF637038	—	—	—	EF107500	—	—	EF107341		—	—	EF107282	EF107445	—
Eleutherodactylus marnockii	—	DQ283102	—	DQ283101	—	—	—	—	—	DQ283502	—	—	EF107463	DQ284151	—	EF107300	EF493399	DQ283817	DQ282677	EF107238	EF107388	EF493476
Eleutherodactylus nitidus	—	EU186730	—	DQ283316	—	—	—	—	—	DQ283647	—	—	—	DQ284316	—	—		DQ283959	DQ282807	—	—	—
Eleutherodactylus planirostris	—	DQ283107	DQ283107	DQ283107	GQ345246	GQ345246	GQ345246	—	HQ831615	DQ283629	—	—	—	DQ284294	HQ831975	HQ831774	EF493396	DQ283937	DQ282788	—	—	DQ282964

Taxon	**tRNAPhe**	**12S**	**tRNAVal**	**16S (1)**	**16S (2)**	**tRNALeu**	**ND1**	**COI**	**cytb**	**28S**	**C—MYC 2**	**C—MYC 3**	**CXCR4**	**H3a**	**POMC**	**RAG1 (1)**	**RAG1 (2)**	**Rho**	**SIAH**	**SLC8A1**	**SLC8A3**	**Tyr**
Eleutherodactylus thorectes	—	EF493384	EF493384	EF493384	EF493384	—	—	—	HQ831649	—	—	—	—	—	HQ832000	HQ831831	EF493416	—	—	—	—	EF493473
Eleutherodactylus tonyi	—	EF493790	—	EF493602	—	—	—	—	HQ831643	—	—	—	—	—	HQ831994	HQ831824		—	—	—	—	—
Engystomops petersi	—	FJ668190	FJ668190	FJ668190	—	—	—	—	—	—	—	—	—	—	—	GQ375543		FJ668238	—	—	—	—
Engystomops pustulosus	—	DQ337242	DQ337242	DQ337248	—	—	—	FJ766700	—	—	—	—	EF107462	—	—	EF107299		—	—	EF107237	EF107387	—
Espadarana prosoblepon	—	AY843574	AY843574	AY843574	—	—	AY286065	FJ766592	AY843796	AY844206	AY819170	AY8192650	AY364193	—	AY819085	EU663453		AY364404	AY844776	AY948834	AY948896	—
Eupsophus roseus	—	AY843587	AY843587	AY843587	JX204054	JX204054	JX204054	DQ502852	AY843808	—	—	—	—	DQ284120	KC604074	KC604032		AY844560	AY844786	—	—	AY844036
Fejervarya limnocharis	NC 005055	NC 005055	NC 005055	NC 005055	NC 005055	NC 005055	NC 005055	NC 005055	NC 005055	—	—	—	AB277307	DQ284356	AB526646		AB277335	DQ458271	AY844787	AB277321	AB526674	EU980027
Flectonotus fitzgeraldi ZSM 1610 2006 + KU 192399	—	AY819355	KR270401	KR270401 + KR270417	AY819486	AY819486	AY819486	—	—	—	AY819189	AY819265	GQ345177	—	KR270361	DQ679274	KR138395	—	—	GQ345224	GQ345322	KR270388
Flectonotus pygmaeus MHNLS 17478 + KU 184958	—	DQ679232	KR270402	KR270402 + KR270418	DQ679341	DQ679341	DQ679341	—	—	—	—	—	—	—	DQ679310	DQ679275		—	—	—	—	—

Taxon	tRNAPhe	12S	tRNAVal	16S (1)	16S (2)	tRNALeu	ND1	COI	cytb	28S	C—MYC 2	C—MYC 3	CXCR4	H3a	POMC	RAG1 (1)	RAG1 (2)	Rho	SIAH	SLC8A1	SLC8A3	Tyr
Fritziana cf. *fissilis* 1 MNRJ 62845	KR559916	KR559916	KR270403	KR270403 + KR270419				MG099269					KR138406			MG099362				KR138384		KR270389
Fritziana cf. *fissilis* 2 CFBH 28886		MG099095	MG099192	MG099192				MG099300								MG099386					MG099464	
Fritziana goeldii Go I (MNRJ 44592 + CFBH 30938)		MG099091	KR270406	KR270406 + KR270424				MG099296					KR138409		KR270364	MG099382					KR270379	
Fritziana goeldii Go II MNRJ 53758	MG099057	MG099057	MG099161	MG099161				MG099262								MG099359					MG099442	
Fritziana goeldii Go III CFBH 10910		MG099017	MG099125	MG099125				MG099222								MG099327						
Fritziana ohausi Oh I CFBH 16287		MG099113	MG099208	MG099208				MG099315								MG099401					MG099480	
Fritziana ohausi Oh II MZUSP 139225 (CTMZ 04627)		MG099074		MG099174	KC844945	KC844945	KC844945	MG099279							JN157629		KC844991				MG099451	
Fritziana ohausi Oh III CFBH 7611	MG099010	MG099010	MG099119	MG099119				MG099215								MG099320					MG099407	
Fritziana sp. CS 1 I CFBH 24810	MG099046	MG099046	MG099154	MG099154				MG099251								MG099348					MG099432	
Fritziana sp. CS 1 II	MG099	MG0990	MG099	MG09				MG099								MG09938					MG099	

Taxon	tRNAPhe	12S	tRNAVal	16S (1)	16S (2)	tRNALeu	ND1	COI	cytb	28S	C—MYC 2	C—MYC 3	CXCR4	H3a	POMC	RAG1 (1)	RAG1 (2)	Rho	SIAH	SLC8A1	SLC8A3	Tyr
MZUFV 11721	098	98	195	9195				302								8					467	
Fritziana sp. CS 1 III CFBH 30747		MG099076	MG099176	MG099176				MG099281								MG099371					MG099453	
Fritziana sp. CS 1 IV MZUSP 133700	MG099071	MG099071		JN157634	KC844943	KC844943	KC844943	MG099276							JN157627	KC844990						
Fritziana sp. CS 2 MCNAM 12341	MG099070	MG099070	MG099170	MG099170				MG099275								MG099368					MG099449	
Fritziana mitus CFBH 8273		MG099012	MG099121	MG099121				MG099217								MG099322					MG099409	
Fritziana mitus (CFBH 5726 + MZUSP 135461)		AY843589	AY843589	AY843589	KC844944	KC844944	KC844944	MG099277	AY843809	AY844215					JN157628	AY844379		AY844562	AY844788		MG099405	AY844038
Fritziana tonimi MNRJ 34921		KR559918	KR270405	KR270405 + KR270423				MG099255					KR138408		KR270363	MG099352				KR138386	KR270378	KR270391
Fritziana ulei MNRJ 44622		KR559917	KR270404	KR270404 + KR270421				MG099261					KR138407		KR270362	KR138422	KR138396			KR138385	KR270377	KR270390
Gastrotheca aguaruna KU 212026	—	—	—	KF723438	KF723462	KF723462	KF723462	—	—	—	—	—	—	—	KF723484	KF723505		—	—	—	—	—
Gastrotheca albolineata MNRJ 54401	—	KR559919	KR270407	KR270407 + KR270	KC844949	KC844949	KC844949	—	—	—	—	—	KR138410	—	KR270365	KR138423	KR138397	—	—	KR138387	KR270380	KR270392

Taxon	tRNAPhe	12S	tRNAVal	16S (1)	16S (2)	tRNALeu	ND1	COI	cytb	28S	C—MYC 2	C—MYC 3	CXCR4	H3a	POMC	RAG1 (1)	RAG1 (2)	Rho	SIAH	SLC8A1	SLC8A3	Tyr
				425																		
Gastrotheca antoniiochoai MUSM 27944	—	—	—	JN157622	KC844950	KC844950	KC844950	—	—	—	—	—	—	—	KC844972	KC844993		—	—	—	—	—
Gastrotheca aratia KU 212056	—	—	—	KF723443	KF723467	KF723467	KF723467	—	—	—	—	—	—	—	KF723489	KF723510		—	—	—	—	—
Gastrotheca argenteovirens KU 181168	DQ679233	DQ679233	—	DQ679383	DQ679342	DQ679342	DQ679342	—	—	—	—	—	—	—	DQ679311	—		—	—	—	—	—
Gastrotheca atympana MHNSM 18692	—	DQ679234	—	DQ679384	DQ679343	DQ679343	DQ679343	—	—	—	—	—	—	—	DQ679312	DQ679276		—	—	—	—	—
Gastrotheca aureomaculata KU 181194	—	DQ679235	—	DQ679385	DQ679344	DQ679344	DQ679344	—	—	—	—	—	—	—	—	DQ679277		—	—	—	—	—
Gastrotheca christiani FML 2881	DQ679236	DQ679236	—	DQ679386	DQ679345	DQ679345	DQ679345	—	—	—	—	—	—	—	DQ679313	DQ679278		—	—	—	—	—
Gastrotheca chrysosticta LM 58	DQ679237	DQ679237	—	DQ679387	DQ679346	DQ679346	DQ679346	—	—	—	—	—	—	—	—	DQ679279		—	—	—	—	—
Gastrotheca cornuta USNM 572472 + AMNH 107251	—	AY843591	AY843591	AY843591	DQ679347	DQ679347	DQ679347	FJ766706	AY843811	—	—	—	—	—	DQ679314	DQ679280		—	—	—	—	AY844040
Gastrotheca dendronastes KU 181203	DQ679239	DQ679239	—	DQ679389	DQ679348	DQ679348	DQ679348	—	—	—	—	—	—	—	DQ679315	DQ679281		—	—	—	—	—

Taxon	**tRNAPhe**	**12S**	**tRNAVal**	**16S (1)**	**16S (2)**	**tRNALeu**	**ND1**	**COI**	**cytb**	**28S**	**C—MYC 2**	**C—MYC 3**	**CXCR4**	**H3a**	**POMC**	**RAG1 (1)**	**RAG1 (2)**	**Rho**	**SIAH**	**SLC8A1**	**SLC8A3**	**Tyr**
Gastrotheca dunni ICN 10059 + MHUA A 4800	DQ679240	DQ679240	—	KR270426	DQ679349	DQ679349	DQ679349	—	—	—	—	—	—	—	DQ679316	DQ679282		—	—	—	—	—
Gastrotheca ernestoi MNRJ 57129 + MNRJ 64000	KR559920	KR559920	KR270408	KR270408 + KR270427	KC844952	KC844952	KC844952	—	—	—	—	—	KR138411	—	KR270366	KR138424	KR138398	—	—	KR138388	KR270381	KR270393
Gastrotheca espeletia KU203440				KJ489465	KJ489514	KJ489514	KJ489514								KJ489555							
Gastrotheca sp. KU 173171	DQ679241	DQ679241	—	DQ679391	DQ679350	DQ679350	DQ679350	—	—	—	—	—	—	—	DQ679317	DQ679283		—	—	—	—	—
Gastrotheca excubitor MUSM 26280	—	—	—	JN157623	—	—	—	—	—	—	—	—	—	—	—	—		—	—	—	—	—
Gastrotheca flamma 845 + 846	—	**438**	**—**	**504**	**—**	**—**	**—**	**646**	**—**	**—**	**—**	**—**	**632**	**—**	**571**	**778**	**632**	**—**	**—**	**695**	**539**	—
Gastrotheca fissipes ZUFRJ 7901	—	—	—	—	JX262925	JX262925	JX262925	—	—	—	JX262895	—	—	—	—	—		JX262904	JX262911	—	—	—
Gastrotheca fulvorufa CTMZ 07467	—	—	—	KC844929	KC844954	KC844954	KC844954	—	—	—	—	—	—	—	KC844977	KC844997		—	—	—	—	—
Gastrotheca galeata KU 181700	DQ679242	DQ679242	—	DQ679392	DQ679351	DQ679351	DQ679351	—	—	—	—	—	—	—	DQ679318	DQ679284		—	—	—	—	—
Gastrotheca gracilis DCC 006	—	DQ679243	—	—	—	—	—	—	—	—	—	—	—	—	DQ679319	—		—	—	—	—	—

Taxon	tRNAPhe	12S	tRNAVal	16S (1)	16S (2)	tRNALeu	ND1	COI	cytb	28S	C—MYC 2	C—MYC 3	CXCR4	H3a	POMC	RAG1 (1)	RAG1 (2)	Rho	SIAH	SLC8A1	SLC8A3	Tyr
Gastrotheca griswoldi MHNSM 20588 + CORBIDI 7289	—	AM0397216	—	AM039648	—	—	—	**595**	—	—	—	—	**632**	—	571		**598**	—	—	**695**	**539**	—
Gastrotheca griswoldi CORBIDI 16066	**22**	**558**	—	**566**	—	—	—	**646**	—	—	—	—	**632**	—	**493**	**840**	**632**	—	—	**695**	**539**	—
Gastrotheca griswoldi KU 181701	—	DQ679244	—	—	DQ679352	DQ679352	DQ679352	—	—	—	—	—	—	—	DQ679320	—		—	—	—	—	—
Gastrotheca griswoldi CORBIDI 7289				**566**				**595**					**632**		**571**		**598**			**695**	**539**	
Gastrotheca guentheri KU 173112	DQ679245	DQ679245	—	DQ679393	DQ679353	DQ679353	DQ679353	—	—	—	—	—	—	—	DQ679321	DQ679285		—	—	—	—	—
Gastrotheca helenae KU 181070	DQ679246	DQ679246	—	DQ679394	DQ679354	DQ679354	DQ679354	—	—	—	—	—	—	—	DQ679322	DQ679286		—	—	—	—	—
Gastrotheca pseustes 1 QCAZ 45113	—	—	—	KC844923	KC844948	KC844948	KC844948	—	—	—	—	—	—	—	KC844970	—		—	—	—	—	—
Gastrotheca litonedis KU 202690	—	DQ679247	—	DQ679395	DQ679355	DQ679355	DQ679355	—	—	—	—	—	—	—	DQ679323	DQ679287		—	—	—	—	—
Gastrotheca lojana QCAZ 42725 + KU 203546	—	—	—	KC844938	KC844964	KC844964	KC844964	—	—	—	—	—	—	—	KC844988	KJ489595		—	—	—	—	—
Gastrotheca longipes USNM 258905 + GGU 1634	DQ679248	DQ679248	—	DQ679396	DQ679356	DQ679356	DQ679356	—	—	—	—	—	—	—	DQ679324	DQ679288	**632**	—	—	—	—	—

Taxon	tRNAPhe	12S	tRNAVal	16S (1)	16S (2)	tRNALeu	ND1	COI	cytb	28S	C—MYC 2	C—MYC 3	CXCR4	H3a	POMC	RAG1 (1)	RAG1 (2)	Rho	SIAH	SLC8A1	SLC8A3	Tyr
Gastrotheca marsupiata KU 214813 + KU 214814	AY819356	AY819356	—	DQ679397	AY819487	AY819487	AY819487	—	—	—	AY819190	—	—	—	AY819105	DQ679289		—	—	—	—	—
Gastrotheca megacephala JLG 90 + CFBH T377	—	AY843592	AY843592	AY843592	KC844953	KC844953	KC844953	—	—	—	—	—	—	—	KC844976	AY844381 + KC844996		AY844564	AY844790	—	—	—
Gastrotheca microdiscus CFBH T 1250 + CFBH T 3068	—	—	—	KC844932	KC844958	KC844958	KC844958	—	—	—	—	—	—	—	KC844979	KC844999		—	—	—	—	—
Gastrotheca monticola KU 212036 + CORBIDI 15891	AY819357	AY819357	—	DQ679398	AY819488	AY819488	AY819488	—	—	—	AY819191	—	**598**	—	AY819106	DQ679290		—	—	**691**	—	—
Gastrotheca nebulanastes MUSM 27943 + MCZ 265218	—	—	—	JN157625	KC844959	KC844959	KC844959	—	—	—	—	—	—	—	KC844982	KC845001		—	—	—	—	—
Gastrotheca nicefori KU 181071	DQ679249	DQ679249	—	DQ679399	DQ679357	DQ679357	DQ679357	—	—	—	—	—	—	—	DQ679325	DQ679291		—	—	—	—	—
Gastrotheca nicefori MHUAA 5716	—	KR559921	KR270409	KR270409	KR138380	KR138380	KR138380	—	—	—	—	—	—	—	KR270367	—		—	—	—	—	—
Gastrotheca ochoai KU173499	—	DQ679250	—	DQ679400	DQ679358	DQ679358	DQ679358	—	—	—	—	—	—	—	DQ679326	DQ679292		—	—	—	—	—
Gastrotheca oresbios CORBIDI 11076	—	—	—	KJ489461	—	—	KJ489509	—	—	—	—	—	—	—	KJ489552	KJ489588		—	—	—	—	—
Gastrotheca orophylax KU 178568	DQ6792	DQ679251	—	DQ679401	DQ6793	DQ6793	DQ679359	—	—	—	—	—	—	—	DQ6793	DQ679293		—	—	—	—	—

Taxon	**tRNAPhe**	**12S**	**tRNAVal**	**16S (1)**	**16S (2)**	**tRNALeu**	**ND1**	**COI**	**cytb**	**28S**	**C—MYC 2**	**C—MYC 3**	**CXCR4**	**H3a**	**POMC**	**RAG1 (1)**	**RAG1 (2)**	**Rho**	**SIAH**	**SLC8A1**	**SLC8A3**	**Tyr**
	51				59	59									27							
Gastrotheca ovifera KU 185758 + MHNLS 20979	—	DQ679252	—	**567**	DQ679360	DQ679360	DQ679360	—	—	—	—	—	**632**	—	**571**	**931**	**632**	—	—	**695**	**539**	—
Gastrotheca pachachacae MUSM 28492	—	—	—	JN157620	—	—	—	—	—	—	—	—	—	—	KC844983	KC845002		—	—	—	—	—
Gastrotheca peruana KU 181740	DQ679253	DQ679253	—	DQ679402	DQ679361	DQ679361	DQ679361	—	—	—	—	—	—	—	—	—		—	—	—	—	—
Gastrotheca phalarosa CORBIDI11044	—	—	—	KJ489459	KJ489459	KJ489507	KJ489507	—	—	—	—	—	—	—	KJ489551	KJ489585		—	—	—	—	—
Gastrotheca phelloderma CORBIDI 14002	**23**	**556**	—	**517**	—	—	—	**646**	—	—	—	—	**632**	—	**493**	**934**	**632**	—	—	**691**	**350**	—
Gastrotheca plumbea KU 178499	DQ679254	DQ679254	—	DQ679403	DQ679362	DQ679362	DQ679362	—	—	—	—	—	—	—	DQ679328	DQ679294		—	—	—	—	—
Gastrotheca prasina MZUSP 147060 + MZUSP 17460 + 414	—	JX262891	—	—	JX262922	JX262922	JX262922	—	—	—	JX262899	—	**632**	—	**571**	KJ489602	**632**	JX262908	JX262915	—	**539**	—
Gastrotheca pseustes 2 QCAZ 42862 + TNHC 62492	AY326051	AY326051	AY326051	JX564866	JX564866	KC844962	KC844962	JX564866	JX564866	—	—	—	—	—	KC844986	KX208740		—	—	—	—	—
Gastrotheca psychrophila KU 142634	DQ679255	DQ679255	—	DQ679404	DQ679363	DQ679363	DQ679363	—	—	—	—	—	—	—	DQ679329	DQ679295		—	—	—	—	—

Taxon	**tRNAPhe**	**12S**	**tRNAVal**	**16S (1)**	**16S (2)**	**tRNALeu**	**ND1**	**COI**	**cytb**	**28S**	**C—MYC 2**	**C—MYC 3**	**CXCR4**	**H3a**	**POMC**	**RAG1 (1)**	**RAG1 (2)**	**Rho**	**SIAH**	**SLC8A1**	**SLC8A3**	**Tyr**
Gastrotheca sp. L MTR 16228 + MUSM 16228 + G0	—	JX262894	—	1157	—	—	—	KU494441	—	—	JX262898	—	—	—	—	KJ489603		JX262907	JX262914	—	—	—
Gastrotheca rebeccae CORBIDI 08006	—	—	—	KC844937	KC844963	KC844963	KC844963	—	—	—	—	—	—	—	KC844987	—		—	—	—	—	—
Gastrotheca rebeccae CORBIDI 10821	**23**	**556**	—	**518**	—	—	—	—	—	—	—	—	**632**	—	**494**	**934**	**632**	—	—	**691**	**539**	—
Gastrotheca recava MZUSP 147044 + MZUSP 147042	—	JX262890	—	KJ489497	JX262921	JX262921	JX262921	—	—	—	JX262903	—	—	—	—	KJ489604		JX262910	JX262919	—	—	—
Gastrotheca recava 634	**23**	**554**	—	**565**	—	—	—	—	—	—	—	—	**632**	—	**463**	**935**	**632**	—	—	**695**	—	—
Gastrotheca riobambae KU 178468 + KU 203516	—	DQ679256	—	DQ679405	DQ679364	DQ679364	DQ679364	—	—	—	—	—	—	—	KJ489580	DQ679296		—	—	—	—	—
Gastrotheca ruizi KU 200002	—	DQ679257	—	DQ679406	DQ679365	DQ679365	DQ679365	—	—	—	—	—	—	—	—	DQ679297		—	—	—	—	—
Gastrotheca sp. 1 IDLR 4073 (MNCN/ADN 566)	—	—	—	KR270428		—	—	—	KR138416	—	—	—	—	—	—	—		KR270372	—	—	—	—
Gastrotheca sp. 2 MNK 5286 + CBG 1020	—	AY843590	AY843590	AY843590		KC844955	KC844955	—	AY843810	—	—	—	—	DQ284069	—	**429 (1 'N')**		AY844563	AY844789	—	—	AY844039
Gastrotheca sp. 3	—	—	—	KR270429		—	—	—	KR138417	—	—	—	—	—	—	—		KR270373	—	—	—	—
Gastrotheca pseustes 2 QCAZ 22635	—	—	—	KC844935	KC8449	KC8449	KC844961	—	—	—	—	—	—	—	KC8449	—		—	—	—	—	—

Taxon	**tRNAPhe**	**12S**	**tRNAVal**	**16S (1)**	**16S (2)**	**tRNALeu**	**ND1**	**COI**	**cytb**	**28S**	**C—MYC 2**	**C—MYC 3**	**CXCR4**	**H3a**	**POMC**	**RAG1 (1)**	**RAG1 (2)**	**Rho**	**SIAH**	**SLC8A1**	**SLC8A3**	**Tyr**
					61	61									85							
Gastrotheca yacuri QCAZ 21105 + CORBIDI 14194	**23**	**556**	—	KC844939	KC844965	KC844965	KC844965	**646**	—	—	—	—	**632**	—	KC844989	**861**	**632**	—	—	**691**	**351**	—
Gastrotheca turnerorum QCAZ 47299	—	—	—	KC844934	KC844960	KC844960	KC844960	—	—	—	—	—	—	—	KC844984	—		—	—	—	—	—
Gastrotheca elicioi QCAZ 21213	—	—	—	KC844922	KC844947	KC844947	KC844947	—	—	—	—	—	—	—	—	—		—	—	—	—	—
Gastrotheca sp. F CORBIDI 12876	**23**	**557**	—	**569**	—	—	—	**646**	—	—	—	—	**632**	—	**493**	**897**	**633**	—	—	**644**	**346**	—
Gastrotheca sp. G CORBIDI 16614	**23**	**558**	—	**570**	—	—	—	**646**	—	—	—	—	**632**	—	**493**	**907**	**632**	—	—	**691**	**539**	—
Gastrotheca sp. H CORBIDI 9036	—	**510**	—	**568**	—	—	—	**646**	—	—	—	—	**632**	—	**465**	**934**	**632**	—	—	**691**	**539**	—
Gastrotheca sp. I CORBIDI 11776	**23**	**557**	—	KJ489475	KJ489525	KJ489525	KJ489525	**646**	—	—	—	—	**598**	—	KJ489563	**932**	**632**	—	—	**691**	**539**	—
Gastrotheca sp. J CORBIDI 862 + CORBIDI 15070	**23**	**556**	—	**570**	KJ489511	KJ489511	KJ489511	**645**	—	—	—	—	**598**	—	**493**	KJ489590		—	—	**691**	—	—
Gastrotheca sp. K CORBIDI 11523	—	**508**	—	**522**	—	—	—	**605**	—	—	—	—	—	—	—	—		—	—	—	—	—
Gastrotheca pulchra 1058 + G3	**23**	**557**	**71**	**1383**	—	—	—	**646**	—	—	—	—	**584**	—	—	**934**	**632**	—	—	—	**505**	—
Gastrotheca spectabilis CORBIDI 11790	—	—	—	KJ489464	KJ48951	KJ48951	KJ489513	—	—	—	—	—	—	—	KJ4895	KJ489592		—	—	—	—	—

Taxon	tRNAPhe	12S	tRNAVal	16S (1)	16S (2)	tRNALeu	ND1	COI	cytb	28S	C—MYC 2	C—MYC 3	CXCR4	H3a	POMC	RAG1 (1)	RAG1 (2)	Rho	SIAH	SLC8A1	SLC8A3	Tyr
					3	3									54							
Gastrotheca stictopleura CORBIDI 14563	**23**	**556**	—	**568**	—	—	—	**603**	—	—	—	—	**632**	—	**491**	**904**	**632**	—	—	**691**	**539**	—
Gastrotheca stictopleura MTD 45230	DQ679258	DQ679258	—	DQ679407	DQ679366	DQ679366	DQ679366	—	—	—	—	—	—	—	DQ679330	DQ679298		—	—	—	—	—
Gastrotheca testudinea CORBIDI 14824 + QCAZ 16444	**23**	**558**	—	**568**	KC844966	KC844966	KC844966	**646**	—	—	—	—	**632**	—	436	**935**	**459**	—	—	**695**	**539**	—
Gastrotheca testudinea CBG 774	—	—	—	KR270430	—	—	—	—	KR559927	—	—	—	—	—	—	—		—	—	—	—	—
Gastrotheca testudinea CORBIDI 8009	**23**	**555**	—	**570**	—	—	—	**645**	—	—	—	—	**632**	—	**436**	**898**	**543**	—	—	**649**	**539**	—
Gastrotheca trachyceps KU 181189	DQ679259	DQ679259	—	DQ679408	DQ679367	DQ679367	DQ679367	—	—	—	—	—	—	—	DQ679331	DQ679299		—	—	—	—	—
Gastrotheca walkeri Vz 8996	—	DQ679260	—	DQ679409	DQ679368	DQ679368	DQ679368	—	—	—	—	—	—	—	DQ679332	DQ679300		—	—	—	—	—
Gastrotheca walkeri MHNLS 21101				**565**																		
Gastrotheca weinlandii KU 143105	DQ679261	DQ679261	—	DQ679410	DQ679369	DQ679369	DQ679369	—	—	—	—	—	—	—	DQ679333	DQ679301		—	—	—	—	—
Gastrotheca zeugocystis MHNSM 18675	—	DQ679262	—	DQ679411	—	—	—	—	—	—	—	—	—	—	DQ679334	DQ679302		—	—	—	—	—

Taxon	**tRNAPhe**	**12S**	**tRNAVal**	**16S (1)**	**16S (2)**	**tRNALeu**	**ND1**	**COI**	**cytb**	**28S**	**C—MYC 2**	**C—MYC 3**	**CXCR4**	**H3a**	**POMC**	**RAG1 (1)**	**RAG1 (2)**	**Rho**	**SIAH**	**SLC8A1**	**SLC8A3**	**Tyr**
Haddadus binotatus	—	EF493361	EF493361	EF493361	EF493361	—	—	—	GQ345198	DQ283493	GQ345147	GQ345165	GQ345183	DQ284142	GQ345259	GQ345278 + GQ345293	EF493397	DQ283807	GQ345309	GQ345231	GQ345329	DQ282918
Heleophryne purcelli	—	AY843593	AY843593	AY843593	—	—	AY948755	—	AY843812	AY844216	—	—	AY364191	DQ284113	—	AY364221		AY364402	AY844791	AY948833	AY948892	—
Hemiphractus bubalus KU 143107 + KU 178588	—	DQ679263	—	DQ679412	DQ679370	DQ679370	DQ679370	—	—	GQ345134	—	—	GQ345179	—	DQ679335	DQ679303		—	—	GQ345226	GQ345324	—
Hemiphractus elioti MVUP 1927	—	—	—	FJ784476	—	—	—	FJ766707	—	—	—	—	—	—	—	—		—	—	—	—	—
Hemiphractus kaylockae sp. 1 CHP 6397 + EVACC 207 + EVACC 065	—	—	—	KC014933	KC844967	KC844967	KC844967	KC014710	—	—	—	—	—	—	—	—		—	—	—	—	—
Hemiphractus panamensis sp. 2 EVACC 061 + CHP 6670	—	—	—	KC129342	KC844968	KC844968	KC844968	KC014706	—	—	—	—	—	—	—	—		—	—	—	—	—
Hemiphractus helioi MHNCP 9063 + MJH 3689	—	AY843594	AY843594	AY843594	—	—	—	—	AY843813	—	—	—	KR138412	DQ284084	KR270368	KR138425	KR138399	AY844566	AY844792	KR138389	KR270382	KR270394
Hemiphractus proboscideus KU 217513	AY819358	AY819358	—	DQ679413	AY819489	AY819489	AY819489	—	—	—	AY819192	AY819266	—	—	AY819107	DQ679304		—	—	—	—	—
Hemiphractus scutatus USNM 317196	—	DQ679264	—	DQ679414	DQ679371	DQ679371	DQ679371		—	—	—	—		—	DQ679336	DQ679305		—	—			—
Hemiphractus scutatus CORBIDI 10054				**530**				**598**					**629**				**632**			**691**	**539**	

Taxon	**tRNAPhe**	**12S**	**tRNAVal**	**16S (1)**	**16S (2)**	**tRNALeu**	**ND1**	**COI**	**cytb**	**28S**	**C—MYC 2**	**C—MYC 3**	**CXCR4**	**H3a**	**POMC**	**RAG1 (1)**	**RAG1 (2)**	**Rho**	**SIAH**	**SLC8A1**	**SLC8A3**	**Tyr**
Hemiphractus scutatus JMP 2150 (MNCN/AND 46913)	—	KR559923	KR270411	KR270411 + KR270432	—	—	—	KR559926	—	—	—	—	KR138413	—	KR270369	KR138426	KR138400	—	—	KR138390	KR270383	KR270395
Hemiphractus scutatus INPA-H38116				MG011478																		
Hemiphractus sp. 1 CORBIDI 14023	**22**	**556**	—	**532**	—	—	—	**646**	—	—	—	—	**629**	—	**495**	**935**	**581**	—	—	**691**	**539**	—
Hemiphractus sp. 2 CORBIDI 13808	**23**	**557**	—	**450**	—	—	—	—	—	—	—	—	**629**	—	**495**	**890**	**632**	—	—	**691**	**539**	—
Holoaden bradei	—	EF493378	—	EF493366	EF493366	—	—	—	—	—	—	—	—	—	—		EF493449	—	—	—	—	EU186779
Holoaden luederwaldti	—	EU186728	—	EU186710	—	—	—	—	—	—	—	—	—	—	—		EU186747	—	—	—	—	EU186768
Hydrolaetare caparu	—	KC603953	KC603953	KC603954	—	—	—	KC603988	KC603975	—	—	—	—	—	KC604050	KC604031 + KC604035		KC604107	—	—	—	KC604081
Hyla chinensis	AY458593	AY458593	AY458593	AY458593	AY458593	AY458593	AY458593	AY458593	AY458593	—	DQ055761	—	—	HM998949	DQ055789	—		—	—	HM998954	—	—
Hylodes phyllodes	—	DQ283096	DQ283096	DQ502171	—	—	—	DQ502873	DQ502606	DQ503009	—	—	—	DQ284146	—	DQ503367		DQ503253	DQ282674	—	—	DQ282923
Hylorina sylvatica	—	JX204222	JX204222	JX204222	—	—	—	—	—	—	—	—	—	—	—	—		—	—	—	—	—
Hyloxalus	—	DQ5020	DQ502	DQ50	—	—	—	DQ5028	DQ5025	DQ5029	—	—	—	—	—	DQ50332		DQ5032	DQ5030	—	—	DQ5031

Taxon	tRNAPhe	12S	tRNAVal	16S (1)	16S (2)	tRNALeu	ND1	COI	cytb	28S	C—MYC 2	C—MYC 3	CXCR4	H3a	POMC	RAG1 (1)	RAG1 (2)	Rho	SIAH	SLC8A1	SLC8A3	Tyr
chlorocraspedus		80	080	2080				00	11	75						5		12	74			53
Hypodactylus brunneus	—	EF493357	EF493357	EF493357	GQ345248	GQ345248	GQ345248	—	GQ345203	GQ345138	GQ345151	—	GQ345187	GQ345218	GQ345264	GQ345282 + GQ345298	EF493422	—	GQ345315	GQ345235	GQ345333	EF493484
Hypodactylus dolops	—	EF493394	EF493394	EF493394	EF493394	—	—	—	—	—	—	—	—	—	—		EF493414	GQ345304	—	—	—	EF493483
Insuetophrynus acarpicus	—	JX204223	JX204223	JX204223	—	—	—	JX203938	AY691185	—	—	—	—	—	—	JX204088		JX204152	JX204285	—	—	—
Ischnocnema guentheri	—	EF493533	EF493533	EF493533	EF493533	—	—	—	GQ345196	—	—	GQ345163	GQ345181	—	GQ345257	GQ345276 + GQ345291	EF493407	—	GQ345308	GQ345229	GQ345327	EF493510
Ischnocnema juipoca	—	DQ283093	DQ283093	DQ283093	—	—	—	—	—	DQ283495	—	—	—	DQ284143	—	—		DQ283809	—	—	—	DQ282920
Lepidobatrachus laevis	—	DQ283152	DQ283152	DQ283152	AY819475	AY819475	AY819475	—	—	DQ283543	AY819179	AY819258	EF107461	DQ284191	AY819094	EF107298		DQ283851	DQ282707	EF107236	EF107386	—
Leptodactylus latrans	—	AY843688	AY843688	AY843688	—	—	—	—	AY843934	AY844302	—	—	DQ306492	DQ284104	DQ158259	DQ158343 + AY844470		AY844681	AY844890	—	—	—
Leptodactylus melanonotus	AY943224	AY943224 + FJ882762	FJ882762	AY943224 + FJ882762	—	—	AY948760	—	—	—	AY337266	AY337266	AY364194	—	—	AY364224		AY364405	—	AY948838	AY948900	DQ347193
Limnodynastes salmini	—	AY32607	AY326	AY326	—	—	—	—	—	—	—	—	AY3641	—	—	AY36421		AY3644	—	AY5237	AY9488	—

Taxon	tRNAPhe	12S	tRNAVal	16S (1)	16S (2)	tRNALeu	ND1	COI	cytb	28S	C—MYC 2	C—MYC 3	CXCR4	H3a	POMC	RAG1 (1)	RAG1 (2)	Rho	SIAH	SLC8A1	SLC8A3	Tyr
		1	071	071									89			9		00		19	89	
Limnomedusa macroglossa	—	AY843689	AY843689	AY843689	—	—	—	—	AY843935	—	—	—	—	DQ284127	—	AY844471		AY844682	AY844891	—	—	AY844128
Lithodytes lineatus	—	AY326012	AY326012	AY326012	HQ290949	HQ290949	HQ290949	KC604003	JQ321833	AY844303	—	—	—	DQ284112	KC604060	KC604025 + AY844472		AY844683	—	HQ290709	—	AY844129
Lynchius nebulanastes	—	EU186704	EU186704	EU186704	—	—	—	—	—	—	AY819320	—	—	—	AY819154	—		—	—	—	—	—
Lynchius parkeri	—	EU186705	EU186705	EU186705	—	—	—	—	—	—	—	—	—	—	—	—		—	—	—	—	—
Macrogenioglottus alipioi	—	KC593360	KC593360	KC593360	KC593360	KC593353	KC593353	—	FJ685665	—	—	—	—	—	—	KC593355		KC593357	KC593363	—	—	KC593367
Mannophryne trinitatis	—	EU342504	EU342504	DQ502131	—	—	—	DQ502838	DQ502562	—	—	—	—	DQ284108	JX036003	DQ503345		DQ503236	DQ503097	GQ345227	GQ345325	—
Megaelosia goeldii	—	DQ283072	DQ283072	DQ283072	—	—	—	DQ502839	DQ502563	—	—	—	—	DQ284109	—	DQ503346		DQ283797	—	—	—	DQ282911
Melanophryniscus klappenbachi	—	AY843699	AY843699	AY843699	—	—	—	DQ502739	AY843944	AY844306	—	—	—	DQ284060	—	DQ503299		DQ283765	AY844899	—	—	—
Myersiohyla kanaima	—	AY843634	AY843634	AY843634	GQ366307	GQ366307	GQ366307	—	AY843868	—	—	—	GQ365994	—	—	AY844422		AY844617	AY844835	—	—	AY844079
Noblella lochites	—	EU18669	EU186	EU186	—	—	—	—	—	—	—	—	—	—	—		EU1867	—	—	—	—	EU1867

Taxon	tRNAPhe	12S	tRNAVal	16S (1)	16S (2)	tRNALeu	ND1	COI	cytb	28S	C—MYC 2	C—MYC 3	CXCR4	H3a	POMC	RAG1 (1)	RAG1 (2)	Rho	SIAH	SLC8A1	SLC8A3	Tyr
		9	699	699													56					77
Noblella sp. MTD 45180	—	AM039714	—	AM039646	—	—	—	—	—	—	—	—	—	—	—	—		—	—	—	—	—
Nyctimystes infrafrenatus	—	AY843694	AY843694	AY843694		GQ366302	GQ366302	FJ952337	AY843940	AY844304	—	—	GQ365990	—	—	AY844474		AY844687	—	—	—	—
Nymphargus bejaranoi	—	AY843576	AY843576	AY843576	—	—	—	—	AY843798	AY844208	EU663314	—	—	DQ284066	EU663239	EU663522		—	AY844777	—	—	AY844029
Odontophrynus achalensis	—	DQ283248	DQ283248	DQ283248	—	—	—	—	—	DQ283611	—	—	—	DQ284273	—	—		DQ283918	DQ282773	—	—	—
Oreobates cruralis	—	EU186666	EU186666	EU186666	EU186666	—	—	—	—	—	—	—	—	—	—		EU186743	—	—	—	—	EU186764
Oreobates saxatilis	—	JF809931	—	JF809962	—	—	—	—	—	—	—	—	—	DQ284091	—		JF809910	DQ283788	DQ282661	—	—	JF809888
Paratelmatobius cardosoi	—	EU224404	EU224404	EU224404	—	—	—	—	—	—	—	—	—	—	—	—		—	—	—	—	—
Phrynopus auriculatus	—	EF493708	EF493708	EF493708	—	—	—	—	—	—	—	—	—	—	—	—		—	—	—	—	—
Phrynopus bracki	—	EF493709	EF493709	EF493709	EF493709	—	—	—	GQ345202	GQ345137	GQ345150	GQ345168	GQ345186	GQ345217	GQ345263	GQ345281 + GQ345297	EF493421	GQ345303	GQ345314	GQ345234	GQ345332	EF493507
Phyllodytes luteolus	—	AY843721	AY843721	AY843721	GQ366314	GQ366314	GQ366314	—	AY843966	AY844324	—	—	—	—	GQ366043	AY844494		AY844708	AY844913	—	—	AY844150

Taxon	tRNAPhe	12S	tRNAVal	16S (1)	16S (2)	tRNALeu	ND1	COI	cytb	28S	C—MYC 2	C—MYC 3	CXCR4	H3a	POMC	RAG1 (1)	RAG1 (2)	Rho	SIAH	SLC8A1	SLC8A3	Tyr
Physalaemus cuvieri	—	AY843729	AY843729	AY843729	JX204060	JX204060	JX204060	—	AY843975	AY844330	AY819181	—	—	—	AY819096	AY844499		AY844717	AY844922	—	—	—
Physalaemus gracilis	AY943229	AY943229	—	AY943242	AY943242	—	—	—	—	DQ283728	—	—	—	—	—	—		DQ284022	DQ282875	—	—	—
Phyzelaphryne miriamae	—	EU186689	EU186689	EU186689	EU186689	—	—	—	—	—	—	—	—	—	—		EU186753	—	—	—	—	EU186774
Pleurodema brachyops	—	AY843733	AY843733	AY843733	—	—	—	—	AY843979	—	—	—	—	DQ284111	—	AY844503		AY844721	AY844926	—	—	—
Pleurodema diplolister	—	JQ937187	JQ937187	JQ937187	—	—	—	KC603986	KC603981	—	—	—	—	—	KC604052	HQ634173		JQ937153	JQ937222	—	—	KC604080
Pristimantis cruentus	—	EF493697	EF493697	EF493697 + FJ84557	FJ882747	FJ882747	FJ882747	FJ766786	—	—	—	—	AY948792	GQ345216	—	AY948935		—	GQ345313	AY948836	AY948898	—
Pristimantis curtipes	—	EF493513	EF493513	EF493513	AY819473	AY819473	AY819473	—	—	—	AY819177	AY819256	—	—	AY819092	DQ679272	EF493435	—	—	—	—	EF493497
Pristimantis gaigeae	—	—	—	FJ84490	—	—	—	FJ766792	—	—	AY211290	AY211290	—	—	—	—		—	—	—	—	—
Pristimantis ridens	—	EF493355	EF493355	EF493355	EF493355	—	—	FJ766808	—	—	AY211306	AY211306	—	—	—	—		—	—	—	—	—
Pristimantis simonsii	—	EU186665	EU186665	EU186665	EU1866	—	—	—	—	—	AY8193	AY819314	—	—	AY8191	—		—	—	—	—	—

Taxon	tRNAPhe	12S	tRNAVal	16S (1)	16S (2)	tRNALeu	ND1	COI	cytb	28S	C—MYC 2	C—MYC 3	CXCR4	H3a	POMC	RAG1 (1)	RAG1 (2)	Rho	SIAH	SLC8A1	SLC8A3	Tyr
					65						21				55							
Proceratophrys avelinoi	—	DQ283038	DQ283038	DQ283038	—	—	—	—	FJ685671	—	—	—	—	DQ284065	—	FJ685711		DQ283769	—	—	—	DQ282903
Pseudis minuta	—	—	AY843739	—	GQ366339	GQ366339	GQ366339	—	AY843985	AY844336	—	—	GQ366028	—	GQ366070	AY844505		—	AY844929	—	—	—
Pseudopaludicola falcipes	—	AY843741	AY843741	AY843741	—	—	—	KC520684	KC593350	—	—	—	—	DQ284117	KC604051	HQ634171 + AY844507		AY844728	AY844930	KC593352	—	KC520700
Psychrophrynella guillei	—	AY843720	AY843720	AY843720	—	—	—	—	—	AY844323	—	—	—	DQ284371	—	—		—	—	—	—	DQ282995
Psychrophrynella wettsteini	—	EU186696	EU186696	EU186696	GQ345250	GQ345250	GQ345250	—	—	GQ345139	GQ345153	—	GQ345189	GQ345219	GQ345266	GQ345284 + GQ345300	EU186755	—	GQ345316	GQ345237	GQ345335	EU186776
Ranoidea caerulea	AY326038	AY326038	AY326038	AY326038		AY819531	AY819531	AY883980	AY843938	—	AY819234	345 (6 indels)	AY948783	—	AY819149	AY323767	EF493446	AY844685	AY844893	AY948821	AY948877	AY844131
Rheobates palmatus	—	EU342508	EU342508	EU342508	HQ290967	HQ290967	HQ290967	DQ502925	HQ290545	—	—	—	—	—	HQ290845	—		DQ503271	—	HQ290725	—	DQ503172
Rhinella arenarum	—	AY843573	AY843573	AY843573	JX204061	JX204061	JX204061	—	AY843795	AY844205	—	—	GQ345189	DQ284103	DQ158271	DQ158354 + AY844370		AY844547	AY844775	—	—	—
Rhinoderma darwinii	—	AY364357	—	AY364378	—	—	AY523783	DQ5028	DQ5025	DQ2836	—	—	AY3641	DQ2843	—	AY364222		AY3644	DQ2828	AY5237	AY9488	—

Taxon	**tRNAPhe**	**12S**	**tRNAVal**	**16S (1)**	**16S (2)**	**tRNALeu**	**ND1**	**COI**	**cytb**	**28S**	**C—MYC 2**	**C—MYC 3**	**CXCR4**	**H3a**	**POMC**	**RAG1 (1)**	**RAG1 (2)**	**Rho**	**SIAH**	**SLC8A1**	**SLC8A3**	**Tyr**
								58	89	54			92	20				03	13	33	95	
Rupirana cardosoi	—	KC603955	KC603955	KC603956	—	—	—	KC603987	KC603964	—	—	—	—	—	KC604049	KC604012 + KC604034		KC604106	—	—	—	KC604078
Scinax staufferi	—	AY843761	AY843761	AY843761	GQ366340	GQ366340	GQ366340	—	AY844006	—	—	—	GQ366029	—	GQ366071	AY844523		AY844748	—	—	—	AY844183
Sclerophrys brauni	—	FJ882822	FJ882822	FJ882822		FJ882822	—	—	—	DQ283726	—	—	DQ306514	—	DQ158279	EF107331		DQ284021	DQ282873	EF107272	EF107435	DQ283011
Scythrophrys sawayae	—	EU224417	EU224417	EU224417	—	—	—	—	—	DQ283500	—	—	—	DQ284149	—	—		DQ283815	—	—	—	DQ282926
Smilisca baudinii	—	AY843762	AY843762	AY843762	DQ388762	DQ388762	DQ388762	—	AY844007	—	DQ388738	—	—	—	DQ388720	DQ830932		AY844749	AY844946	—	—	—
Sooglossus thomasseti	—	DQ283452	DQ283452	DQ283452	—	—	—	—	AY341742	DQ283755	—	—	AY364187	DQ284425	—	AY323778		DQ284042	—	AY948828	AY948884	DQ347167
Stefania ackawaio VUB 3546	—	—	—	JQ742214	—	—	JQ742276	—	—	—	—	—	KX399824	—	—	KX399786		—	—	—	—	—
Stefania ayangannae VUB 3542	—	—	—	JQ742212	—	—	JQ742380	—	—	—	—	—	KX399822	—	—	KX399784		—	—	—	—	—
Stefania coxi ROM 39478	—	DQ679265	—	DQ679415	DQ679372	DQ679372	JQ742350 + DQ679372	—	—	—	—	—	KX399838	—	DQ679337	DQ679306		—	—	—	—	—

Taxon	tRNAPhe	12S	tRNAVal	16S (1)	16S (2)	tRNALeu	ND1	COI	cytb	28S	C—MYC 2	C—MYC 3	CXCR4	H3a	POMC	RAG1 (1)	RAG1 (2)	Rho	SIAH	SLC8A1	SLC8A3	Tyr
Stefania evansi IRSNB 16738 + AMNH A 164211	—	AY843767	AY843767	AY843767	—	—	KX399773	—	—	AY844353	—	—	KX399850	—	—	KX399812		AY844755	AY844950	—	—	AY844189
Stefania ginesi IRSNB 16736	—	—	—	KU958591	—	—	—	—	—	—	—	—	KU958636	—	—	KU958617		—	—	—	—	—
Stefania riae VUB 3754	—	—	—	JQ742172	—	—	JQ742343	—	—	—	—	—	KU958620	—	—	KU958601		—	—	—	—	—
Stefania riveroi IRSNB 15703	—	—	—	JQ742177	—	—	JQ742348	—	—	—	—	—	KU958621	—	—	KU958602		—	—	—	—	—
Stefania roraimae VUB 3311	—	—	—	JQ742205	—	—	JQ742374	—	—	—	—	—	KX399817	—	—	KX399778		—	—	—	—	—
Stefania satelles IRSNB 16728	—	—	—	KU958583	—	—	—	—	—	—	—	—	KU958628	—	—	KU958609		—	—	—	—	—
Stefania scalae KU 181122 + MHNLS 17152	—	DQ679267	DQ679267	KR270413 + KR270434	DQ679374	DQ679374	JQ742362 + DQ679374	—	—	—	—	—	KR138415	—	KR270371	KR138428 + DQ679309	KR138402	—	—	KR138392	KR270385	KR270397
Stefania schuberti MNHN 2002 692 + IRSNB 16732	—	AY843768	AY843768	AY843768	—	—	—	—	AY844013	AY844354	—	—	KU958632	—	—	AY844528		AY844756	AY844951	—	—	—
Stefania sp. 1 LM 1056	DQ679266	DQ679266	—	DQ679417	DQ679373	DQ679373	DQ679373	—	—	—	—	—	GQ345178	GQ345211	DQ679338	DQ679308		—	—	GQ345225	GQ345323	—
Stefania sp. 2 IRSNB 16734	—	—	—	KU958589	—	—	KU958598	—	—	—	—	—	KU9586	—	—	KU958615		—	—	—	—	—

Taxon	tRNAPhe	12S	tRNAVal	16S (1)	16S (2)	tRNALeu	ND1	COI	cytb	28S	C—MYC 2	C—MYC 3	CXCR4	H3a	POMC	RAG1 (1)	RAG1 (2)	Rho	SIAH	SLC8A1	SLC8A3	Tyr
													34									
Stefania sp. 3 VUB 3755	—	—	—	JQ742175	—	—	JQ742346	—	—	—	—	—	KU958625	—	—	KU958606		—	—	—	—	—
Stefania sp. 4 IRSNB 16730	—	—	—	KU958585	—	—	KU958594	—	—	—	—	—	KU958630	—	—	KU958611		—	—	—	—	—
Stefania sp. 5 IRSNB 16727	—	—	—	KU958582	—	—	KU958593	—	—	—	—	—	KU958627	—	—	KU958608		—	—	—	—	—
Stefania sp. 6 VUB 3266	—	—	—	JQ742181	—	—	JQ742352	—	—	—	—	—	KX399829	—	—	KX399791		—	—	—	—	—
Stefania sp. 7 VUB 3548	—	—	—	JQ742201	—	—	JQ742273	—	—	—	—	—	KX399826	—	—	KX399788		—	—	—	—	—
Stefania sp. 8 ROM 42811 + ROM 39465	—	—	—	JQ742209	—	—	JQ742377	—	—	—	—	—	KX399825	—	—	KX399780		—	—	—	—	—
Stefania sp. 9 VUB 3555	—	—	—	JQ742195	—	—	JQ742366	—	—	—	—	—	KX399834	—	—	KX399796		—	—	—	—	—
Stefania sp. 10 IRSNB 14595 + ROM 39450 + IRSNB 14586	—	AY819359	KR270412	JQ742193	AY819490	AY819490	AY819490	—	—	—	AY819193	AY819267	KR138414	—	KR270370	KR138427	KR138401	—	—	KR138391	KR270384	KR270396
Stefania woodleyi VUB 3282	—	—	—	JQ742185	—	—	JQ742356	—	—	—	—	—	KX399814	—	—	KX399775		—	—	—	—	—
Strabomantis biporcatus	—	EU18669	EU186	EU186	GQ3452	GQ3452	GQ34524	—	GQ3452	—	GQ3451	—	GQ3451	—	GQ3452	GQ34528	EU1867	—	—	GQ3452	GQ3453	EU1867

Taxon	**tRNAPhe**	**12S**	**tRNAVal**	**16S (1)**	**16S (2)**	**tRNALeu**	**ND1**	**COI**	**cytb**	**28S**	**C—MYC 2**	**C—MYC 3**	**CXCR4**	**H3a**	**POMC**	**RAG1 (1)**	**RAG1 (2)**	**Rho**	**SIAH**	**SLC8A1**	**SLC8A3**	**Tyr**
		1	691	691	49	49	9		04		52		88		65	3	54			36	34	75
Strabomantis bufoniformis	—	DQ283165	DQ283165	DQ283165	—	—	—	FJ766635	—	DQ283555	—	—	—	DQ284203	—	—		—	DQ282718	—	—	DQ282942
Telmatobius truebae	AY819348	AY819348	—	DQ679378	AY819478	AY819478	AY819478	—	—	—	AY819182	AY819260	—	—	AY819097	DQ679271		—	—	—	—	—
Thoropa miliaris	—	DQ283331	DQ283331	DQ283331	—	—	—	DQ502874	FJ685662	—	—	—	—	DQ502369	—	FJ685702		—	—	—	—	JX298241
Trachycephalus typhonius	—	AY549362	AY549362	AY549362	GQ366341	GQ366341	GQ366341	—	EU034077	AY844322	AY819217	AY819291	GQ366030	—	AY819132	EU034147		AY364396	AY844912	AY948824	AY948880	DQ347161
Uperoleia laevigata	—	DQ283221	DQ283221	DQ283221	—	—	—	—	—	—	—	—	EF107474	DQ284251	—	EF107310		DQ283898	DQ282758	EF107251	EF107410	—
Yunganastes ashkapara	—	FJ438807	—	EU192233 + FJ438796	—	—	—	—	—	—	—	—	—	—	—		JF809919	—	—	—	—	JF809898
Yunganastes fraudator	—	JF809938	—	FJ539065	—	—	—	—	—	—	—	—	—	—	—		JF809916	—	—	—	—	JF809895
Zachaenus parvulus	—	KC593362	KC593362	KC593362	—	—	—	—	—	—	—	—	—	—	—	—		—	—	—	—	—

Appendix II. Specimens, voucher/field number codes, localities, and list of sequences generated in this study. Numbers in bold face refer to available base pairs per marker and sample. Acronyms follow Frost (2018) except for CORBIDI (Centro de Ornitologia y Biodiversidad) and GGU (Giussepe Gagliardi Urrutia field series). Other codes correspond to specimens collected by Iuri Ribeiro Dias or Caio Vinicius and deposited at Universidade Estadual de Santa Cruz, Bahia, Brazil.

Genus	**Species**	**Voucher/ Field number**	**Locality**	**tRNAPhe**	**12S**	**tRNAVal**	**16S (1)**	**COI**	**CXCR4**	**POMC**	**RAG1 (1)**	**RAG1 (2)**	**SLC8A1**	**SLC8A3**
Gastrotheca	*flamma*	845	Brazil: Bahia State: Santa Teresinha Municipality: Serra da Jibóia	—	**438**	—	**504**	**646**	**632**	—	**778**	**632**	**695**	**539**
Gastrotheca	*flamma*	846	Brazil: Bahia State: Santa Teresinha Municipality: Serra da Jibóia	—	—	—	**555**	—	—	**571**	—	—	—	—
Gastrotheca	*flamma*	AM 01		—	—	—	**555**	—	—	—	—	—	—	—
Gastrotheca	*flamma*	AM 11		—	—	—	**555**	—	—	—	—	—	—	—
Gastrotheca	*flamma*	844		—	—	—	**518**	—	—	—	—	—	—	—
Gastrotheca	*flamma*	809		—	—	—	**555**	—	—	—	—	—	—	—
Gastrotheca	*flamma*	810		—	—	—	**505**	—	—	—	—	—	—	—
Gastrotheca	*flamma*	458		—	—	—	**514**	—	—	—	—	—	—	—
Gastrotheca	*flamma*	AM 03		—	—	—	**473**	—	—	—	—	—	—	—
Gastrotheca	*griswoldi*	CORBIDI 7289	Peru: Pasco Department: Oxapampa Province: Santa Barbara	—	—	—	—	**595**	**632**	**571**	—	**598**	**695**	**539**
Gastrotheca	*griswoldi*	CORBIDI 16066	Peru: Huanuco Department: Huanuco Province: Achupampa (9° 44' 30.228"S, 75° 57' 20.2314"W, 3287 m)	**22**	**558**	—	**566**	**646**	**632**	**493**	**840**	**632**	**695**	**539**
Gastrotheca	*griswoldi*	CORBIDI 15355	Peru: Pasco Department : Pasco Province: Unidad Minera El Porvenir (10°36'16.52"S, 76°10'59.28"W, 4540 m)	—	—	—	**565**	—	—	—	—	—	—	—

Genus	**Species**	**Voucher/ Field number**	**Locality**	**tRNAPhe**	**12S**	**tRNAVal**	**16S (1)**	**COI**	**CXCR4**	**POMC**	**RAG1 (1)**	**RAG1 (2)**	**SLC8A1**	**SLC8A3**
Gastrotheca	*longipes*	GGU 1634	Peru: Loreto Department	—	—	—	—	—	—	—	—	**632**	—	—
Gastrotheca	*monticola*	CORBIDI 15891	Peru: Amazonas Department: Centro Poblado 2 de Mayo (2379 m)	—	—	—	—	—	**598**	—	—	—	**691**	—
Gastrotheca	*monticola*	MUSM	Peru: Lambayeque Department: Lambayeque Province: Salas district: Penachi	—	—	—	566	—	—	—	—	—	—	—
Gastrotheca	*nicefori*	MHNLS 22595	Venezuela: Estado Barinas: Minas de feldespato, carretera Barinitas-Santo Domingo			—	567	—	—	—	—	—	—	—
Gastrotheca	*nicefori*	MHNLS 22596	Venezuela: Estado Barinas: Minas de feldespato, carretera Barinitas-Santo Domingo	—	—	—	567	—	—	—	—	—	—	—
Gastrotheca	*ovifera*	MHNLS 20979	Venezuela: Estado Yaracuy: Municipio Bolívar: Sierra de Aroa (Sector El Silencio)	—	—	—	**567**	—	**632**	**571**	**931**	**632**	**695**	**539**
Gastrotheca	*ovifera*	MHNLS 21001	Venezuela: Estado Yaracuy: Municipio Bolivar: Sierra de Aroa (Pico El Tigre: Quebrada de la Toma) (10°25'40.4"S, 68°48'21.3"W, 1563 m)	—	—	—	**591**	—	—	—	—	—	—	—
Gastrotheca	*ovifera*	MHNLS 21326	Venezuela: Estado Yaracuy: Municipio Sucre: Sierra de Aroa (Sector La Rondona: Quebrada La Rondona) (10°19'20.8"S, 68°52'24"W, 1180 m)	—	—	—	**589**	—	—	—	—	—	—	—
Gastrotheca	*peruana*	CORBIDI 10434	Peru: Ancash Department: Huari Province (9° 35' 47.798'' S, 77° 2' 6.096'' W, 4343 m)	—	—	—	**567**	—	—	—	—	—	—	—
Gastrotheca	*peruana*	CORBIDI 12848	Peru: La Libertad Department: Santiago de Chuco Province: Quebrada Vira Vira (7°56'56.40''S, 78°12'23.19''W, 3875 m)	—	—	—	**567**	—	—	—	—	—	—	—
Gastrotheca	*phelloderma*	CORBIDI 14002	Peru: La Libertad Department: Pataz Province: Quebrada Mush Mush (Distrito de Porcoy) (8° 3' 38.714"S, 77° 24' 13.203"W,	**23**	**556**	—	**517**	**646**	**632**	**493**	**934**	**632**	**691**	**350**

Genus	**Species**	**Voucher/ Field number**	**Locality**	**tRNAPhe**	**12S**	**tRNAVal**	**16S (1)**	**COI**	**CXCR4**	**POMC**	**RAG1 (1)**	**RAG1 (2)**	**SLC8A1**	**SLC8A3**
			4109 m)											
Gastrotheca	*prasina*	413	Brazil: Bahia State: Macarani Municipality	—	—	—	**572**	—	—	—	—	—	—	—
Gastrotheca	*prasina*	414	Brazil: Bahia State: Macarani Municipality	—	—	—	—	—	**632**	**571**	—	**632**	—	**539**
Gastrotheca	sp. L	G0	Brazil: Bahia State: Camacã Municipality: RPPN Serra Bonita	—	—	—	**1157**	—	—	—	—	—	—	—
Gastrotheca	*rebeccae*	CORBIDI 10821	Peru: Ayacucho Department: La Mar Province: Chiquintirca	**23**	**556**	—	**518**	—	**632**	**494**	**934**	**632**	**691**	**539**
Gastrotheca	*recava*	634	Brazil: Bahia State: Guaratinga Municipality	**23**	**554**	—	**565**	—	**632**	**463**	**935**	**632**	**695**	—
Gastrotheca	*yacuri*	CORBIDI 14194	Peru: Piura Department: Ayabaca Province: Tapal (4° 47´40.29"S, 79° 26´57.78"W, 3330 m)	**23**	**556**	—	—	**646**	**632**	—	**861**	**632**	**691**	**351**
Gastrotheca	sp. F	CORBIDI 12876	Peru: Amazonas Department: Rodriguez de Mendoza Province: La Colpa (6° 22' 53.7"S, 79° 17' 13''W, 2341 m)	**23**	**557**	—	**569**	**646**	**632**	**493**	**897**	**633**	**644**	**346**
Gastrotheca	sp. G	CORBIDI 16614	Peru: Amazonas Department	**23**	**558**	—	**570**	**646**	**632**	**493**	**907**	**632**	**691**	**539**
Gastrotheca	sp. H	CORBIDI 9036	Peru: Huanuco Department: Huanuco Province: Rio Tambo (9° 40' 45.4"S. 75° 47' 16.9" W, 1615 m)	—	**510**	—	**568**	**646**	**632**	**465**	**934**	**632**	**691**	**539**
Gastrotheca	sp. I	CORBIDI 11776	Peru: Amazonas Department: Rodriguez de Mendoza Province: Hornillo (Distrito de Vista Alegre) (6° 8' 30"S, 77° 29' 4.9"W, 3308 m)	**23**	**557**	—	—	**646**	**598**	—	**932**	**632**	**691**	**539**
Gastrotheca	sp. J	CORBIDI 15070	Peru: San Martin Department: Mariscal Caceres Province: Albazo (06° 42' 49'' S, 77° 40' 36.6'' W, 2423 m)	**23**	**556**	—	**570**	**645**	**598**	**493**	—	—	**691**	—
Gastrotheca	sp. K	CORBIDI 11523	Peru: Pasco Department: Pasco Province: Bosque de Sho'llet (10° 37' 49.33''S. 75° 16'	—	**508**	—	**522**	**605**	—	—	—	—	—	—

Genus	Species	Voucher/ Field number	Locality	tRNAPhe	12S	tRNAVal	16S (1)	COI	CXCR4	POMC	RAG1 (1)	RAG1 (2)	SLC8A1	SLC8A3
			58.45''W, 2181 m)											
Gastrotheca	sp.	158	Brazil	—	—	—	**448**	—	—	—	—	—	—	—
Gastrotheca	sp.	213	Brazil	—	—	—	**555**	—	—	—	—	—	—	—
Gastrotheca	sp.	288	Brazil	—	—	—	**505**	—	—	—	—	—	—	—
Gastrotheca	sp.	405	Brazil	—	—	—	**553**	—	—	—	—	—	—	—
Gastrotheca	sp.	631	Brazil	—	—	—	**213**	—	—	—	—	—	—	—
Gastrotheca	*pulchra*	1058	Brazil: Bahia State: Igrapiuna Municipality: Michelin	**23**	**557**	—	—	**646**	**584**	—	**934**	**632**	—	**505**
Gastrotheca	*pulchra*	G3	Brazil: Bahia State: Igrapiuna Municipality: Michelin	—	**942**	**71**	**1383**	—	—	—	—	—	—	—
Gastrotheca	*pulchra*	G1	Brazil	—			**223**	—	—	—	—	—	—	—
Gastrotheca	*pulchra*	G2	Brazil	—	**933**	**71**	**1358**	—	—	—	—	—	—	—
Gastrotheca	*pulchra*	691	Brazil	—			**554**	—	—	—	—	—	—	—
Gastrotheca	*rebeccae*	CORBIDI 10821	Peru: Ayacucho Department: La Mar Province: Chiquintirca	**23**	**556**	—	**518**	—	**632**	**494**	**934**	**632**	**691**	**539**
Gastrotheca	*recava*	634	Brazil: Bahia State: Guaratinga Municipality	**23**	**554**	—	**565**	—	**632**	**463**	**935**	**632**	**695**	—
Gastrotheca	*stictopleura*	CORBIDI 14563	Peru: Huanuco Department: Huanuco Province: Achupampa (9°43'48.87"S, 75°57'4.29"W, 3122 m)	**23**	**556**	—	**568**	**603**	**632**	**491**	**904**	**632**	**691**	**539**
Gastrotheca	*testudinea*	CORBIDI 14824	Peru: Cajamarca Deparment: San Ignacio Province: El Chaupe (5°17'48.8"S, 79°2'11.3"W, 1816 m)	**23**	**558**	—	**568**	**646**	**632**	**436**	**935**	**459**	**695**	**539**
Gastrotheca	*testudinea*	CORBIDI 8009	Peru: Cusco Department: La Convencion Province: CC NN Aendoshiari (12° 48' 15.17" S, 73° 24' 56.229" W, 2286 m)	**23**	**555**	—	**570**	**645**	**632**	**436**	**898**	**543**	**649**	**539**

Genus	**Species**	**Voucher/ Field number**	**Locality**	**tRNAPhe**	**12S**	**tRNAVal**	**16S (1)**	**COI**	**CXCR4**	**POMC**	**RAG1 (1)**	**RAG1 (2)**	**SLC8A1**	**SLC8A3**
Gastrotheca	*walkeri*	MHNLS 20980	Venezuela: Estado Yaracuy: Municipio Bolivar: Sierra de Aroa (Sector El Silencio) (10°25'13.7"S, 68°48'57.7"W, 1447 m)	—	—	—	**587**	—	—	—	—	—	—	—
Gastrotheca	*walkeri*	MHNLS 21769	Venezuela: Estado Miranda: Municipio Zamora: rio Araira (10°30'8.42"S, 66°27'25.98"W, 683 m)	—	—	—	**568**	—	—	—	—	—	—	—
Gastrotheca	*walkeri*	MHNLS 21101	Venezuela: Estado Yaracuy: Municipio Bolivar: Sierra de Aroa (Sector El Silencio) (10°25'13.7"S, 68°48'57.7"W, 1447 m)	—	—	—	**565**	—	—	—	—	—	—	—
Hemiphractus	*helioi*	CORBIDI 9937	Peru: San Martin Department: Picota Province: Chambirillo (Puesto de Control 16, Parque Nacional Cordillera Azul) (07° 04' 08.9'' S, 76° 00' 55.2'' W, 1122 m)	—	—	—	**542**	—	—	—	—	—	—	—
Hemiphractus	*helioi*	CORBIDI 9204	Peru: San Martin Department: Picota Province: Chambirillo (Puesto de Control 16, Parque Nacional Cordillera Azul) (07° 04' 08.9'' S, 76° 00' 55.2'' W, 1122 m)	—	—	—	**542**	—	—	—	—	—	—	—
Hemiphractus	*scutatus*	CORBIDI 10054	Peru: Cusco Department: La Convencion Province: Pagoreni B (Malvinas) (11° 41' 7.9''S, 72° 57' 19.5''W, 472 m)	—	—	—	**530**	**598**	**629**	—	—	**632**	**691**	**539**
Hemiphractus	sp. 1	CORBIDI 14023	Peru: Amazonas Department: Condorcanqui Province: Concesion de Conservacion Pamau Nain (5° 14' 21.8394"S, 78° 10' 56.8914"W, 1095 m)	**22**	**556**	—	**532**	**646**	**629**	**495**	**935**	**581**	**691**	**539**
Hemiphractus	sp. 2	CORBIDI 13808	Peru: Loreto Department: Datem del Maranon Province: Cordillera Escalera	**23**	**557**	—	**450**	—	**629**	**495**	**890**	**632**	**691**	**539**

Figure S1. Tree-alignment + Parsimony: strict consensus of 393 most parsimonious trees of 84 885 steps showing phylogenetic relationships of egg-brooding frogs and outgroup taxa, nferred from the analysis of nuclear and mitochondrial DNA and phenotypic data, and Jackknife proportions.

Figure S2. Similarity-alignment + Parsimony, gaps as fifth state (SAP5th): consensus of 309 most parsimonious trees of 90 684 steps showing phylogenetic relationships of egg-brooding frogs and outgroup taxa, inferred from the analysis of nuclear and mitochondrial DNA and phenotypic data. Numbers above branches are Jackknife proportions.

Figure S3. Similarity-alignment + Parsimony, gaps as binary characters (SAPg): consensus of 1 402 most parsimonious trees of 87 670 steps showing phylogenetic relationships of egg-brooding frogs and outgroup taxa, inferred from the analysis of nuclear and mitochondrial DNA and phenotypic data. Numbers above branches are Jackknife proportions.

Figure S4. Similarity-alignment + Parsimony, gaps as missing data (SAPm): consensus of 1 132 most parsimonious trees of 83 798 steps showing phylogenetic relationships of egg-brooding frogs and outgroup taxa, inferred from the analysis of nuclear and mitochondrial DNA and phenotypic data. Numbers above branches are Jackknife proportions.

Figure S5. Similarity-alignment + Maximum Likelihood, gaps as binary characters (SALg): optimal tree (log likelihood = - 368 553.75), showing phylogenetic relationships of egg-brooding frogs and outgroup taxa, inferred from the analysis of nuclear and mitochondrial DNA and phenotypic data, and Jackknife proportions above 50 %.

Figure S6. Similarity-alignment + Maximum Likelihood, gaps as missing data (SALm): optimal tree (log likelihood = - 348 389.07), showing phylogenetic relationships of egg-brooding frogs and outgroup taxa, inferred from the analysis of nuclear and mitochondrial DNA and phenotypic data, and Jackknife proportions above 50 %.

Figure S7. Comparison between SAP5th (left) and TAP (right) within *Fritziana*.

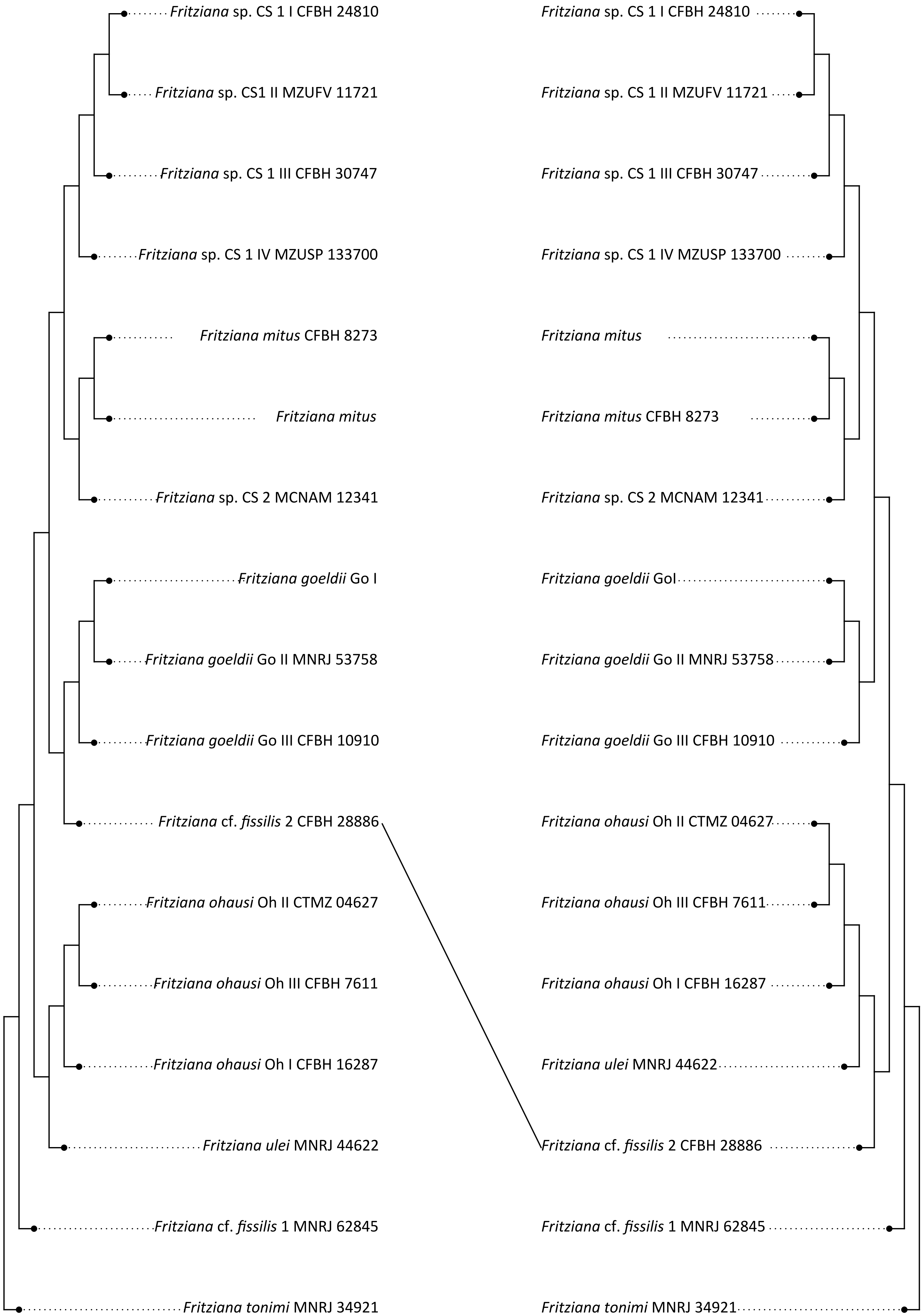


Figure S7 (Continuation). Comparison between SAP5th (left) and TAP (right) within *Gastrotheca*.

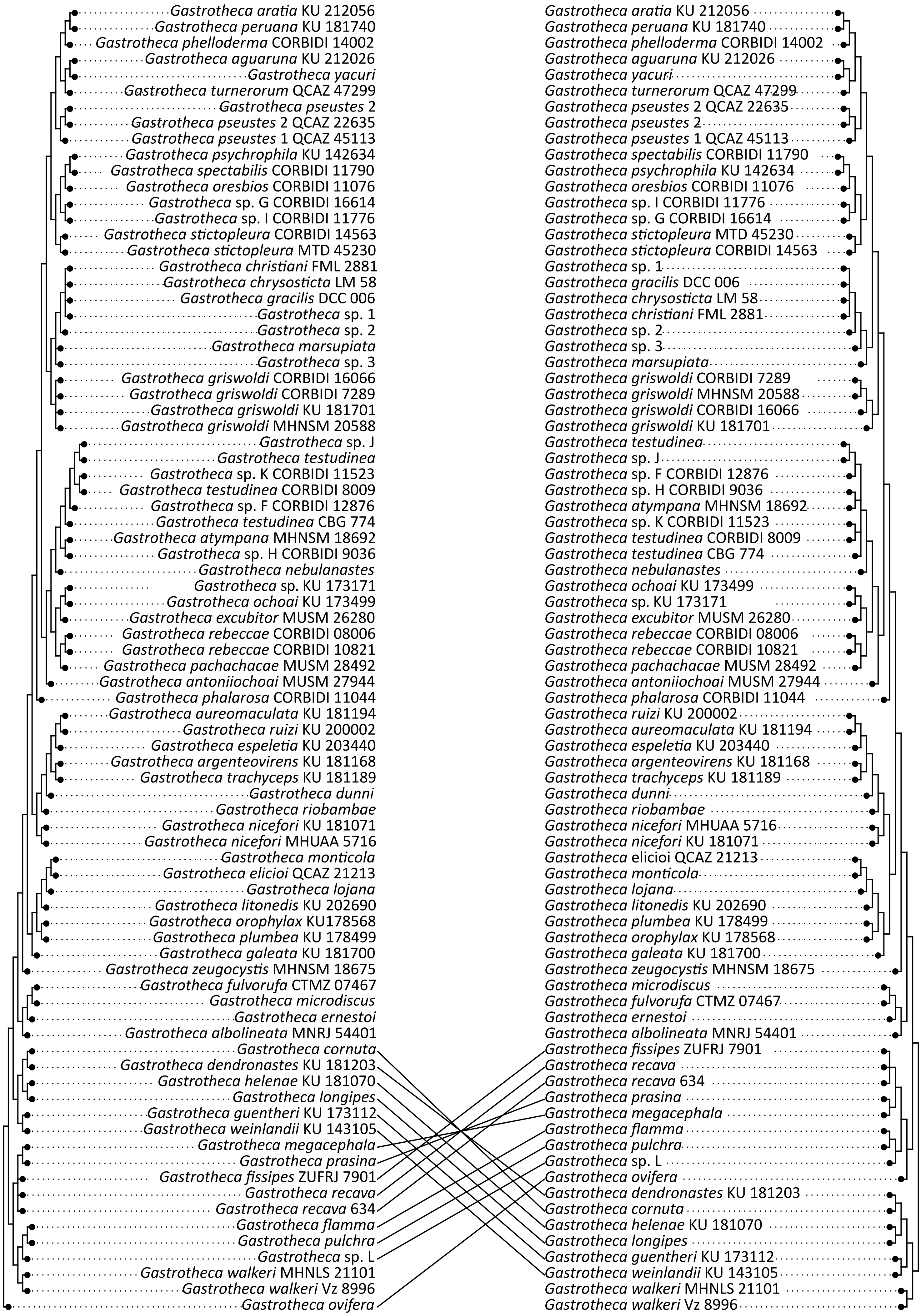


Figure S7 (Continuation). Comparison between SAP5th (left) and TAP (right) within *Hemiphractus*.

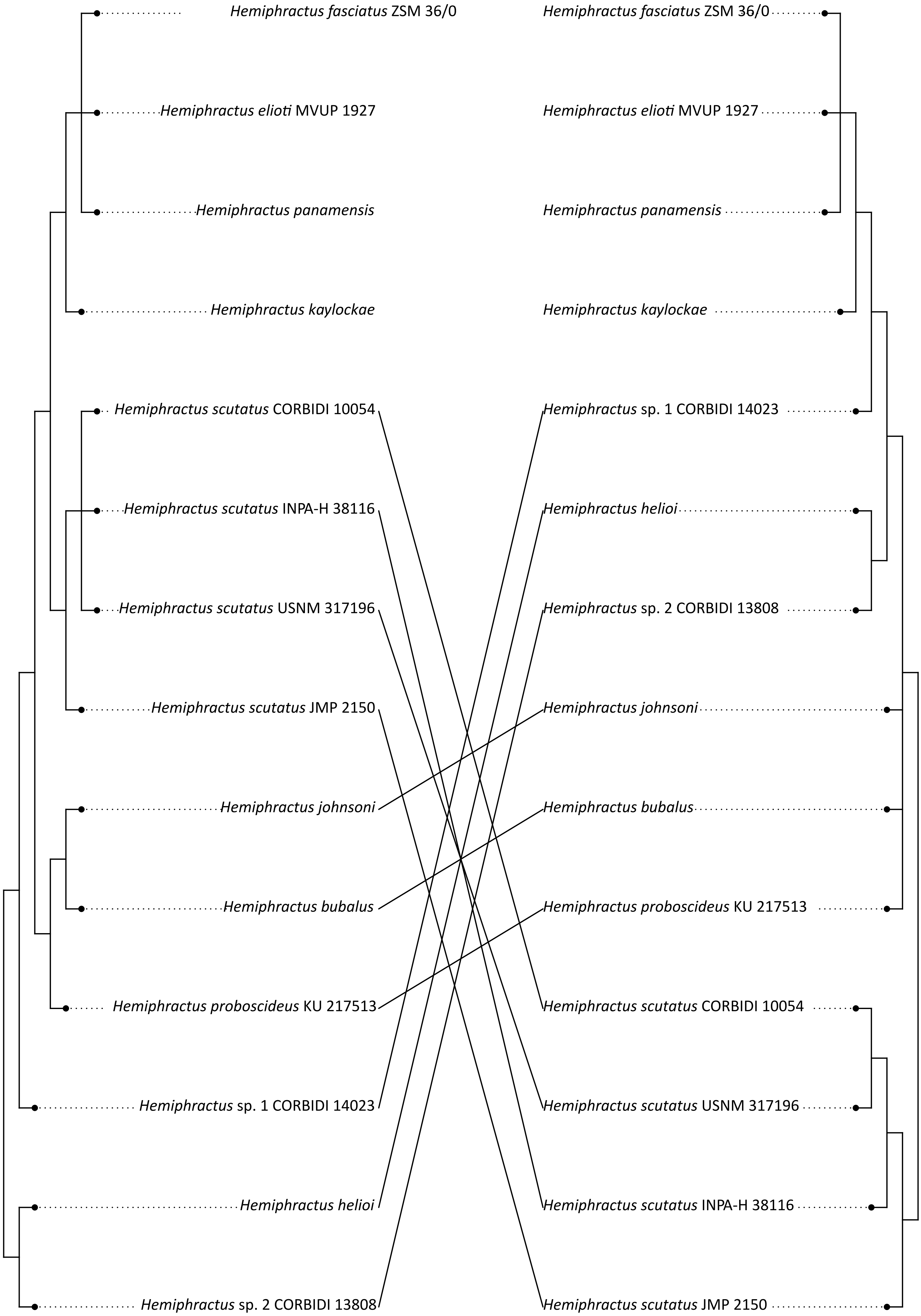


Figure S7 (Continuation). Comparison between SAP5th (left) and TAP (right) within *Stefania*.

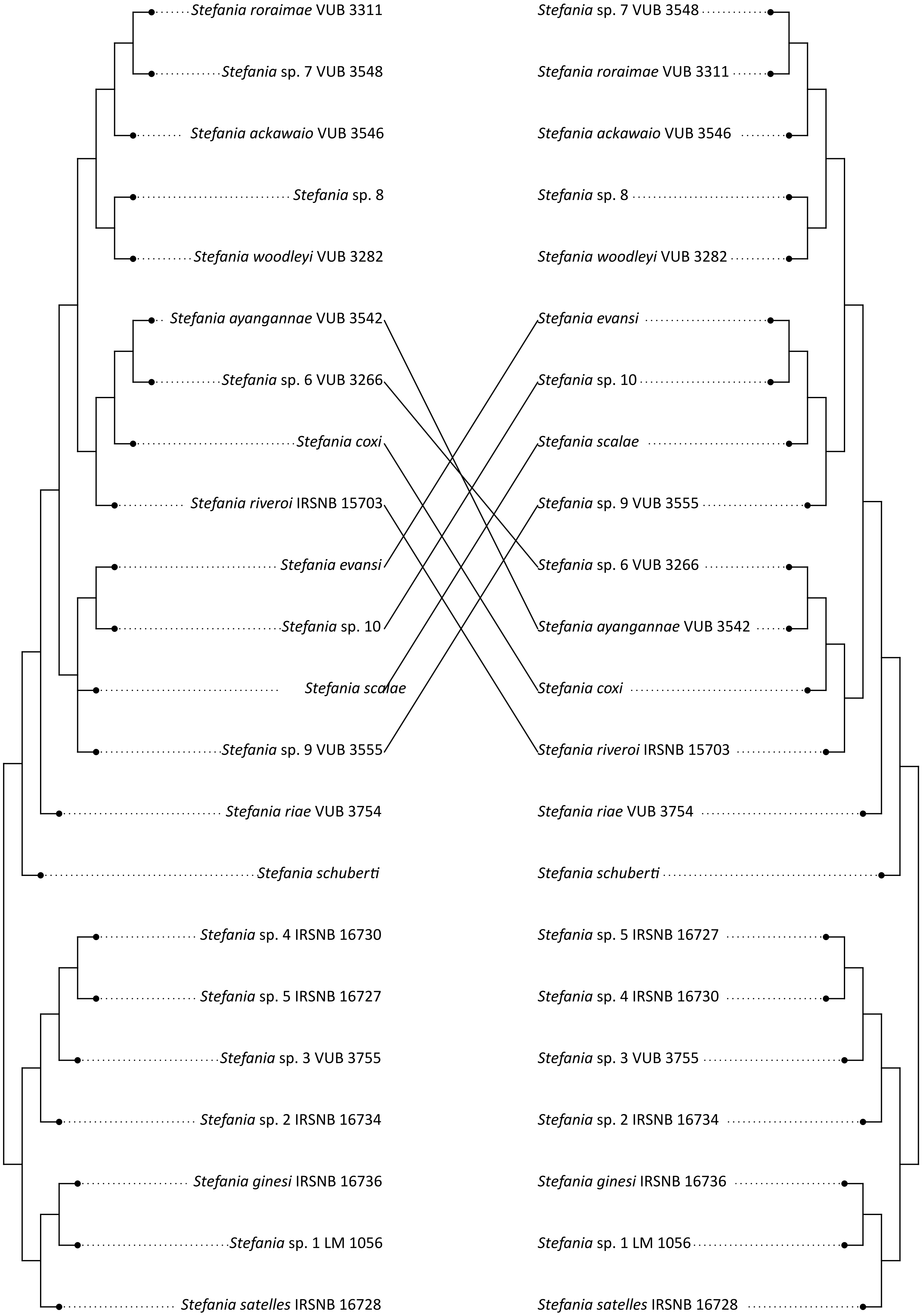


Figure S8. Comparison between SAPg (left) and TAP (right) within *Fritziana*.

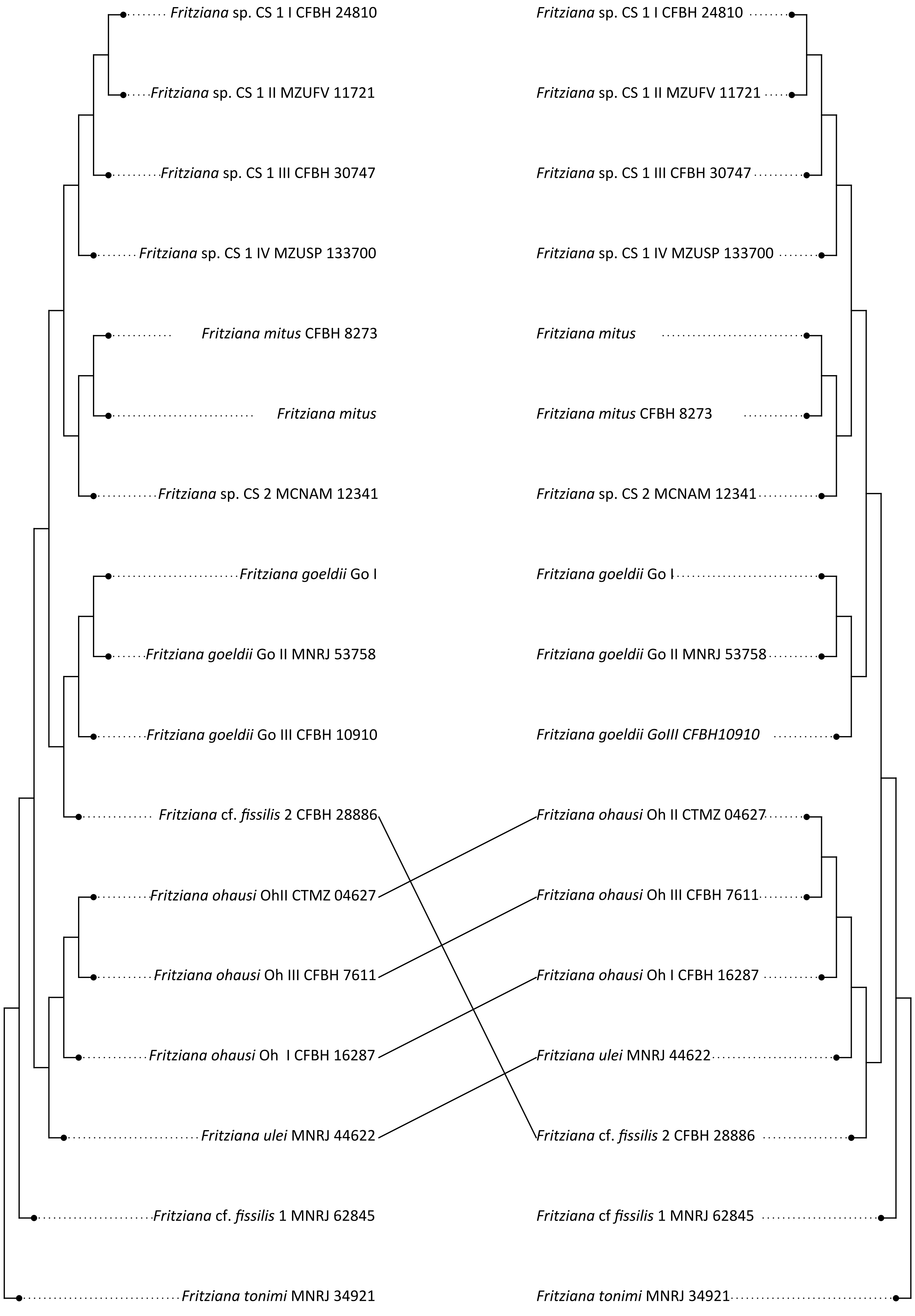


Figure S8 (Continuation). Comparison between SAPg (left) and TAP (right) within *Gastrotheca*.

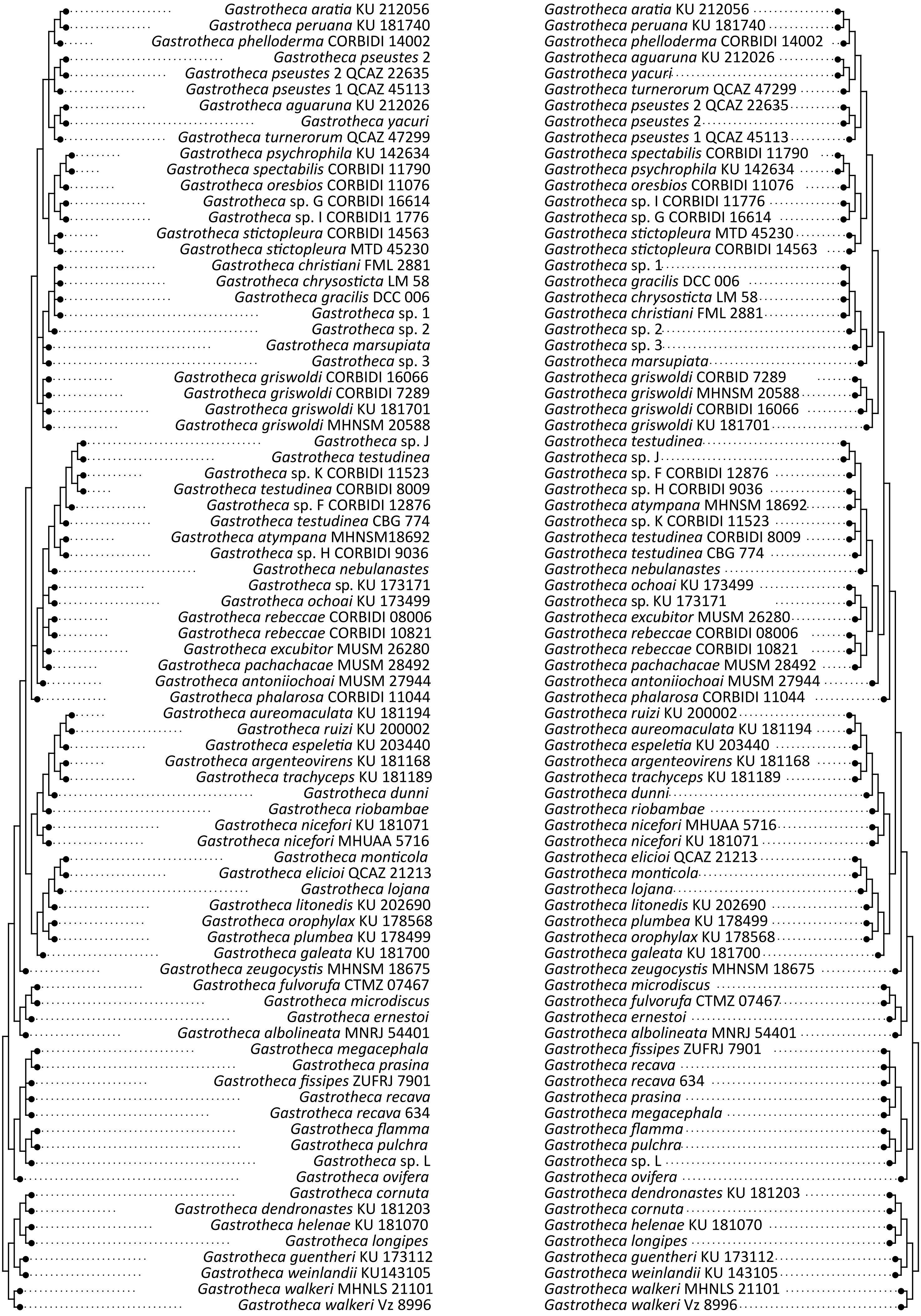


Figure S8 (Continuation). Comparison between SAPg (left) and TAP (right) within *Hemiphractus*.

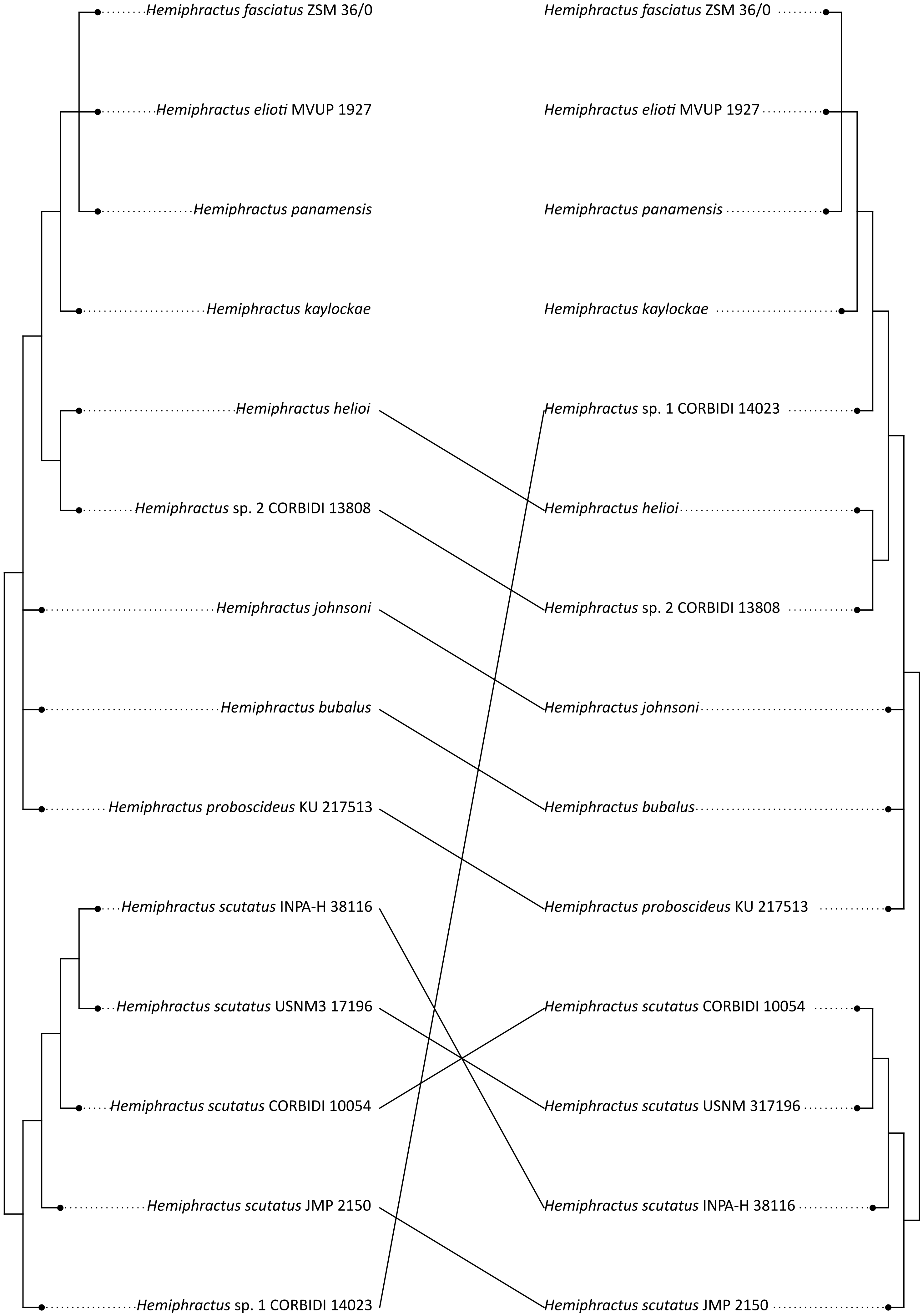


Figure S8 (Continuation). Comparison between SAPg (left) and TAP (right) within *Stefania*.

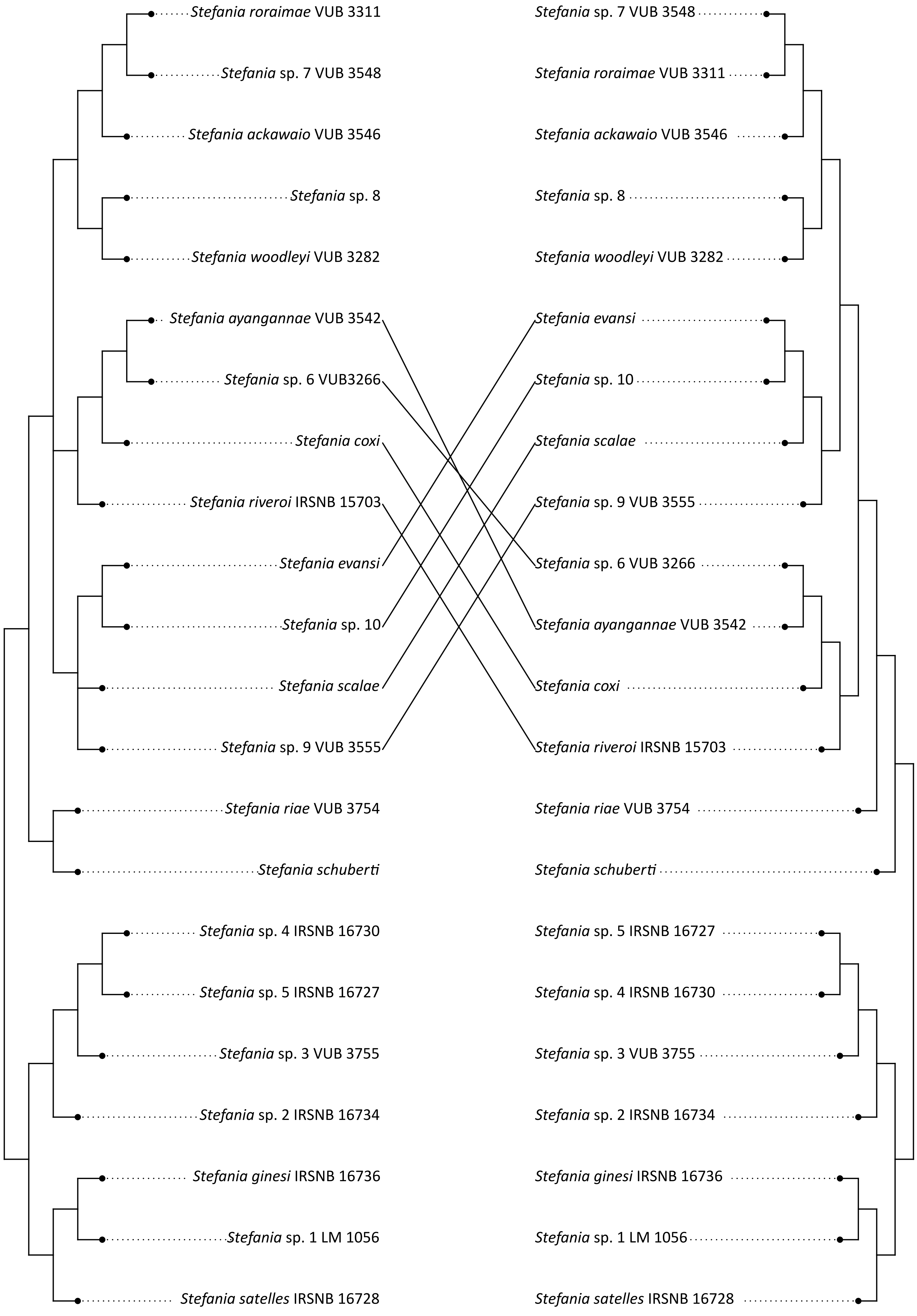


Figure S9. Comparison between SAPm (left) and TAP (right) within *Fritziana*.

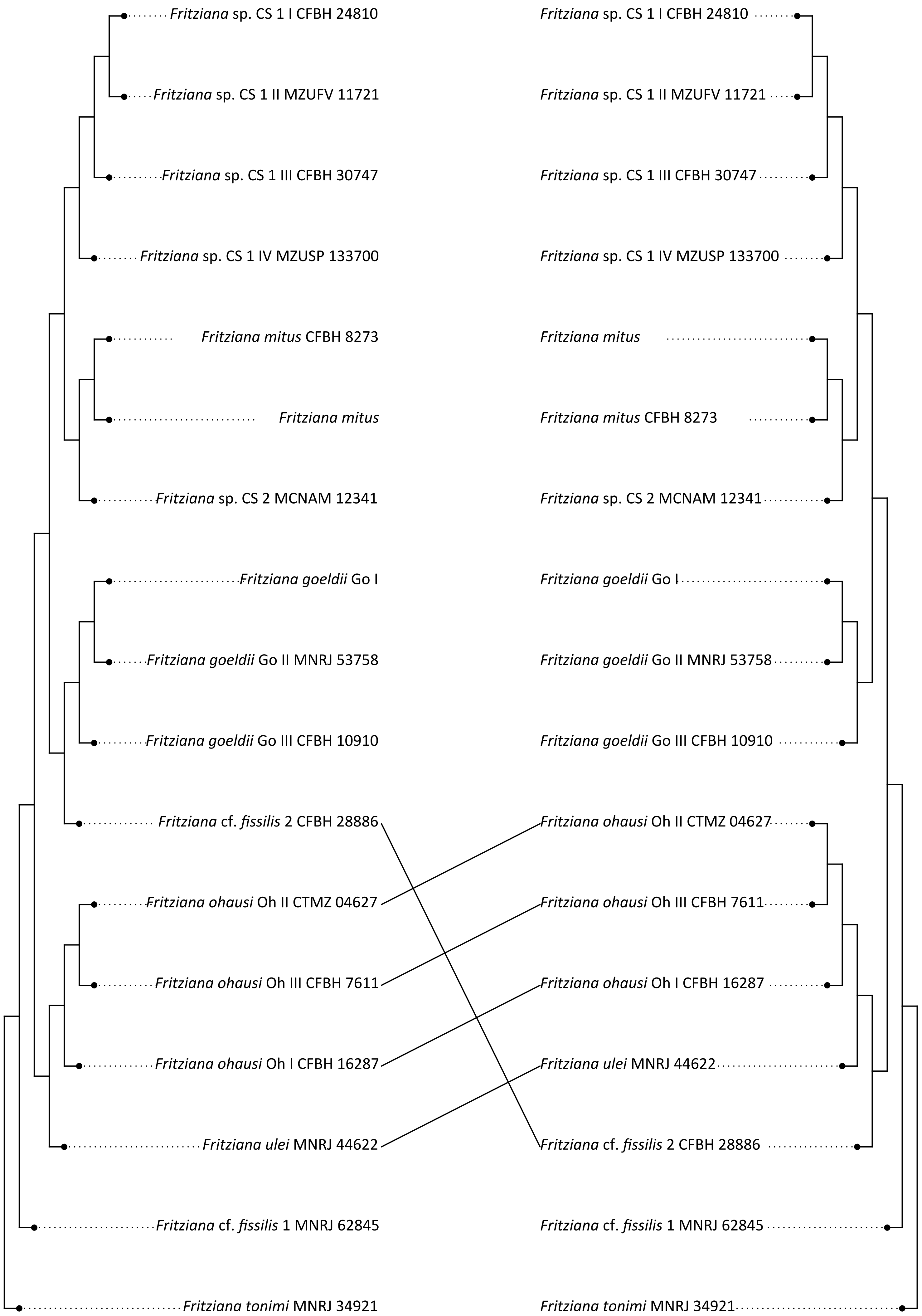


Figure S9 (Continuation). Comparison between SAPm (left) and TAP (right) within *Gastrotheca*.

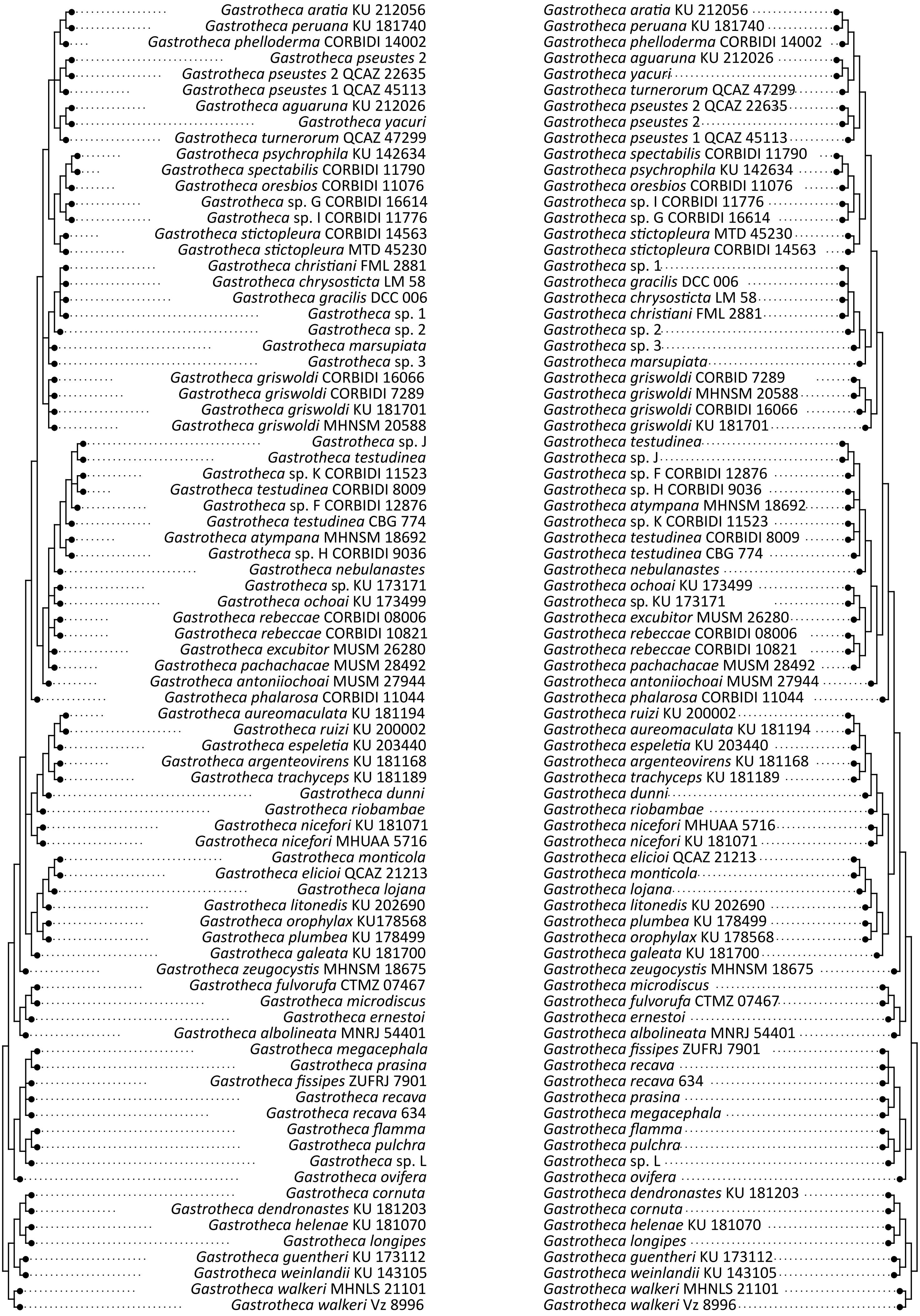


Figure S9 (Continuation). Comparison between SAPm (left) and TAP (right) within *Hemiphractus*.

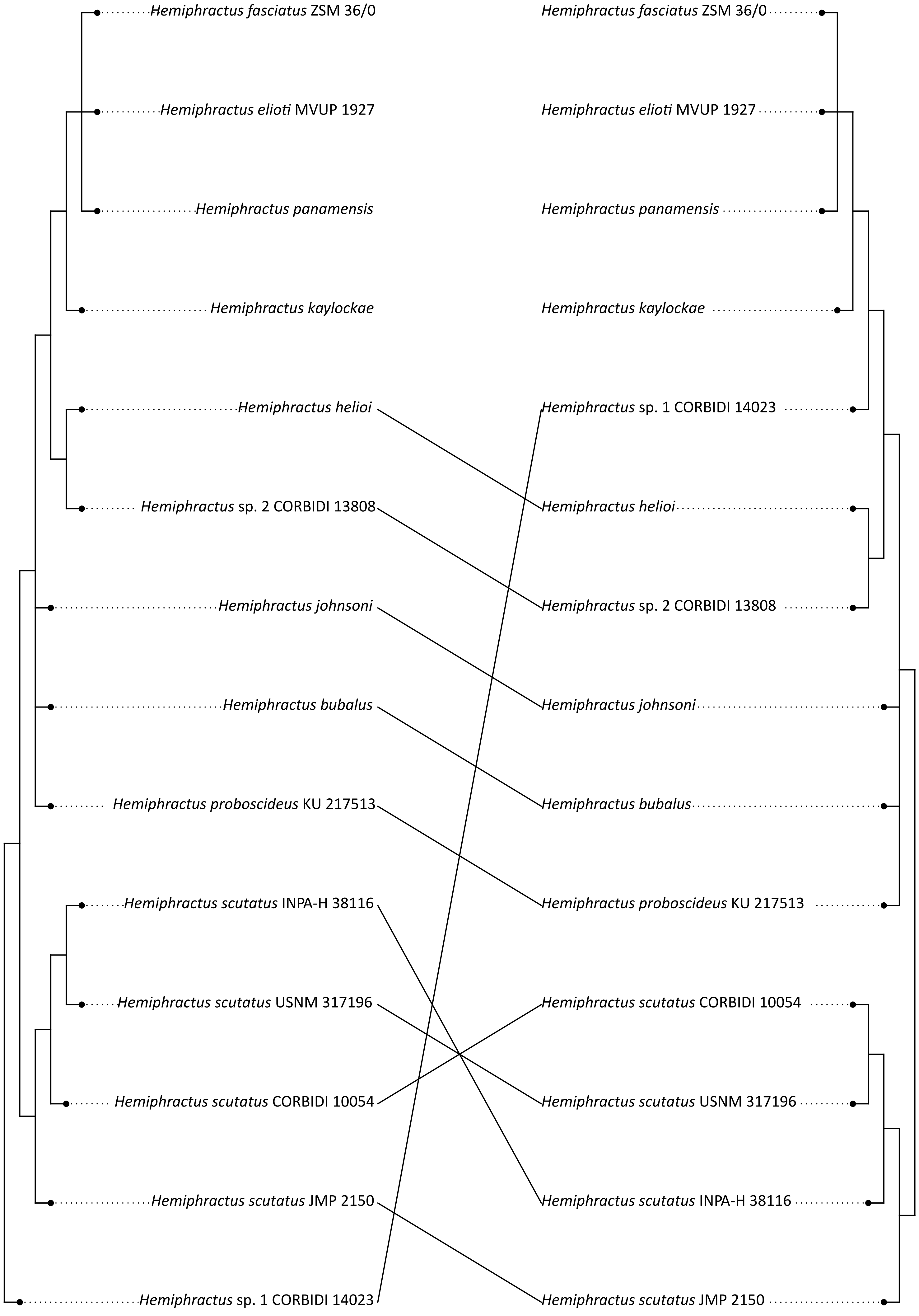


Figure S9 (Continuation). Comparison between SAPm (left) and TAP (right) within *Stefania*.

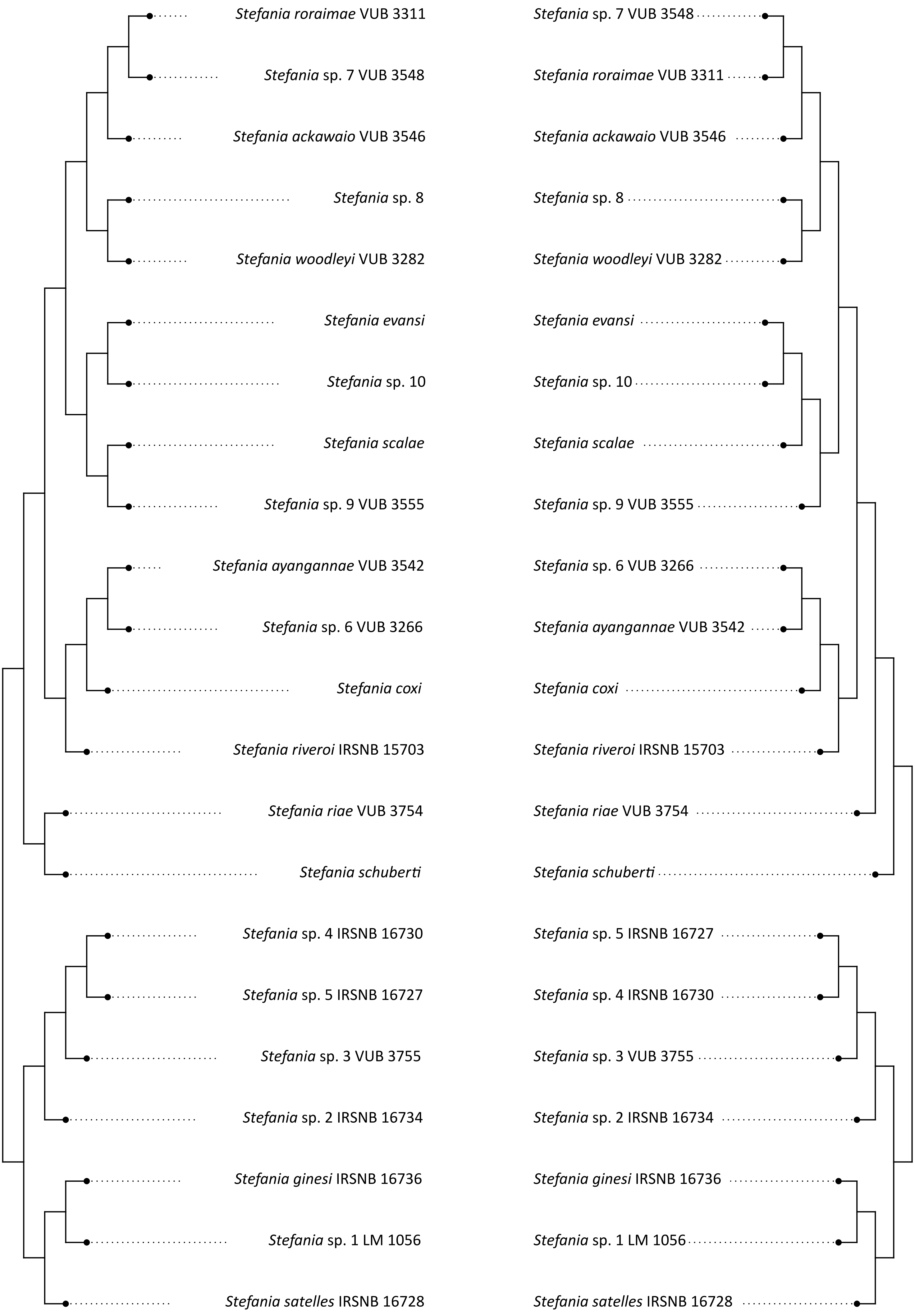


Figure S10. Comparison between SALg (left) and TAP (right) within *Fritziana*.

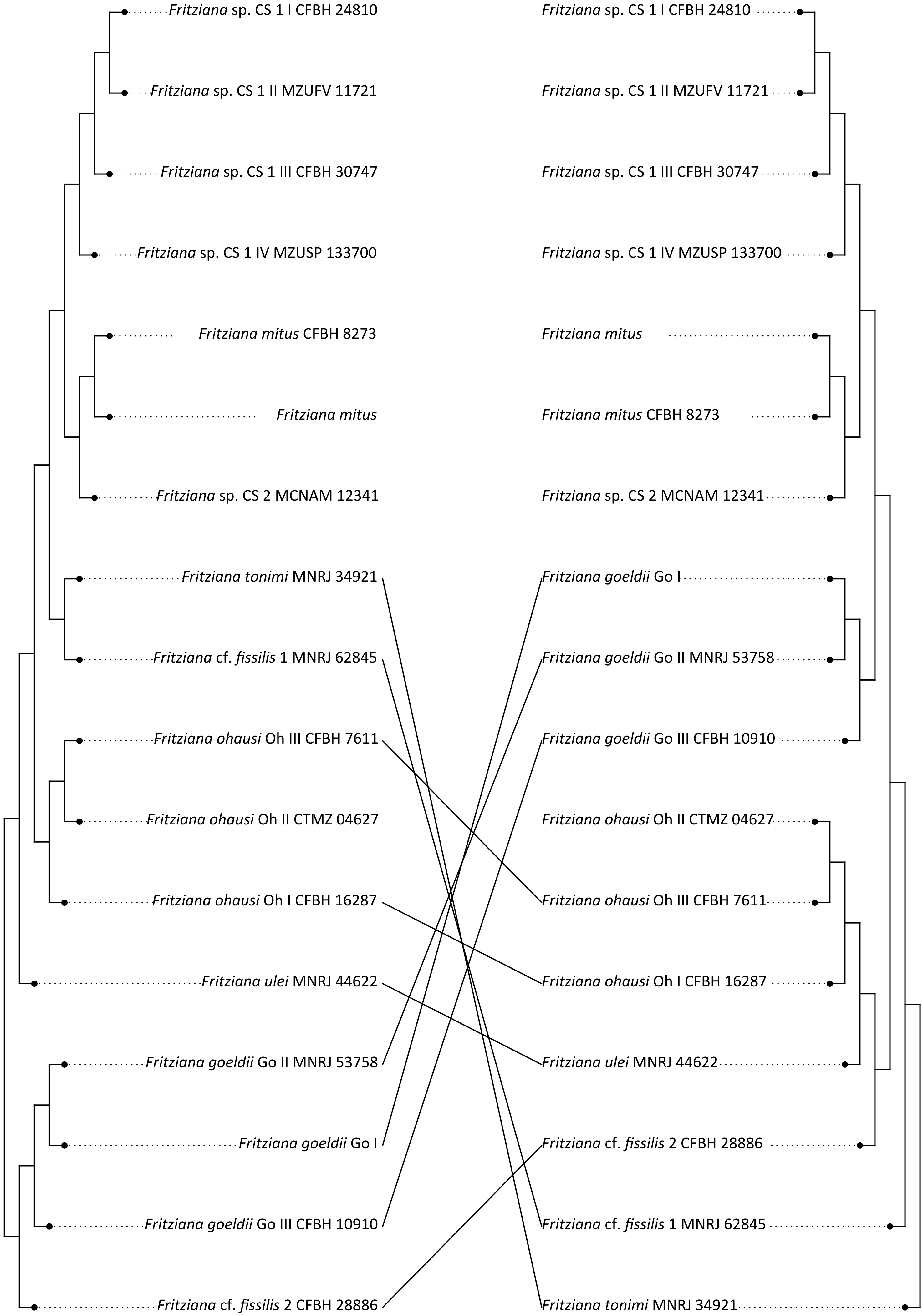


Figure S10 (Continuation). Comparison between SALg (left) and TAP (right) within *Gastrotheca*.

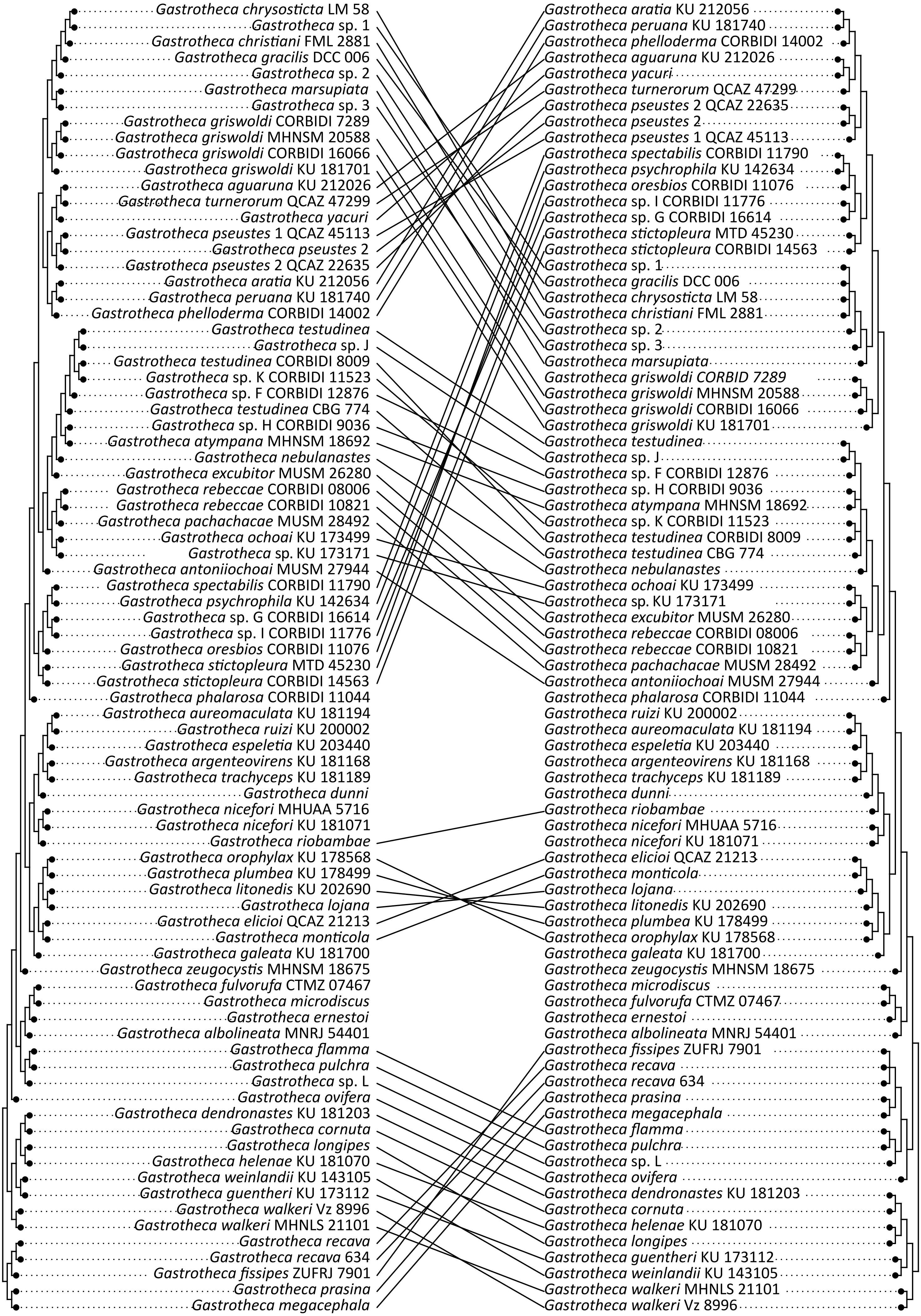


Figure S10 (Continuation). Comparison between SALg (left) and TAP (right) within *Hemiphractus*.

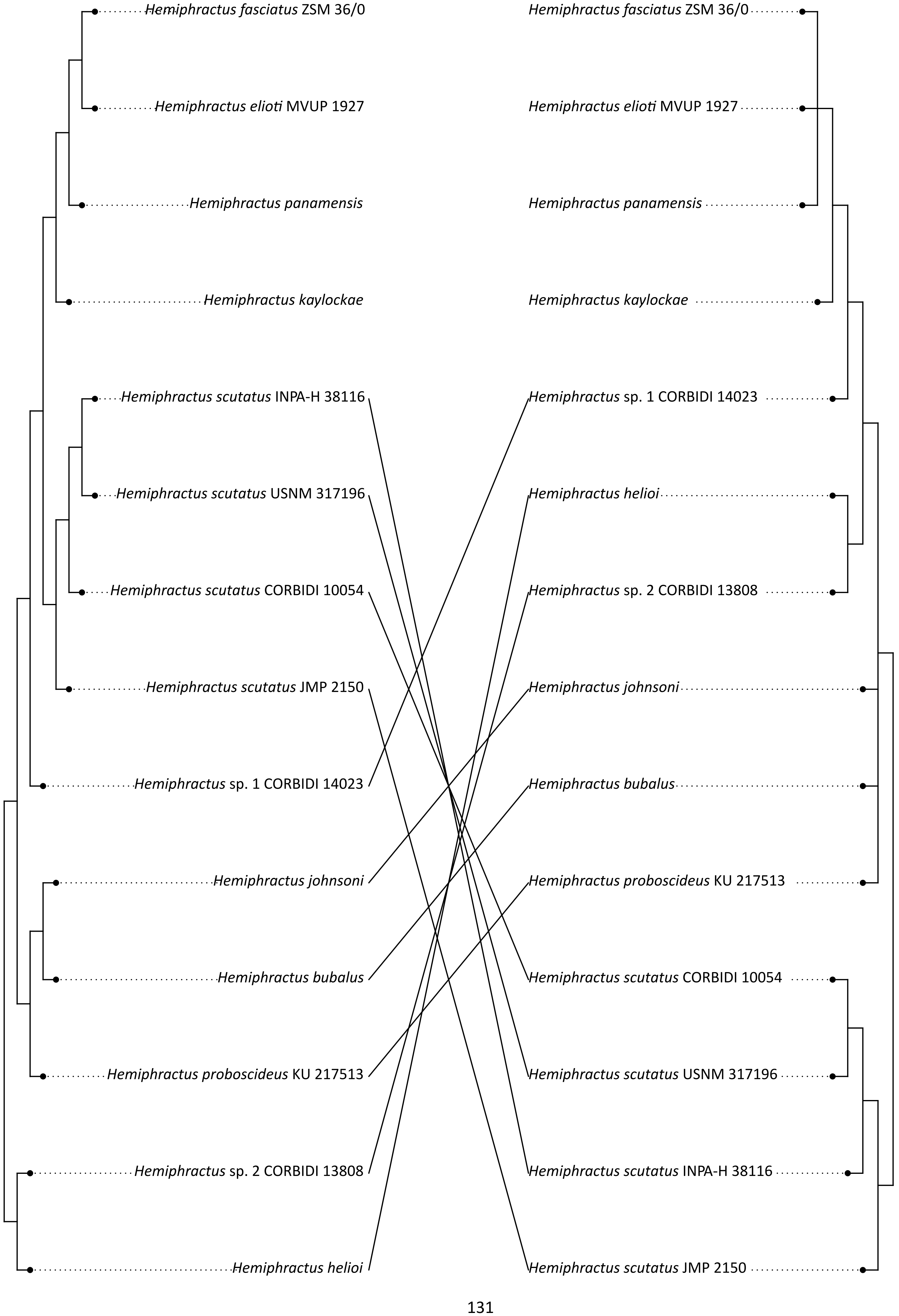


Figure S10 (Continuation). Comparison between SALg (left) and TAP (right) within *Stefania*.

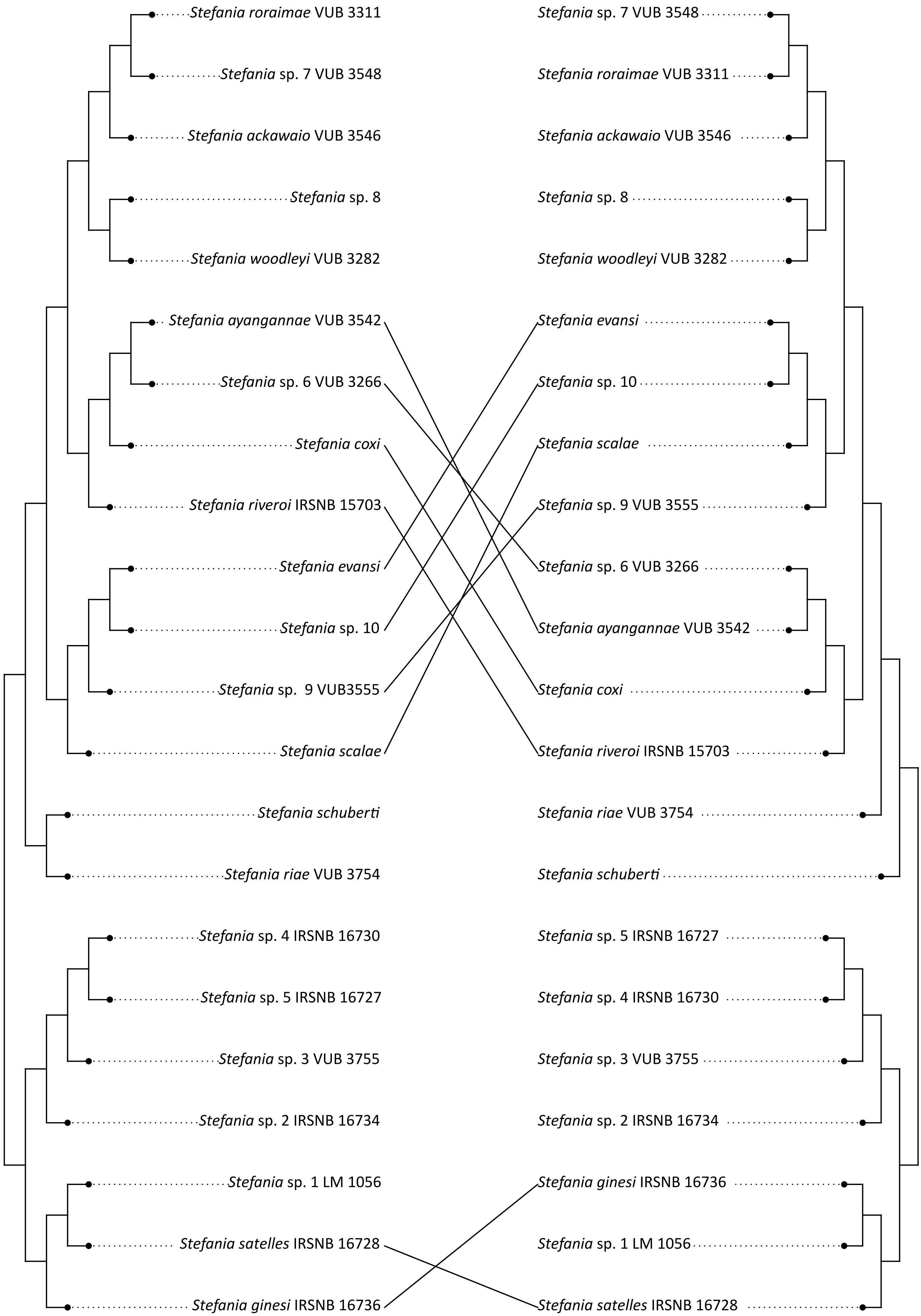


Figure S11. Comparison between SALm (left) and TAP (right) within *Fritziana*.

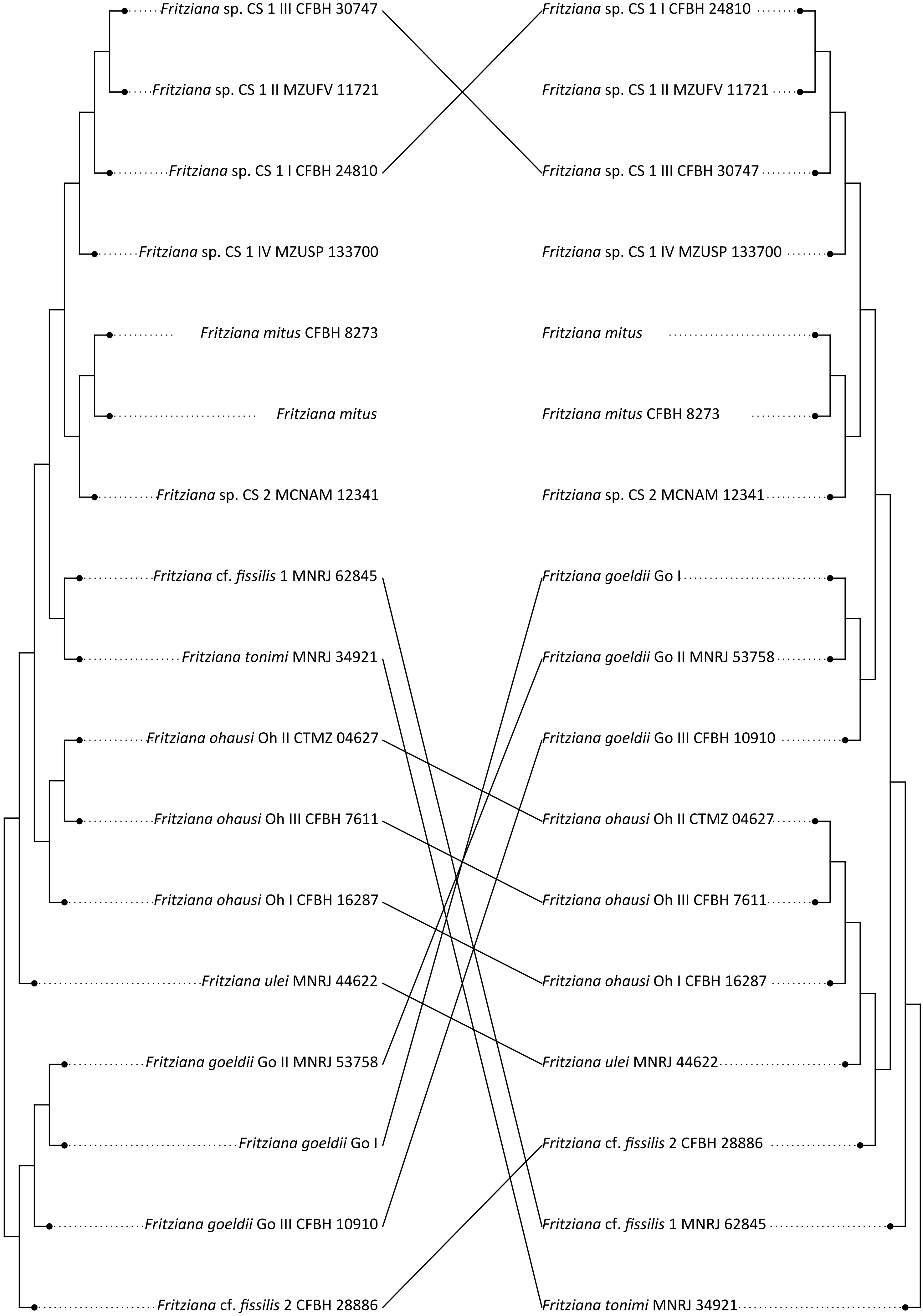


Figure S11 (Continuation). Comparison between SALm (left) and TAP (right) within *Gastrotheca*.

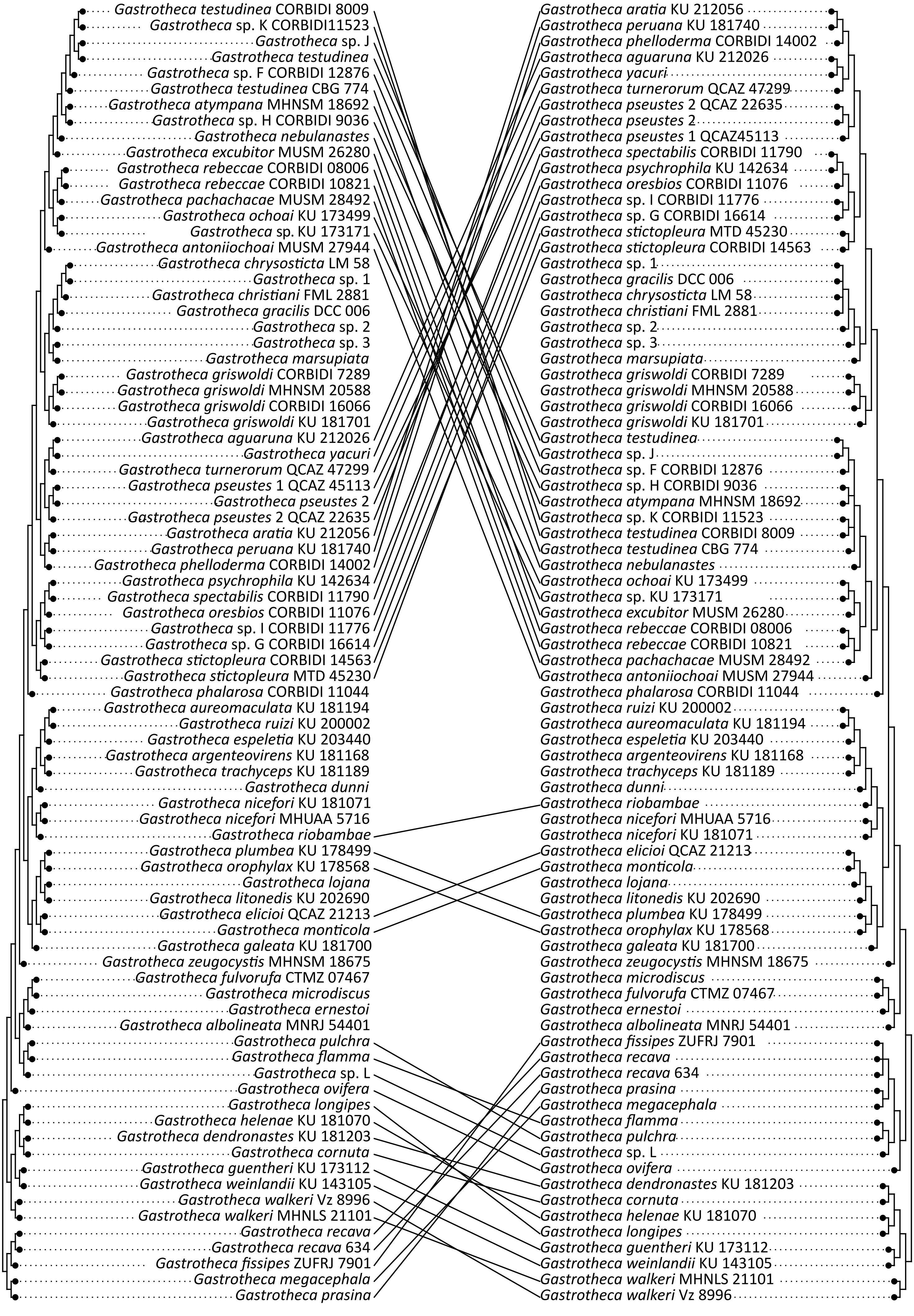


Figure S11 (Continuation). Comparison between SALm (left) and TAP (right) within *Hemiphractus*.

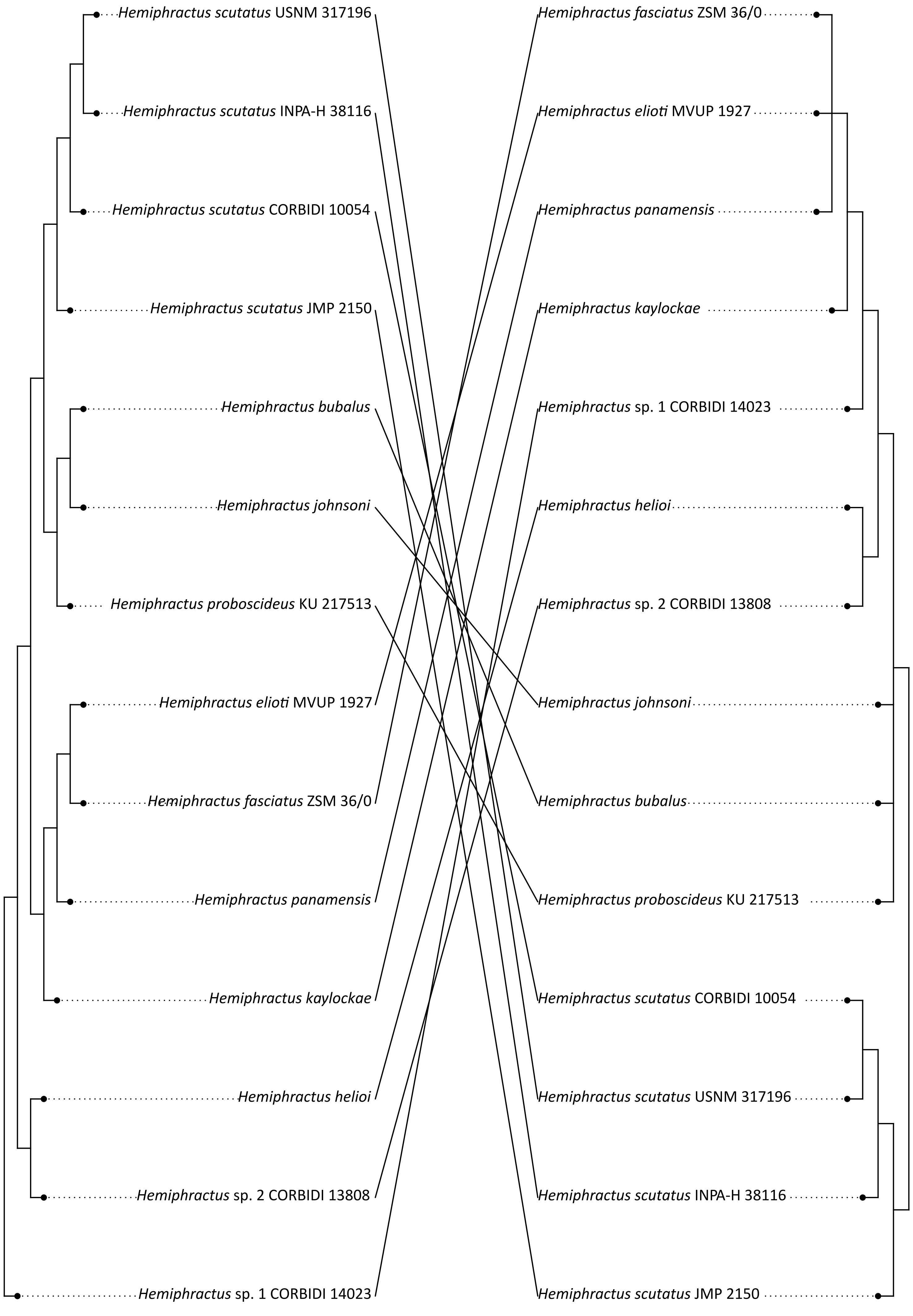


Figure S11 (Continuation). Comparison between SALm (left) and TAP (right) within *Stefania*.

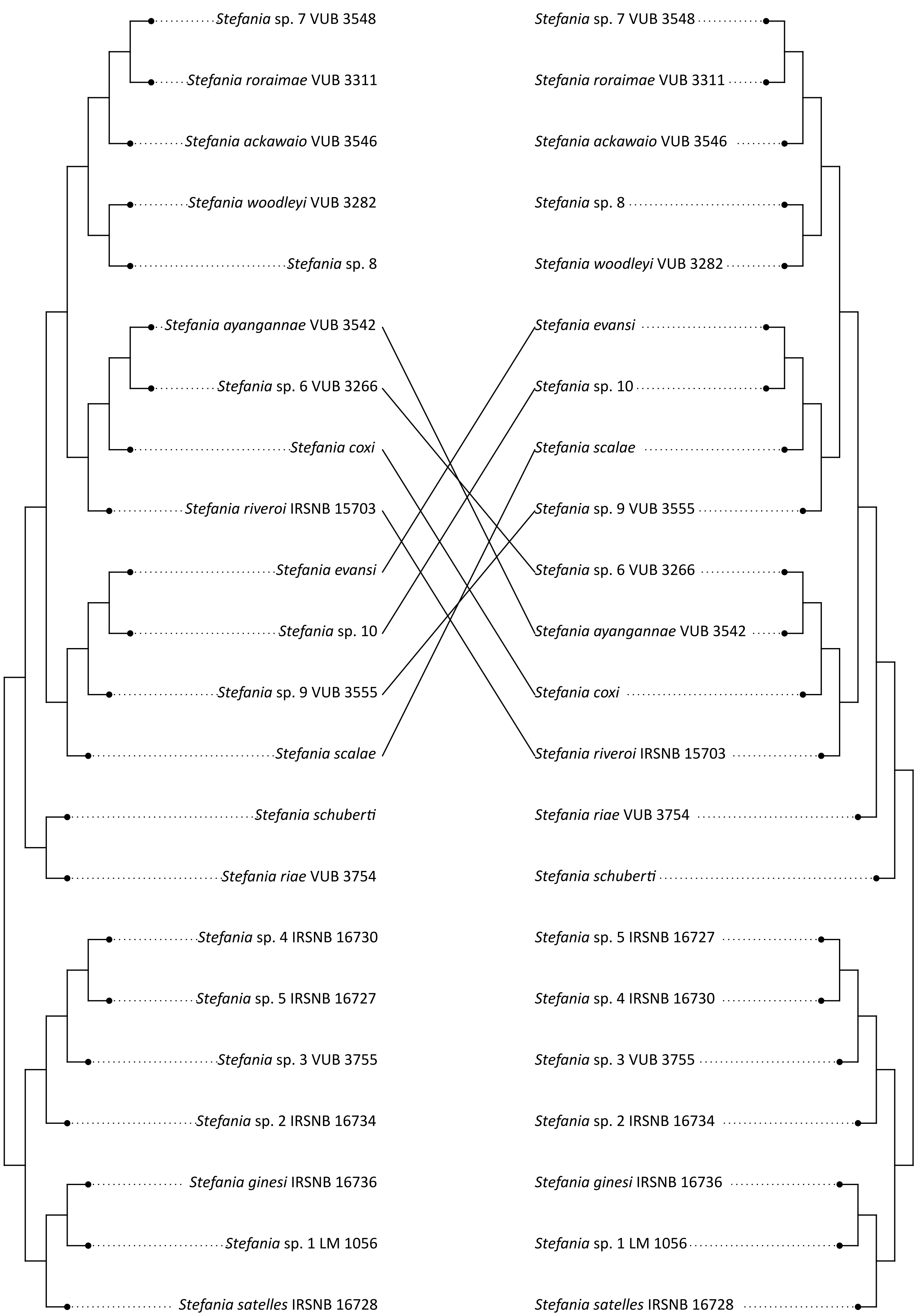
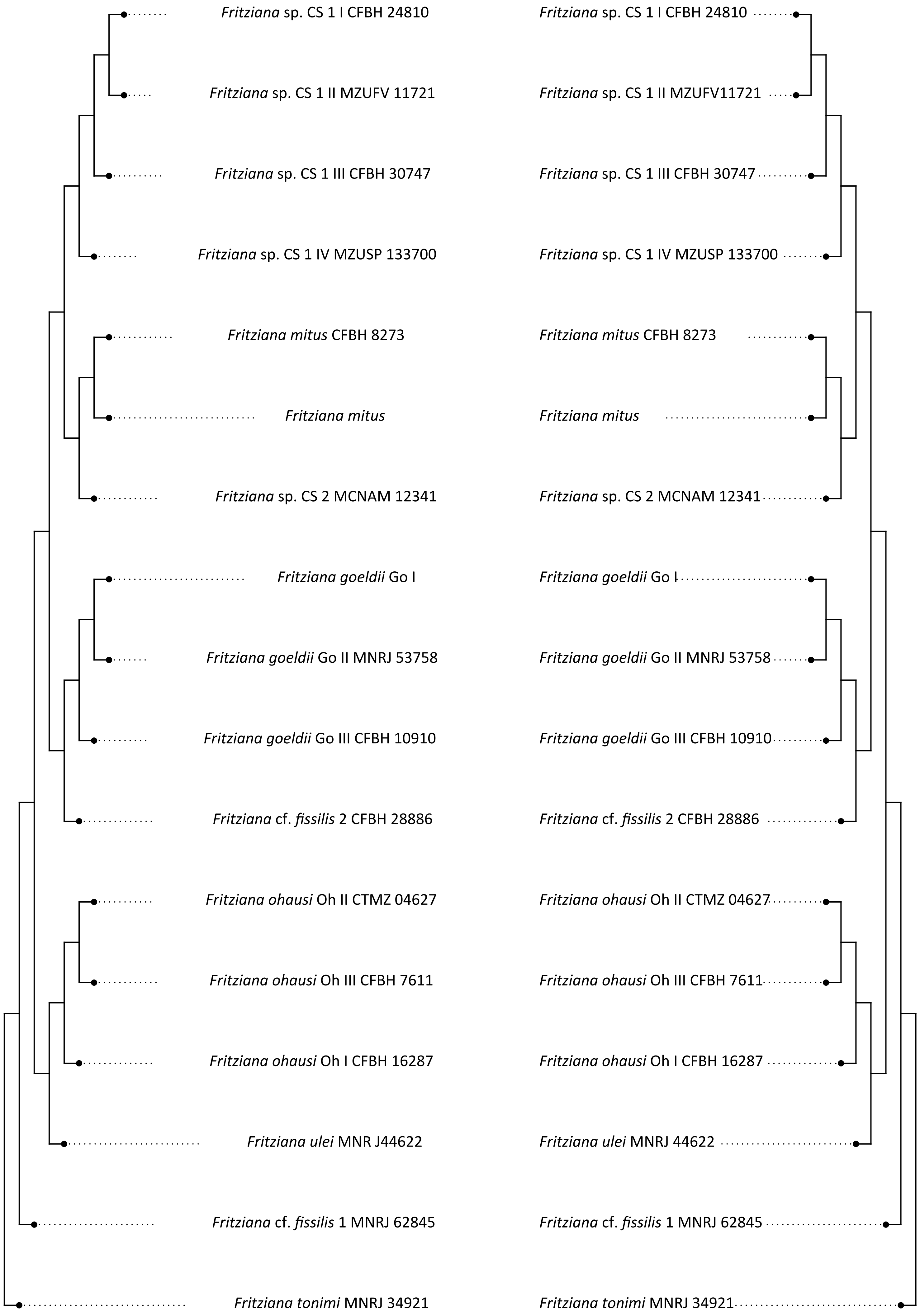


Figure S12. Comparison between SAP5th (left) and SAPg (right) within *Fritziana*.



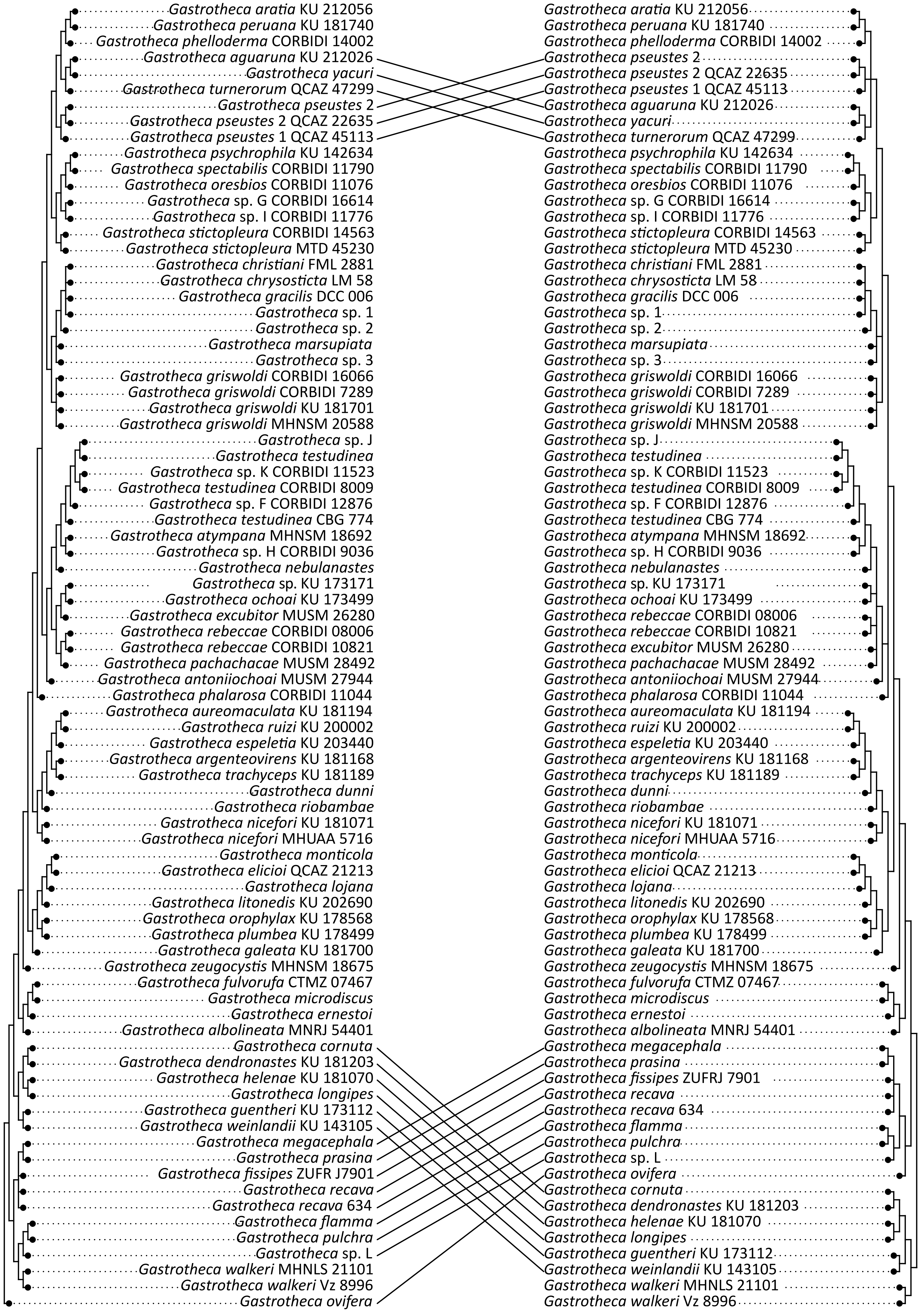


Figure S12 (Continuation). Comparison between SAP5th (left) and SAPg (right) within *Gastrotheca*.

Figure S12 (Continuation). Comparison between SAP5th (left) and SAPg (right) within *Hemiphractus*.

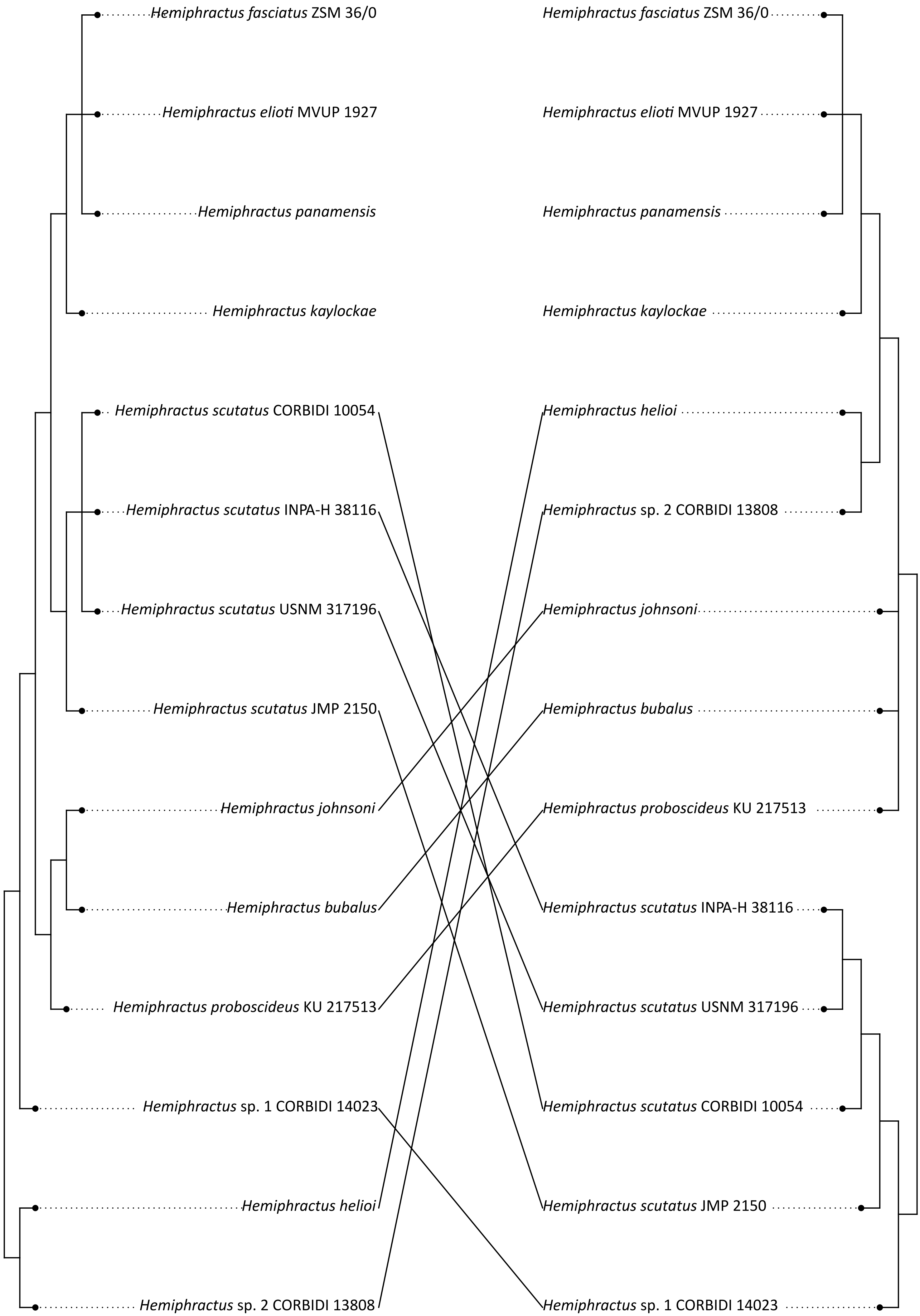


Figure S12 (Continuation). Comparison between SAP5th (left) and SAPg (right) within *Stefania*.

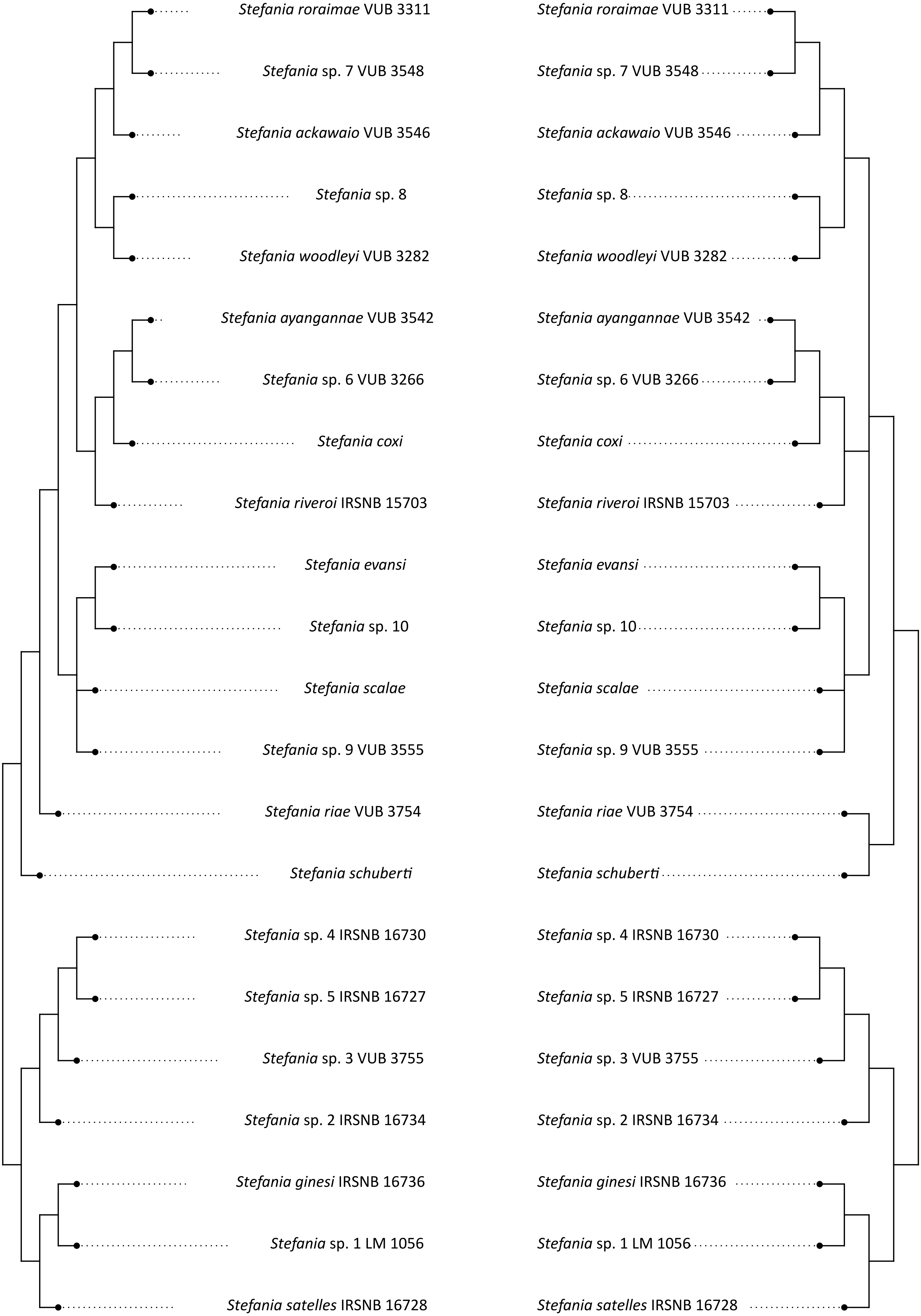


Figure S13. Comparison between SAP5th (left) and SAPm (right) within *Fritziana*.

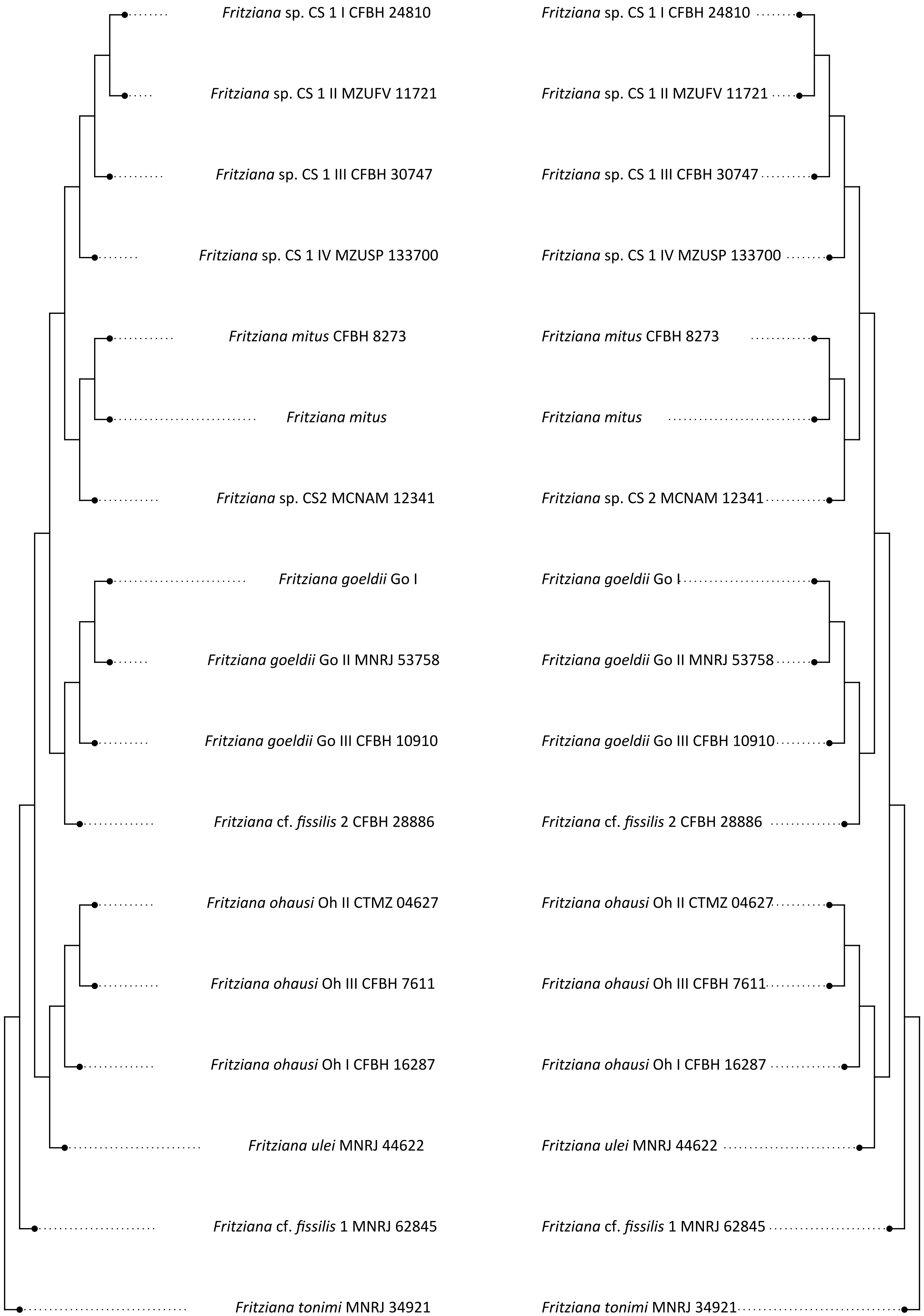


Figure S13 (Continuation). Comparison between SAP5th (left) and SAPm (right) within *Gastrotheca*.

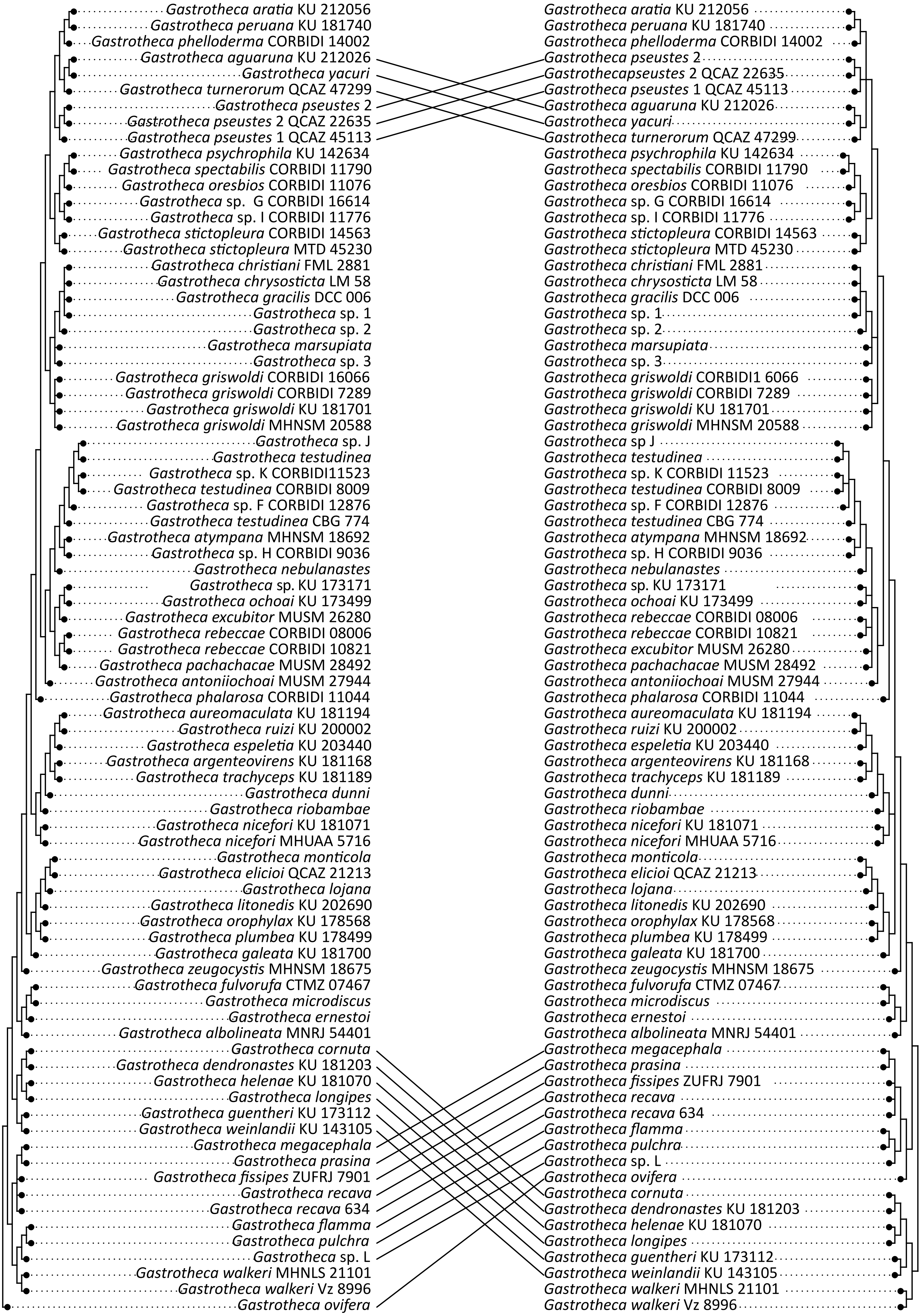


Figure S13 (Continuation). Comparison between SAP5th (left) and SAPm (right) within *Hemiphractus*.

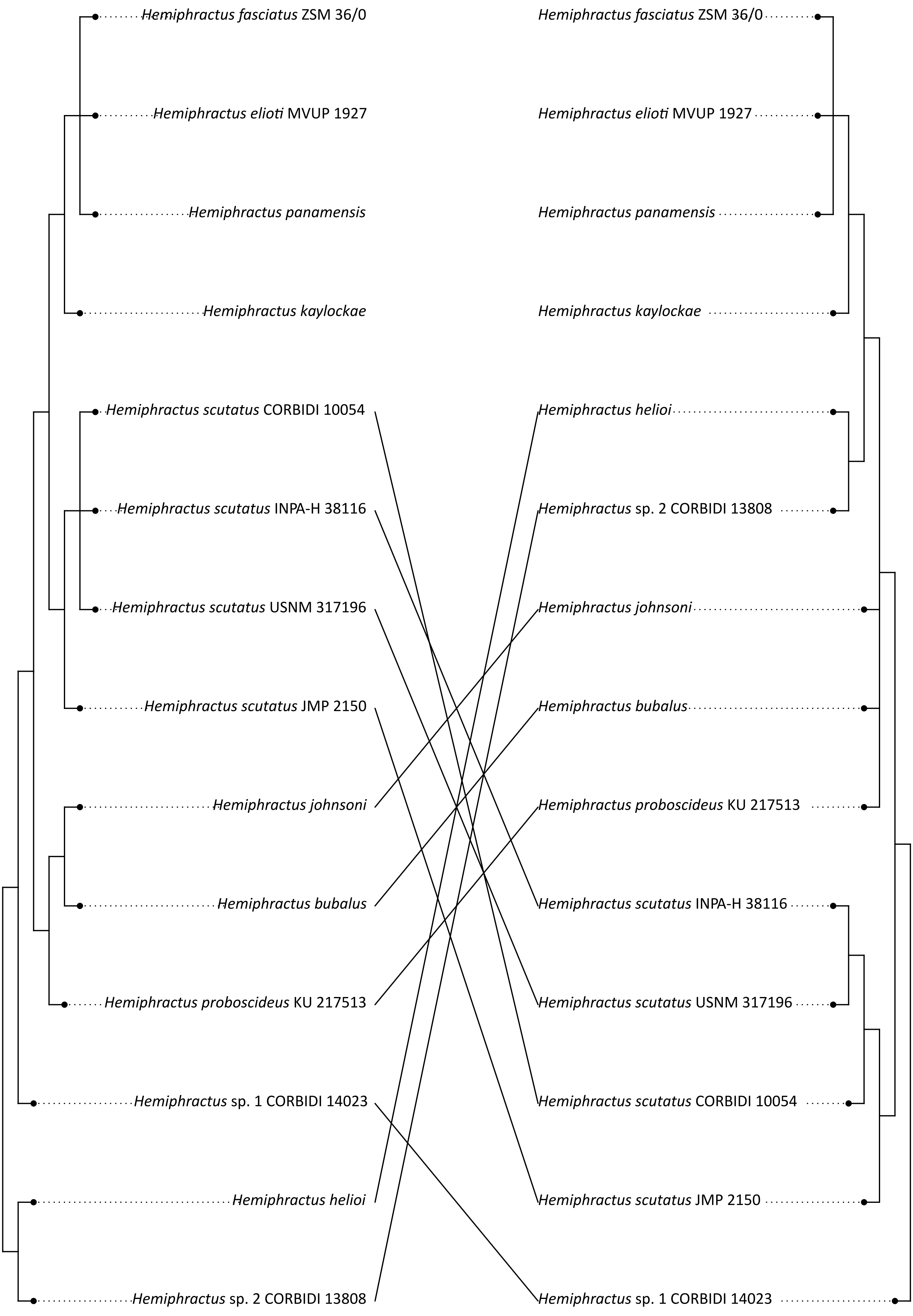


Figure S13 (Continuation). Comparison between SAP5th (left) and SAPm (right) within *Stefania*.

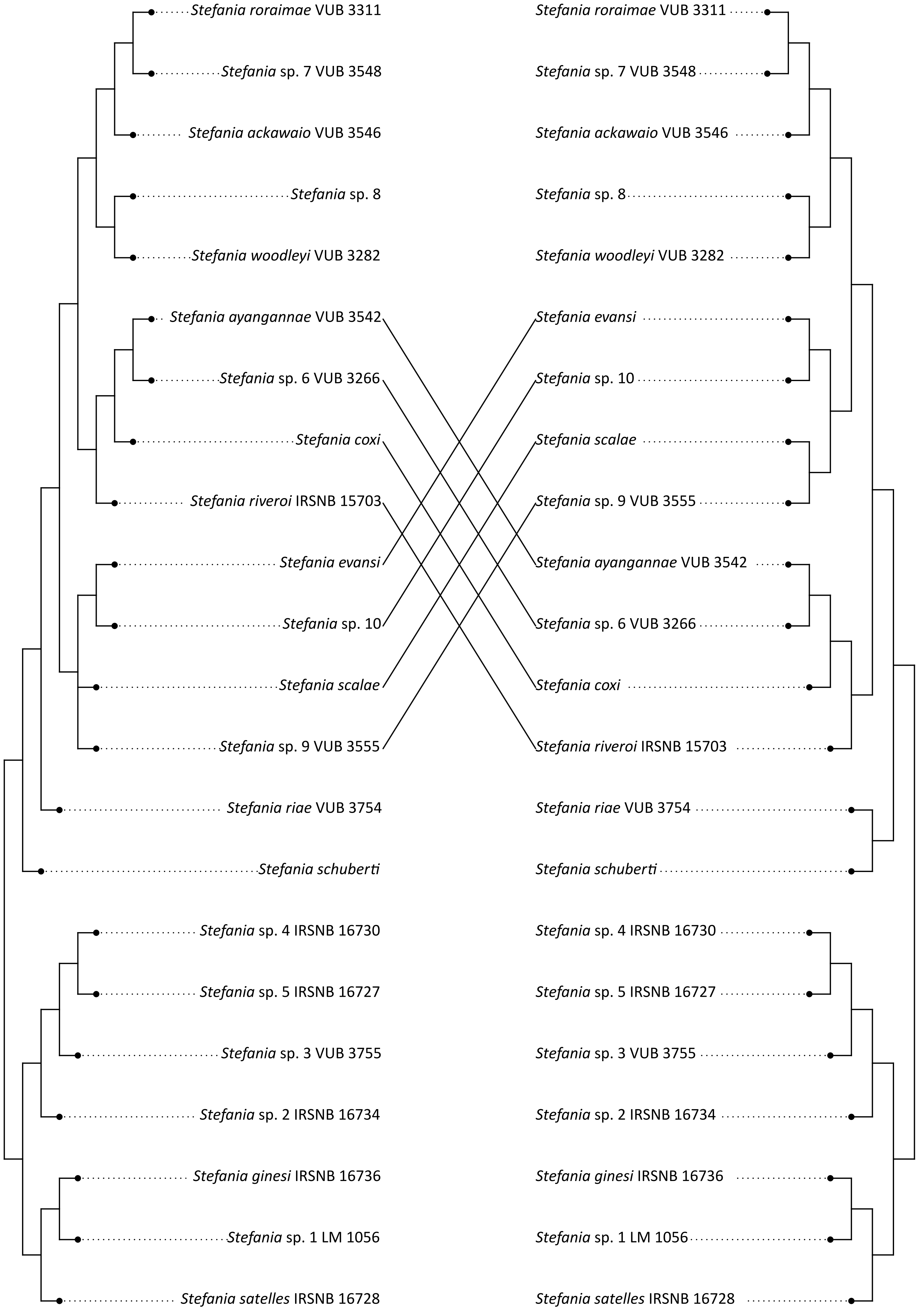


Figure S14. Comparisons between SALg (left) and SAP5th (right) within *Fritziana*.

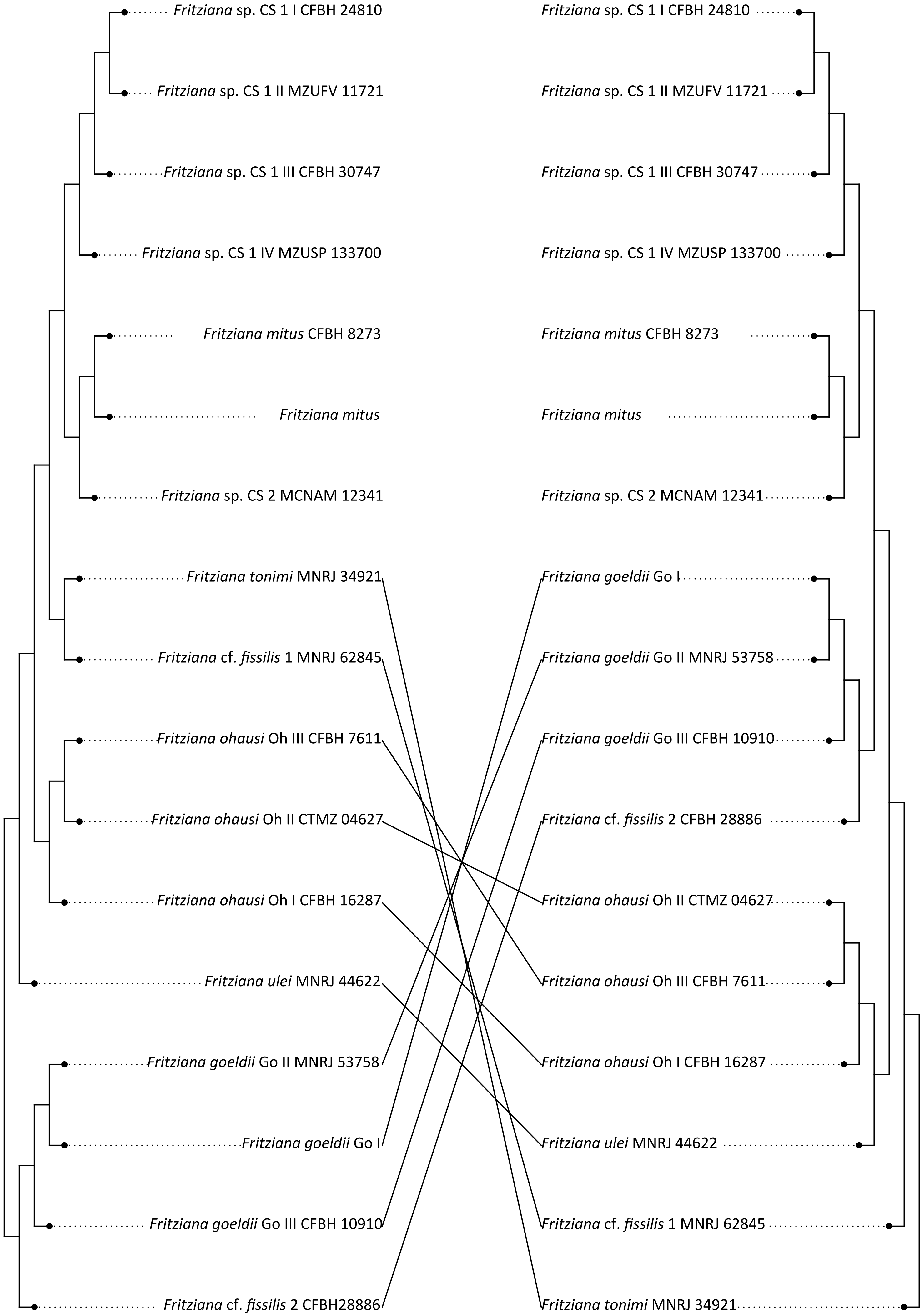


Figure S14 (Continuation). Comparisons between SALg (left) and SAP5th (right) within *Gastrotheca*.

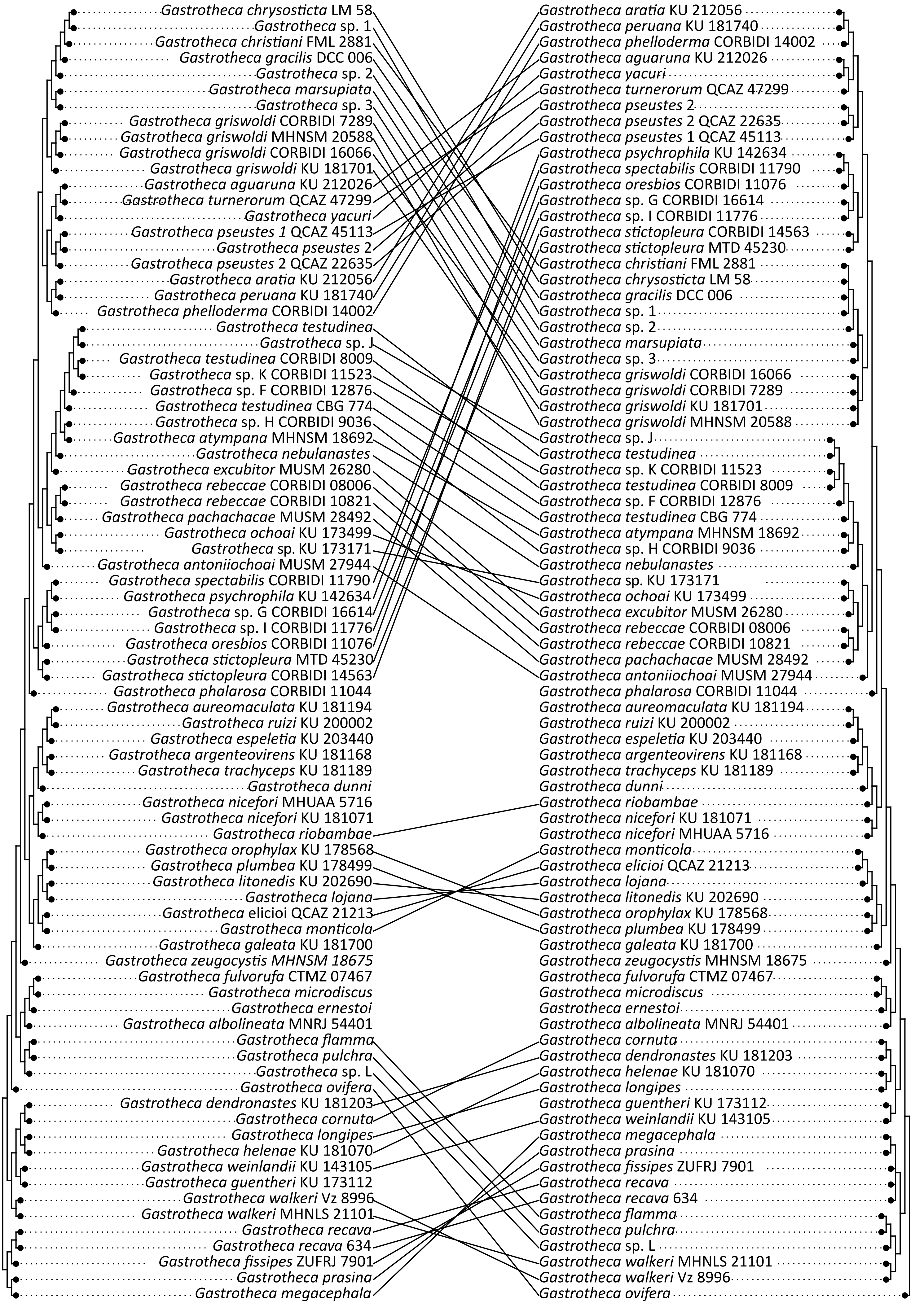


Figure S14 (Continuation). Comparisons between SALg (left) and SAP5th (right) within *Hemiphractus*.

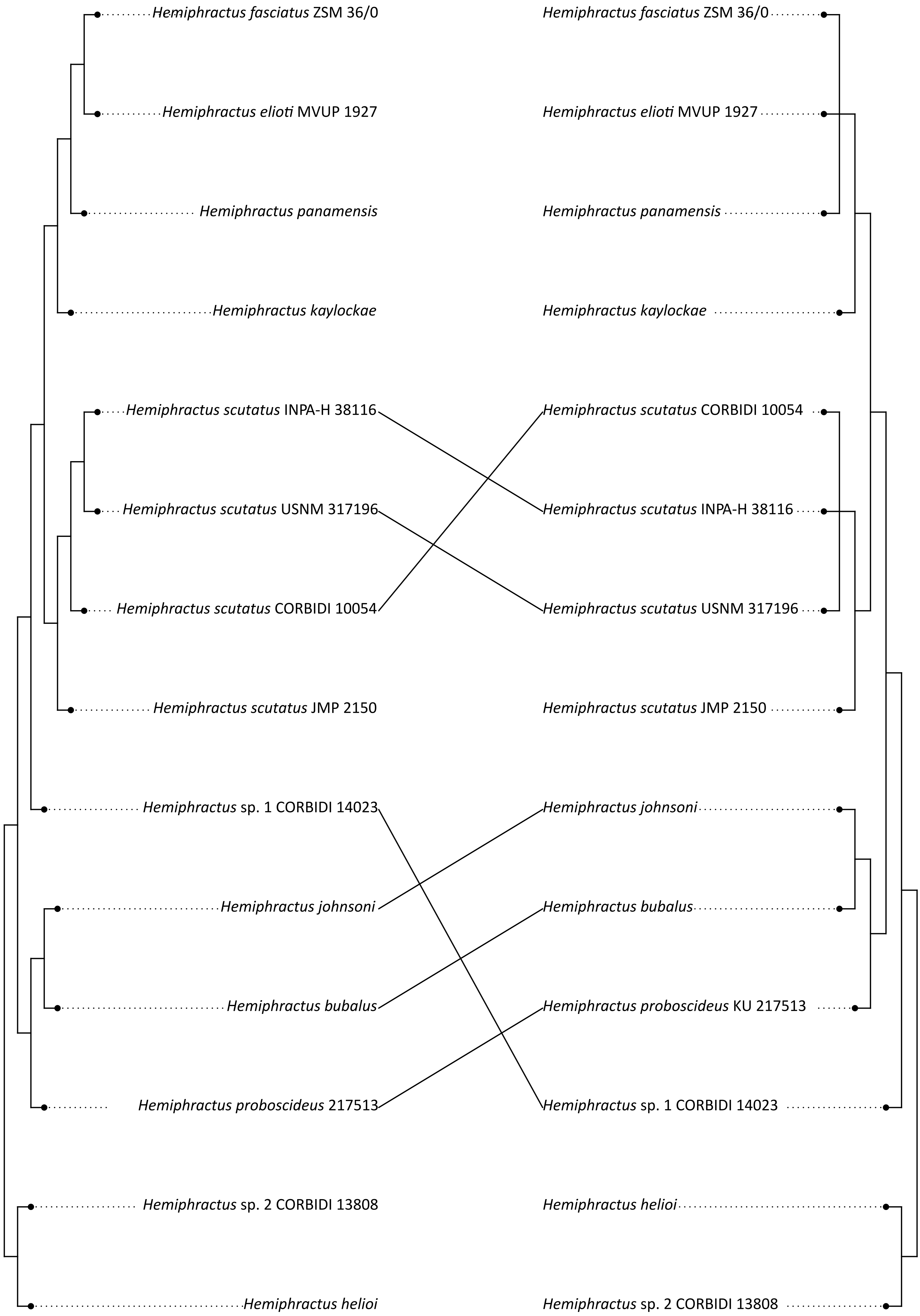


Figure S14 (Continuation). Comparisons between SALg (left) and SAP5th (right) within *Stefania*.

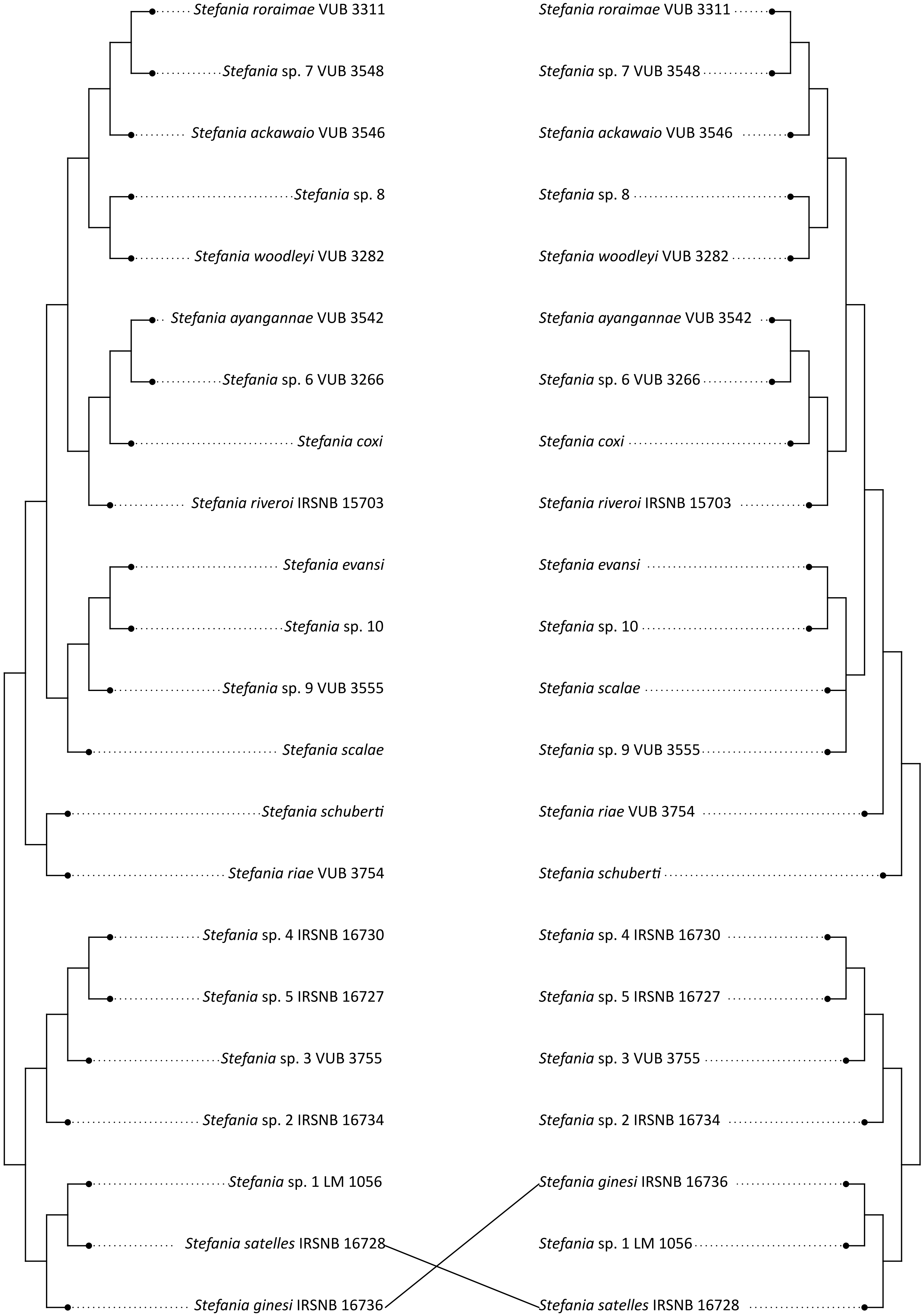


Figure S15. Comparison between SALm (left) and SAP5th (right) within *Fritziana*.

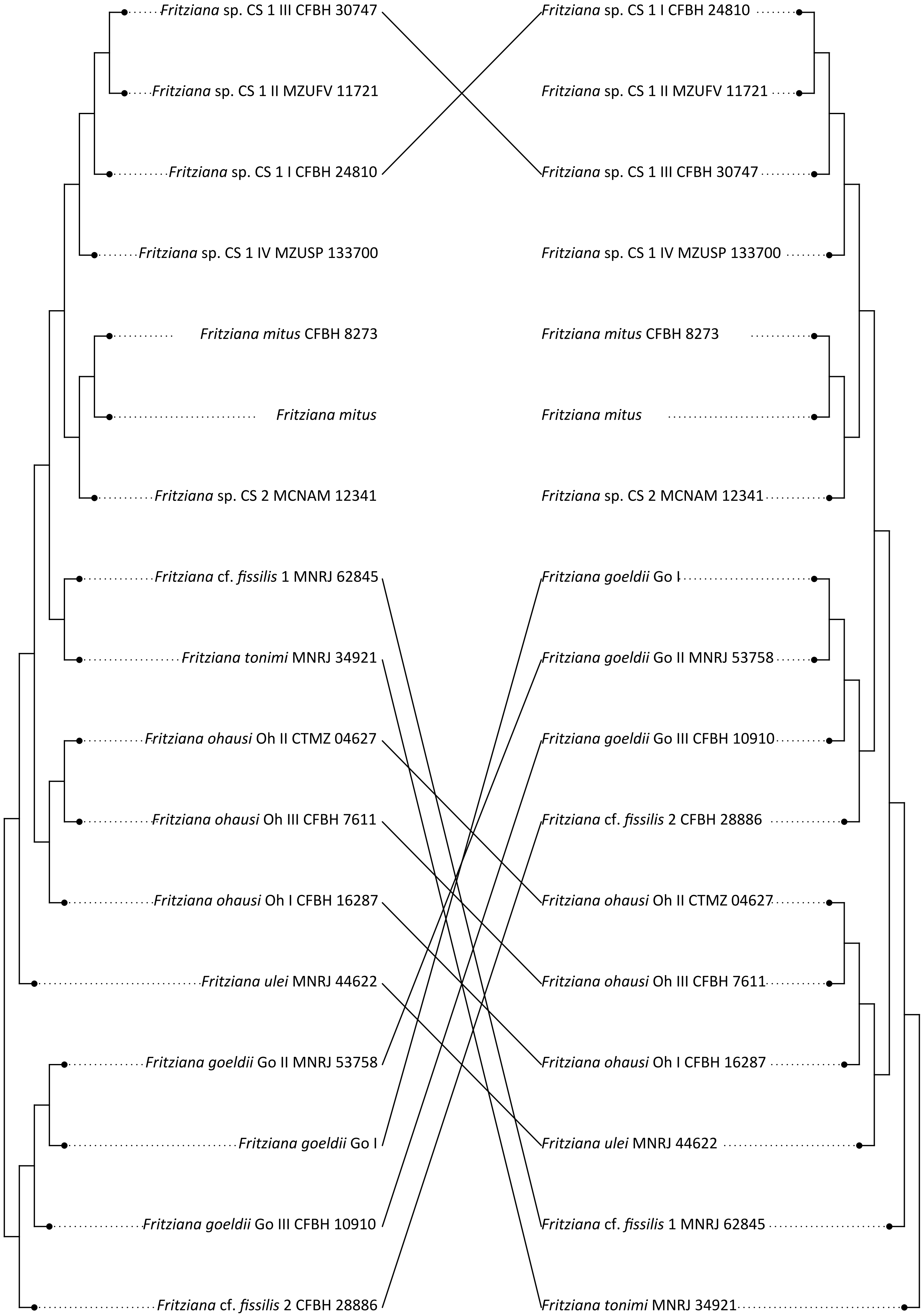


Figure S15 (Continuation). Comparison between SALm (left) and SAP5th (right) within *Gastrotheca*.

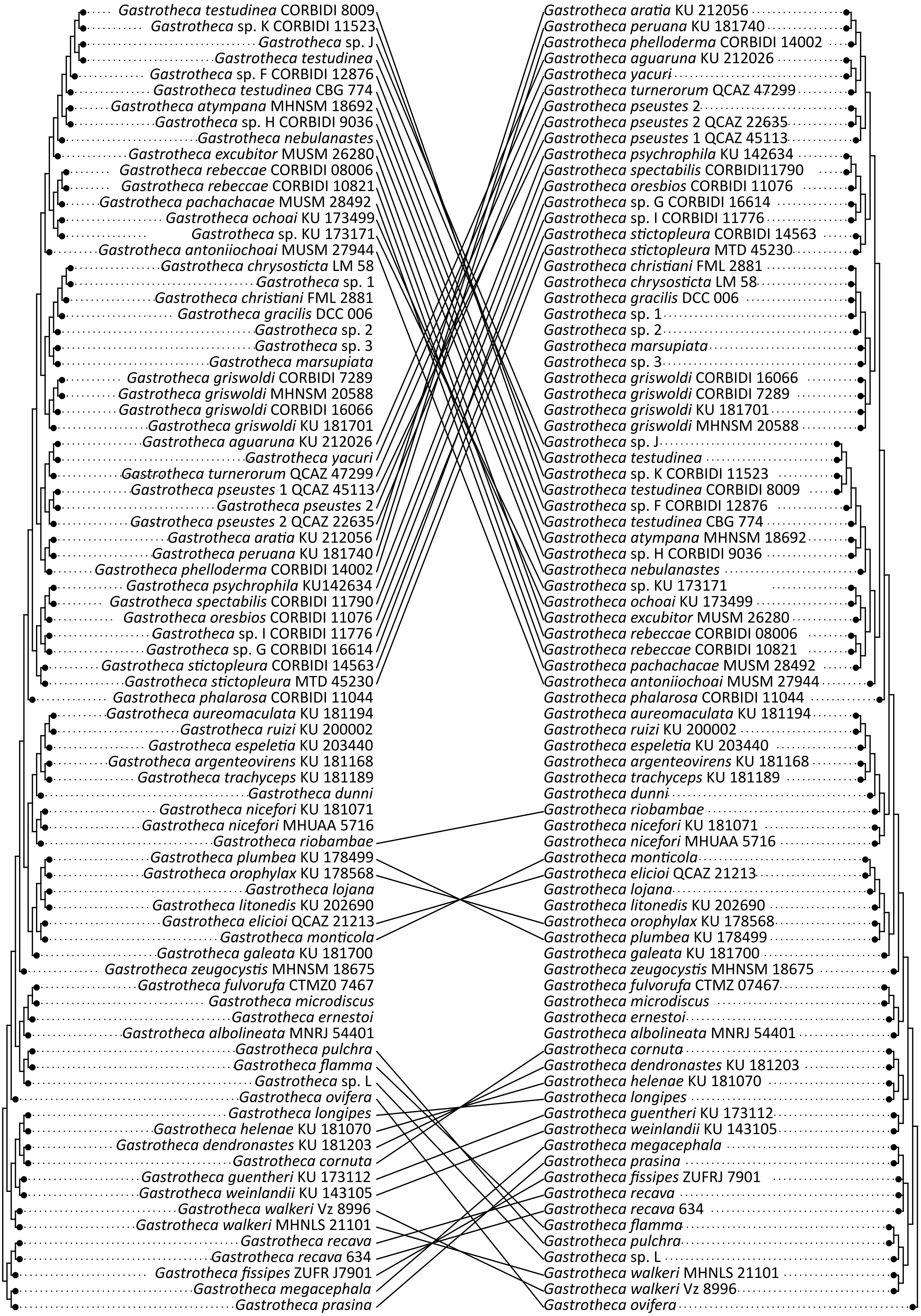


Figure S15 (Continuation). Comparison between SALm (left) and SAP5th (right) within *Hemiphractus*.

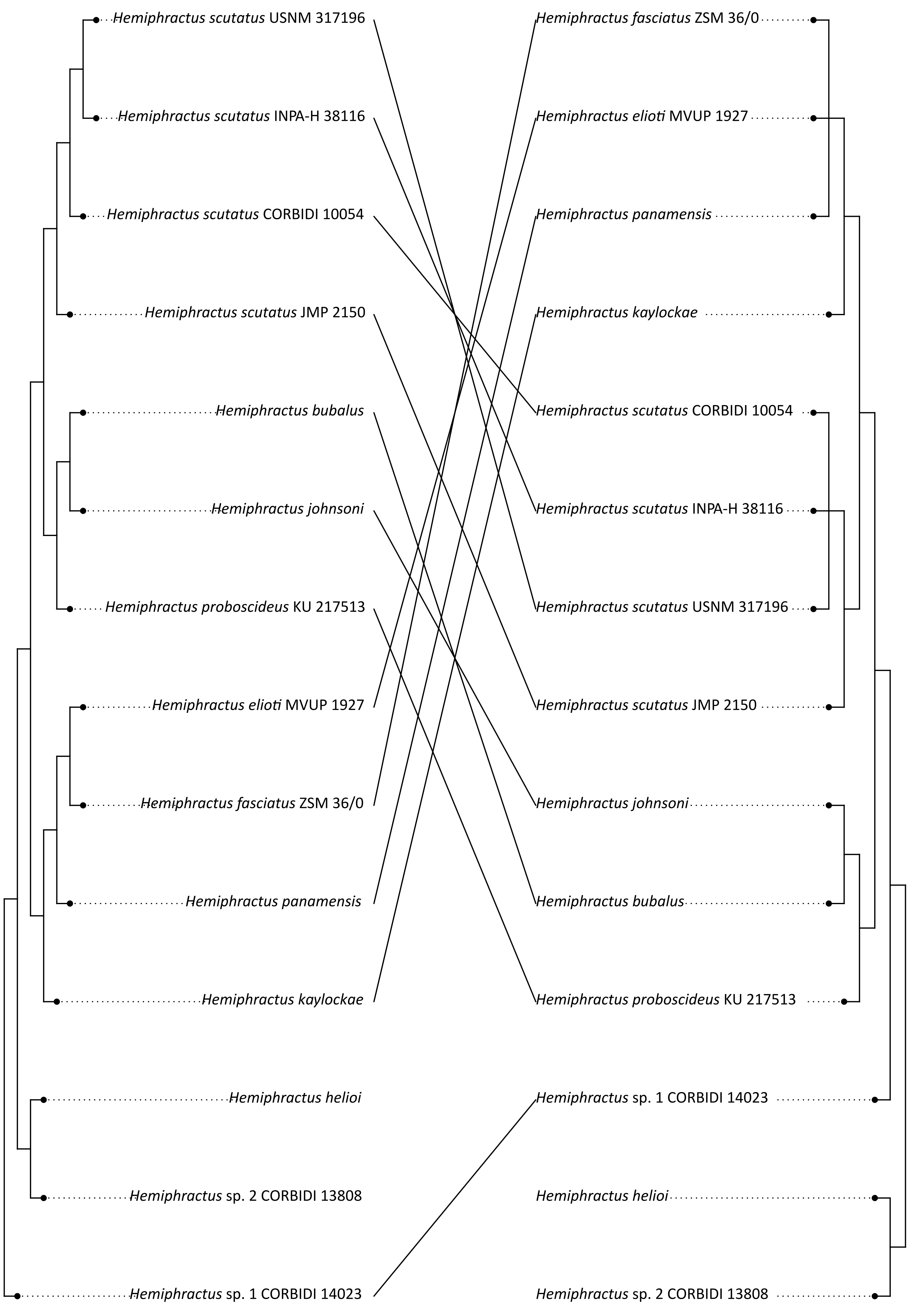


Figure S15 (Continuation). Comparison between SALm (left) and SAP5th (right) within *Stefania*.

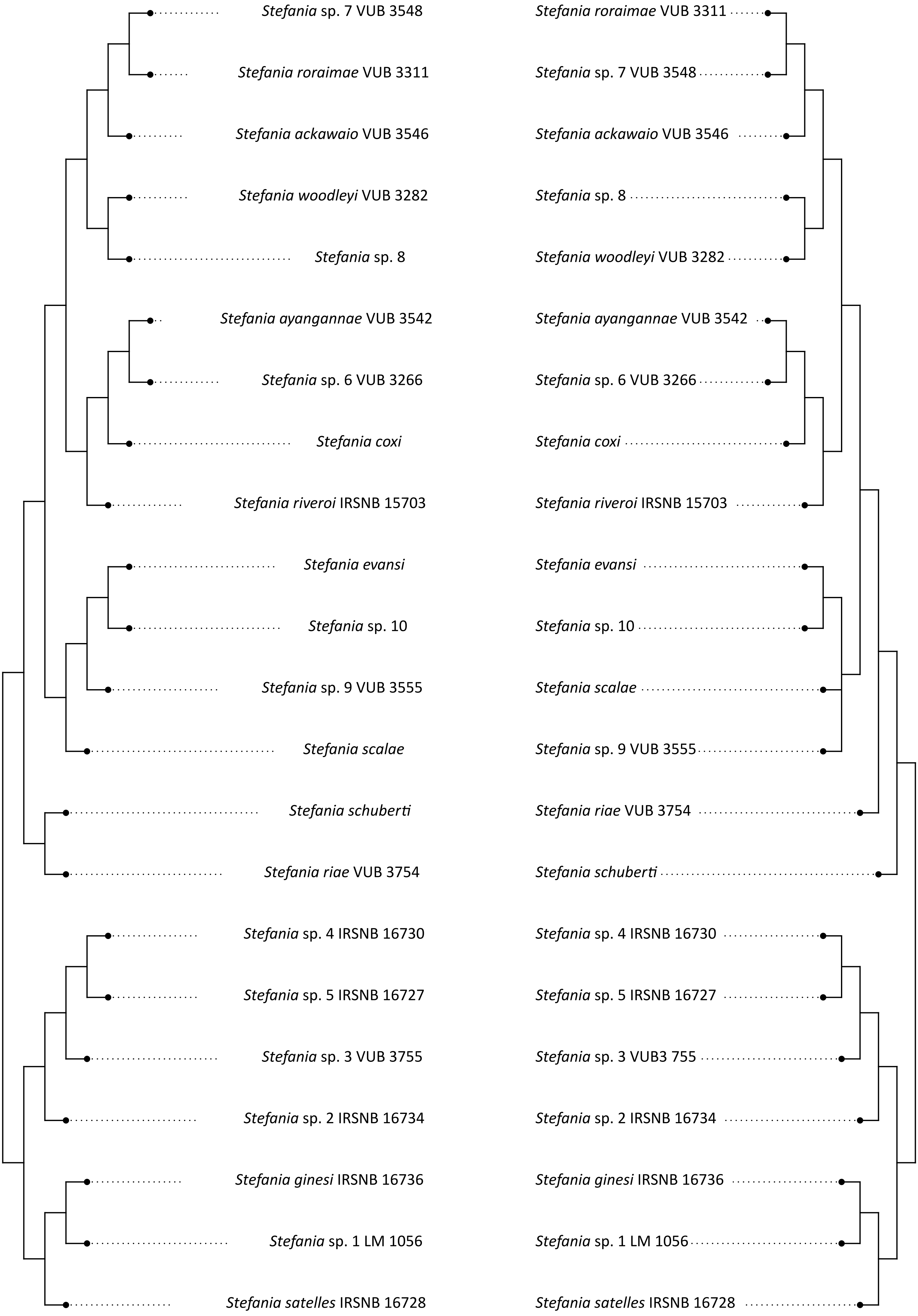


Figure S16. Comparison between SAPg (left) and SAPm (right) within *Fritziana*.

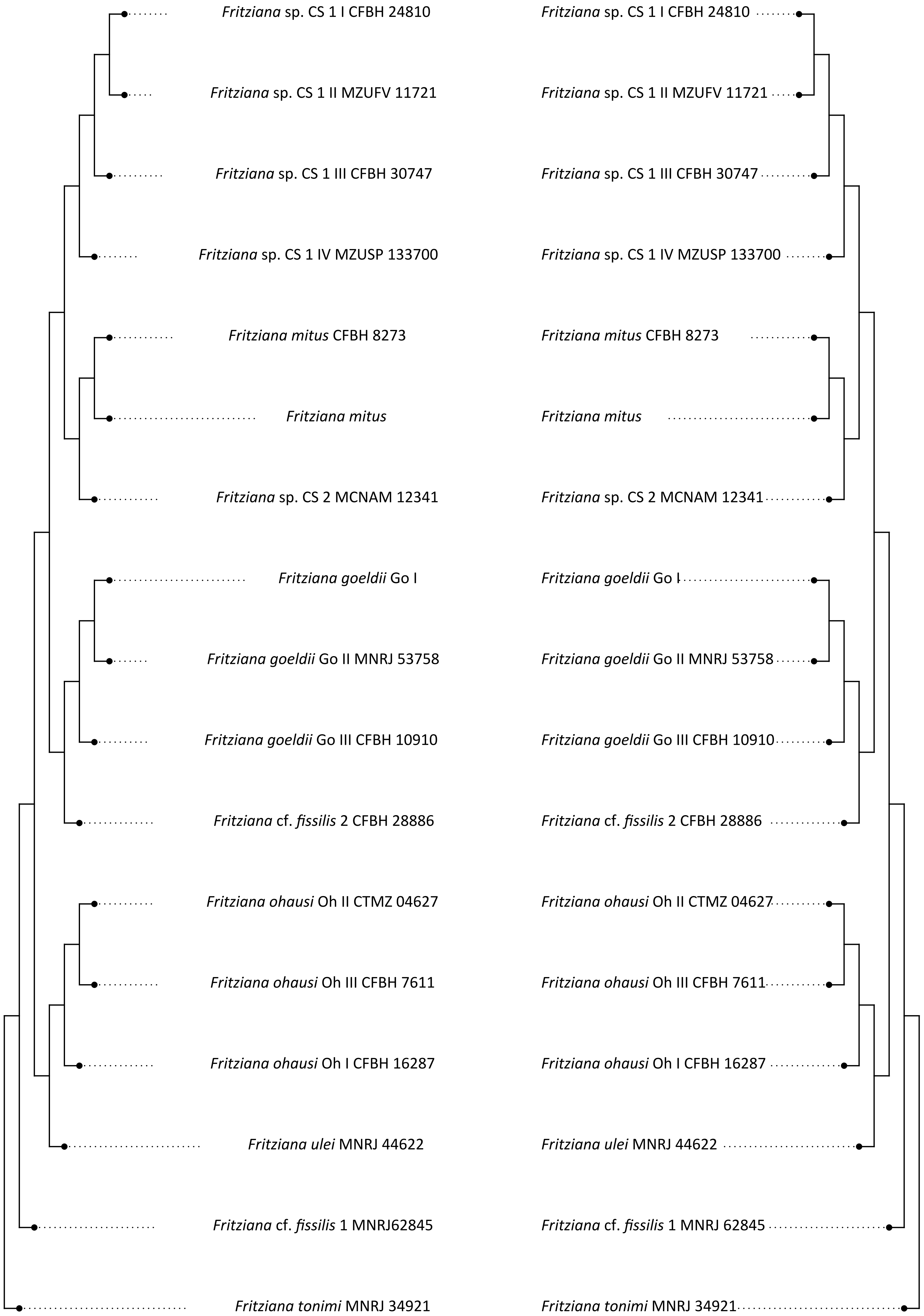


Figure S16 (Continuation). Comparison between SAPg (left) and SAPm (right) within *Gastrotheca*.

Figure S16 (Continuation). Comparison between SAPg (left) and SAPm (right) within *Hemiphractus*.

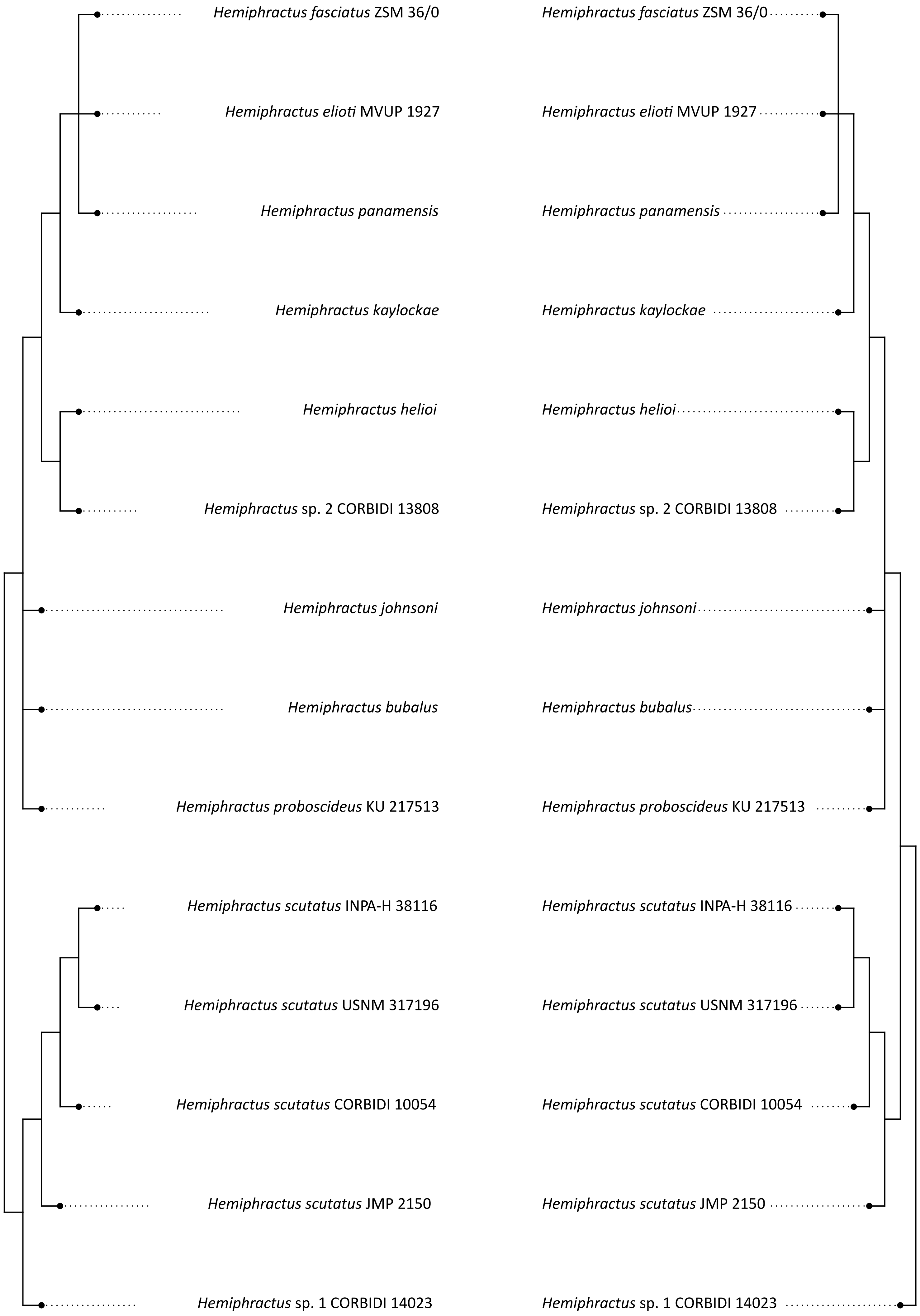


Figure S16 (Continuation). Comparison between SAPg (left) and SAPm (right) within *Stefania*.

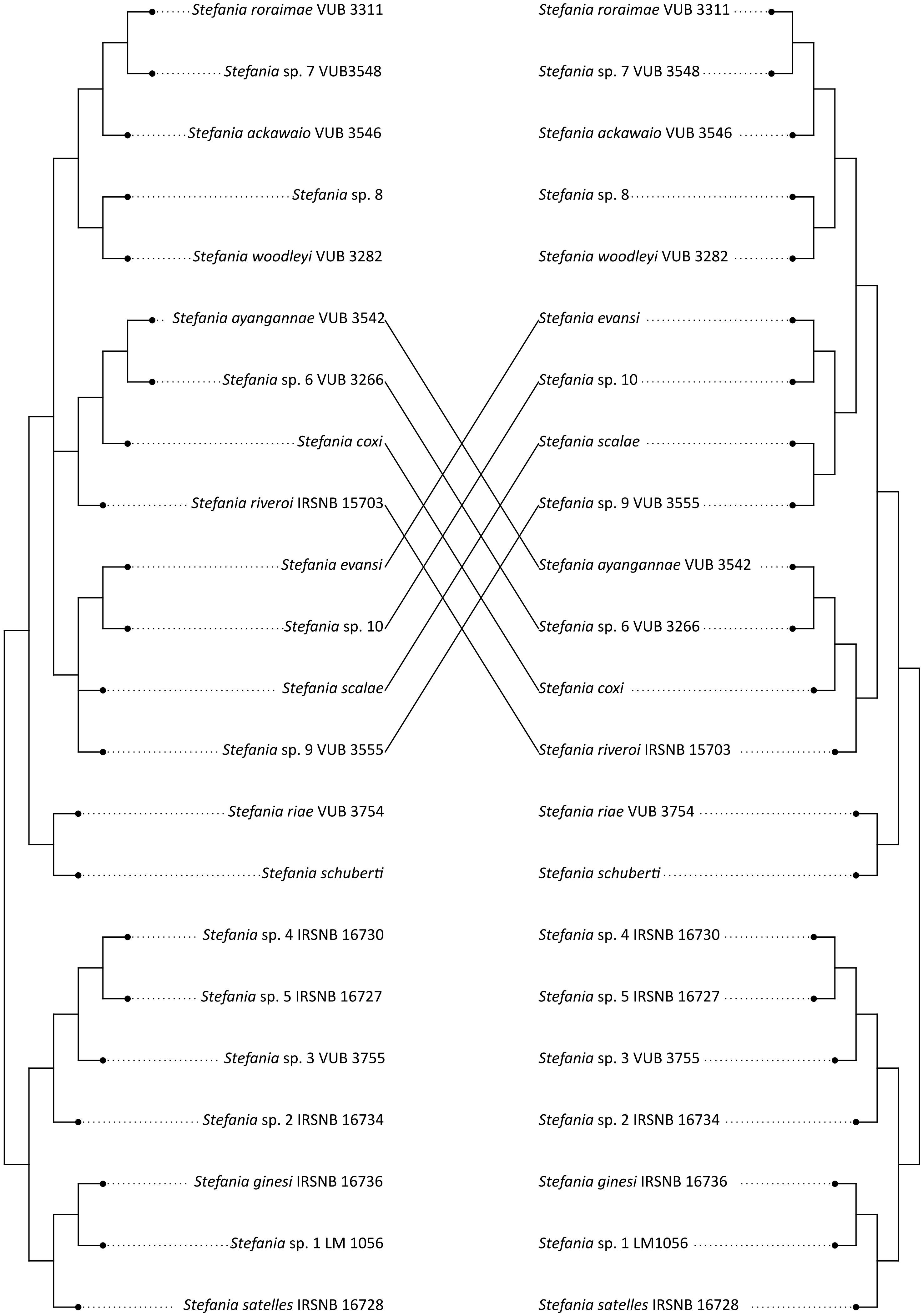


Figure S17. Comparison between SALg (left) and SAPg (right) within *Fritziana*.

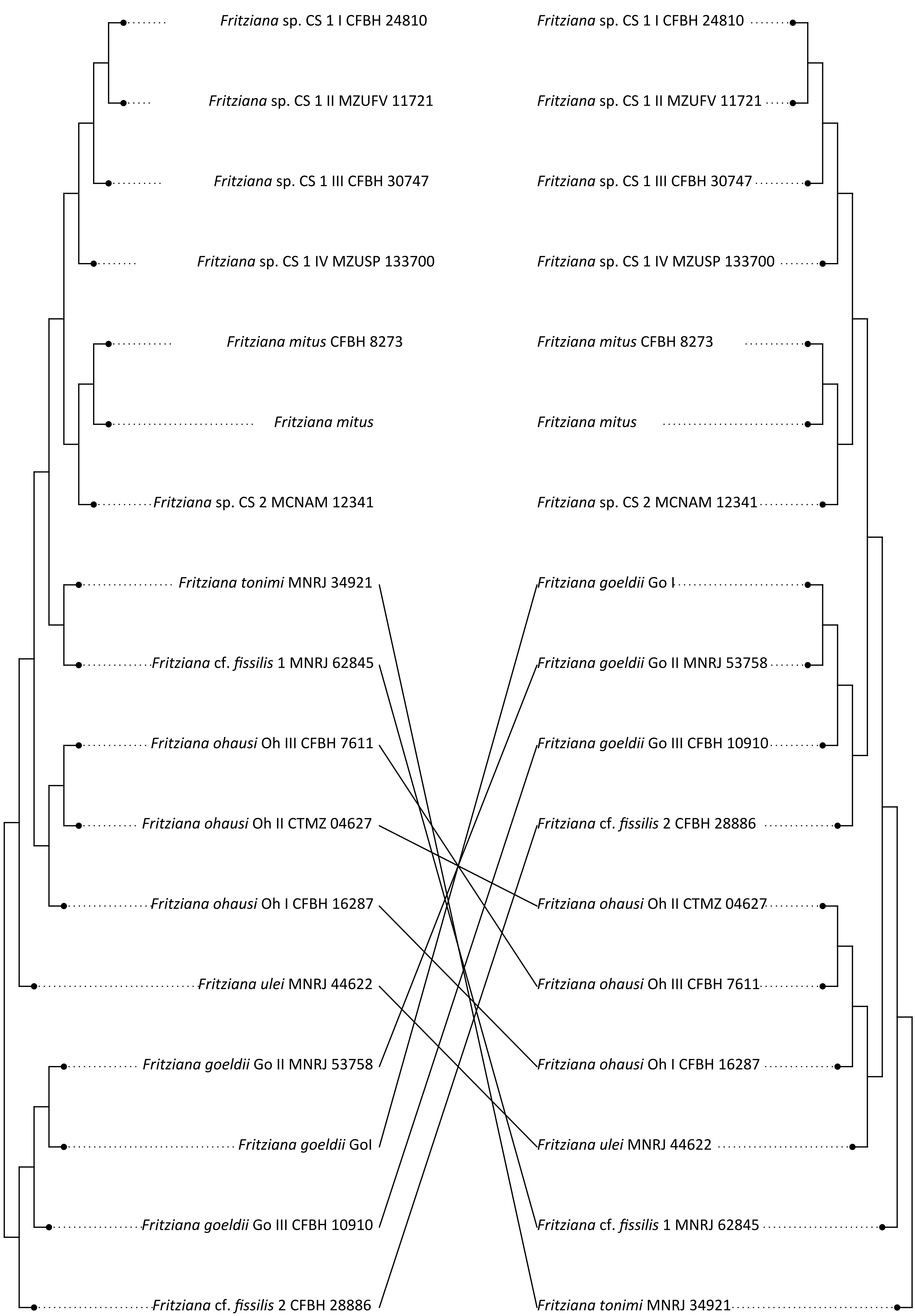


Figure S17 (Continuation). Comparison between SALg (left) and SAPg (right) within *Gastrotheca*.

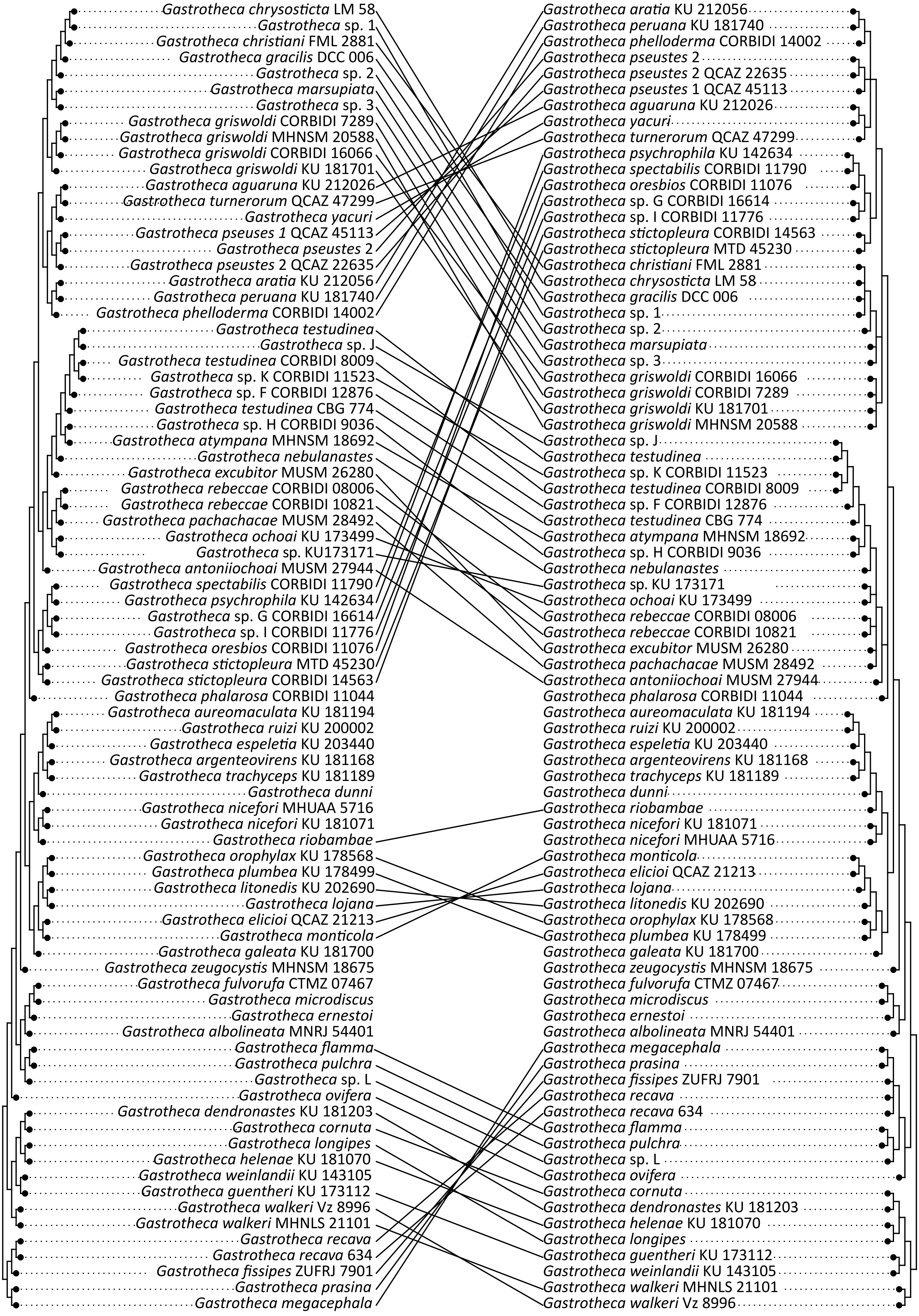


Figure S17 (Continuation). Comparison between SALg (left) and SAPg (right) within *Hemiphractus*.

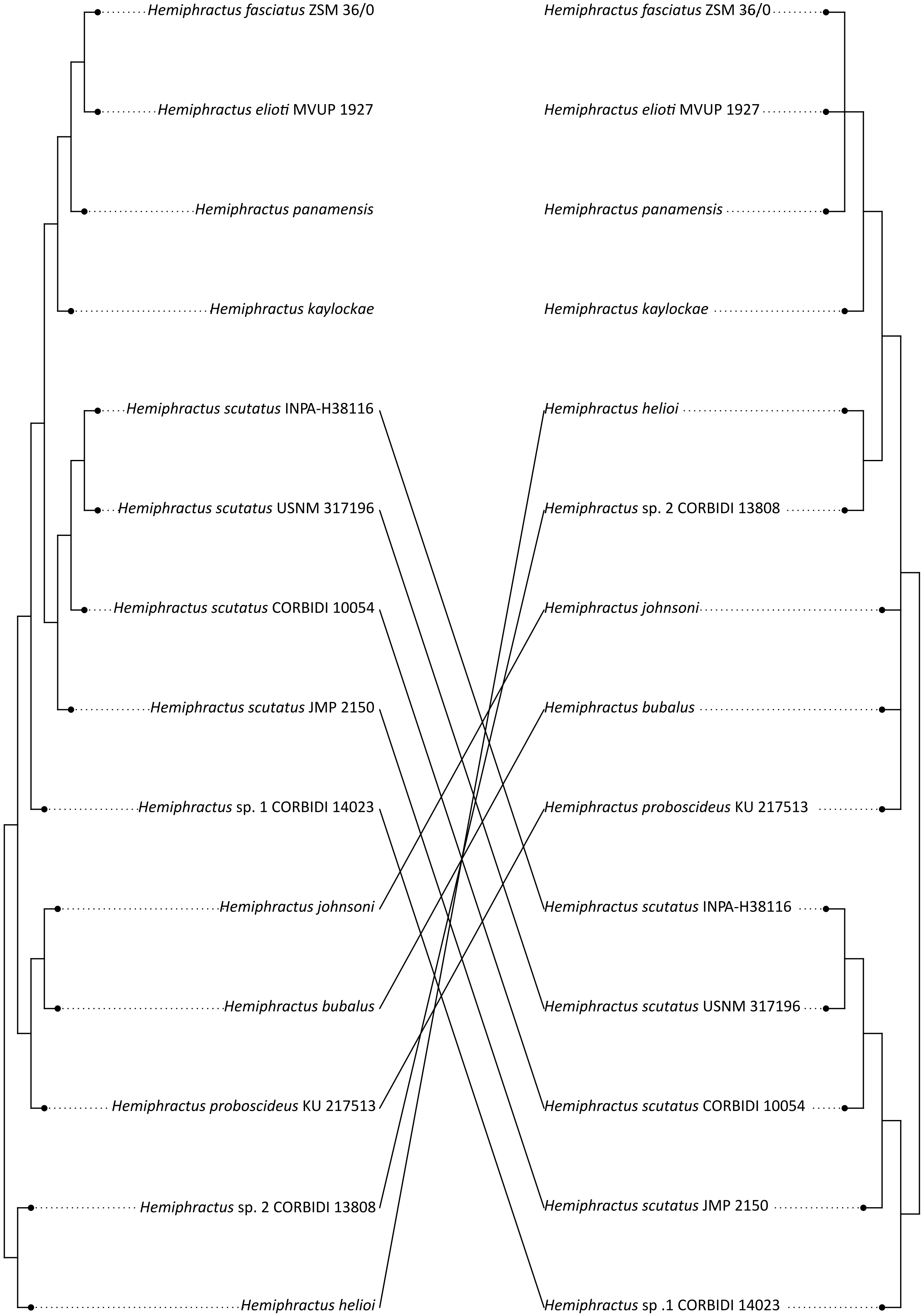


Figure S17 (Continuation). Comparison between SALg (left) and SAPg (right) within *Stefania*.

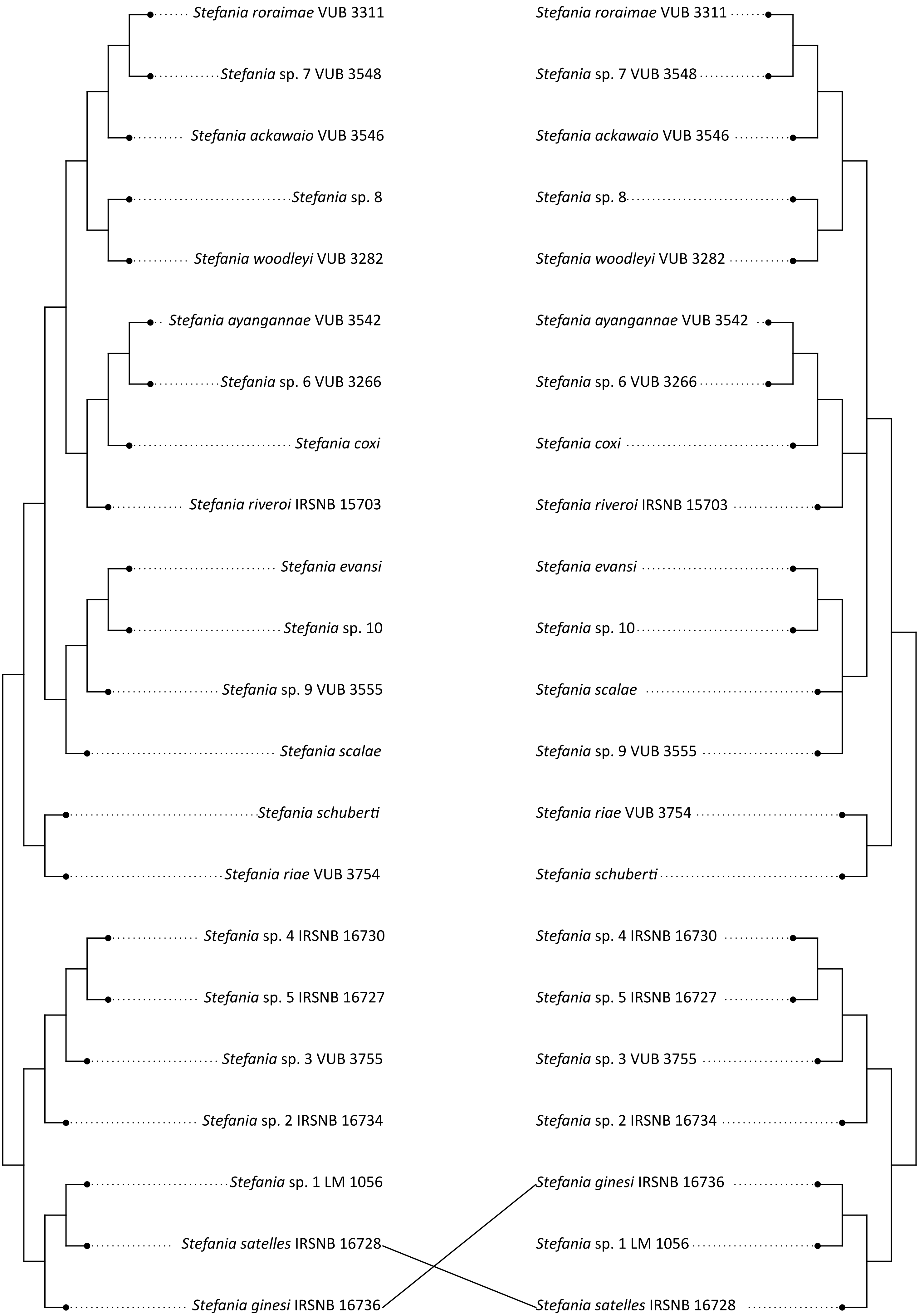


Figure S18. Comparison between SALm (left) and SAPg (right) within *Fritziana*.

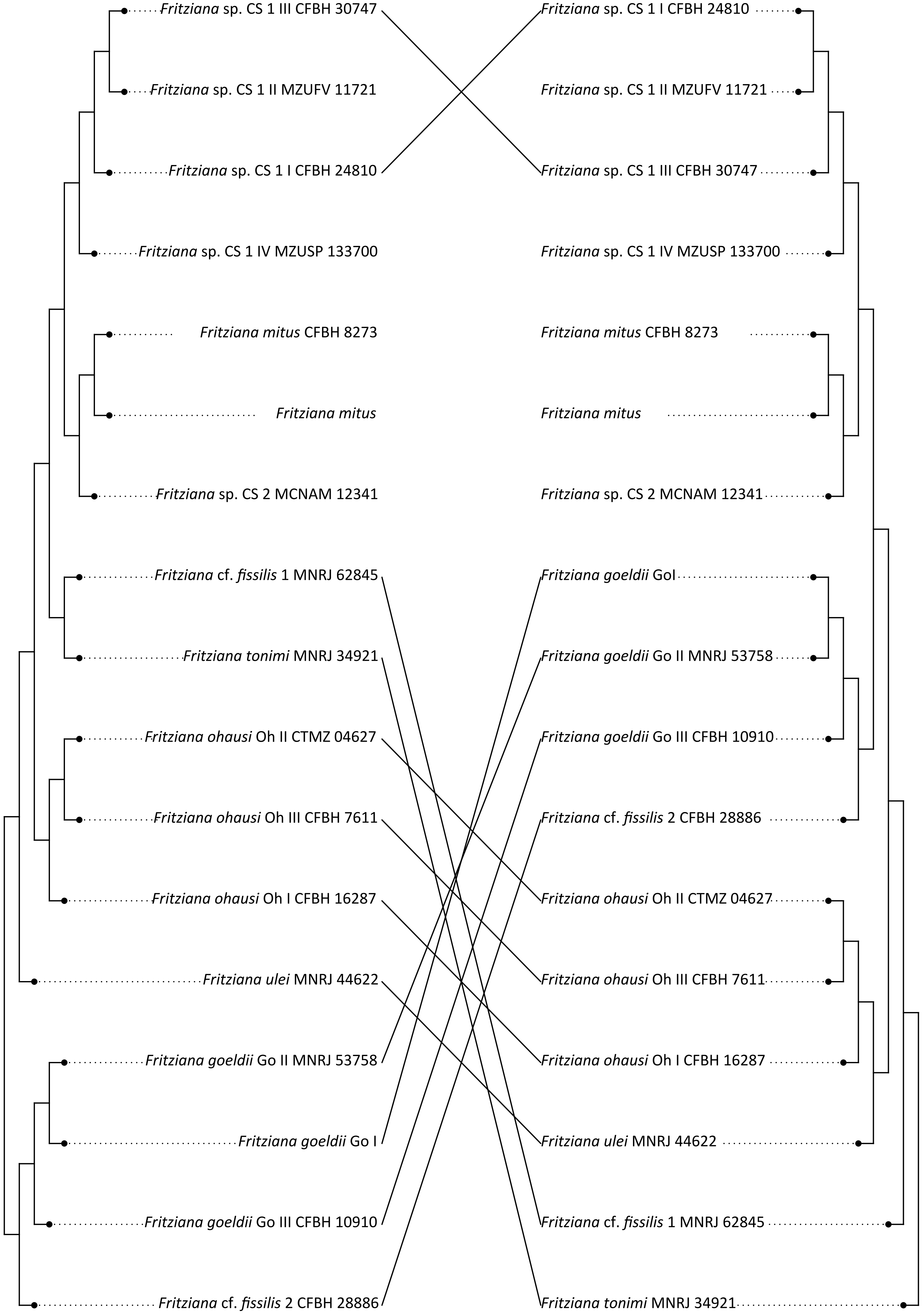


Figure S18 (Continuation). Comparison between SALm (left) and SAPg (right) within *Gastrotheca.*

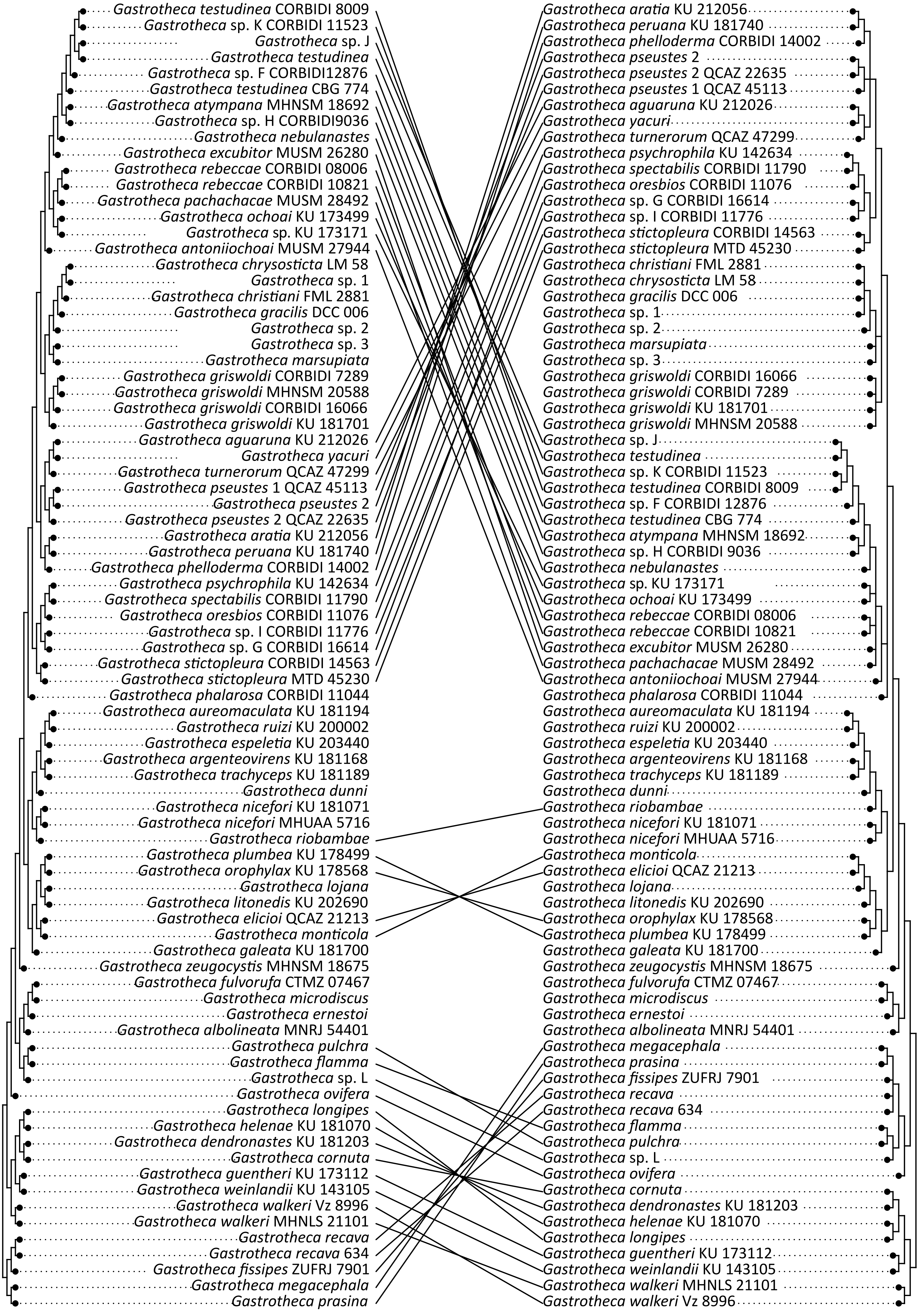


Figure S18 (Continuation). Comparison between SALm (left) and SAPg (right) within *Hemiphractus*

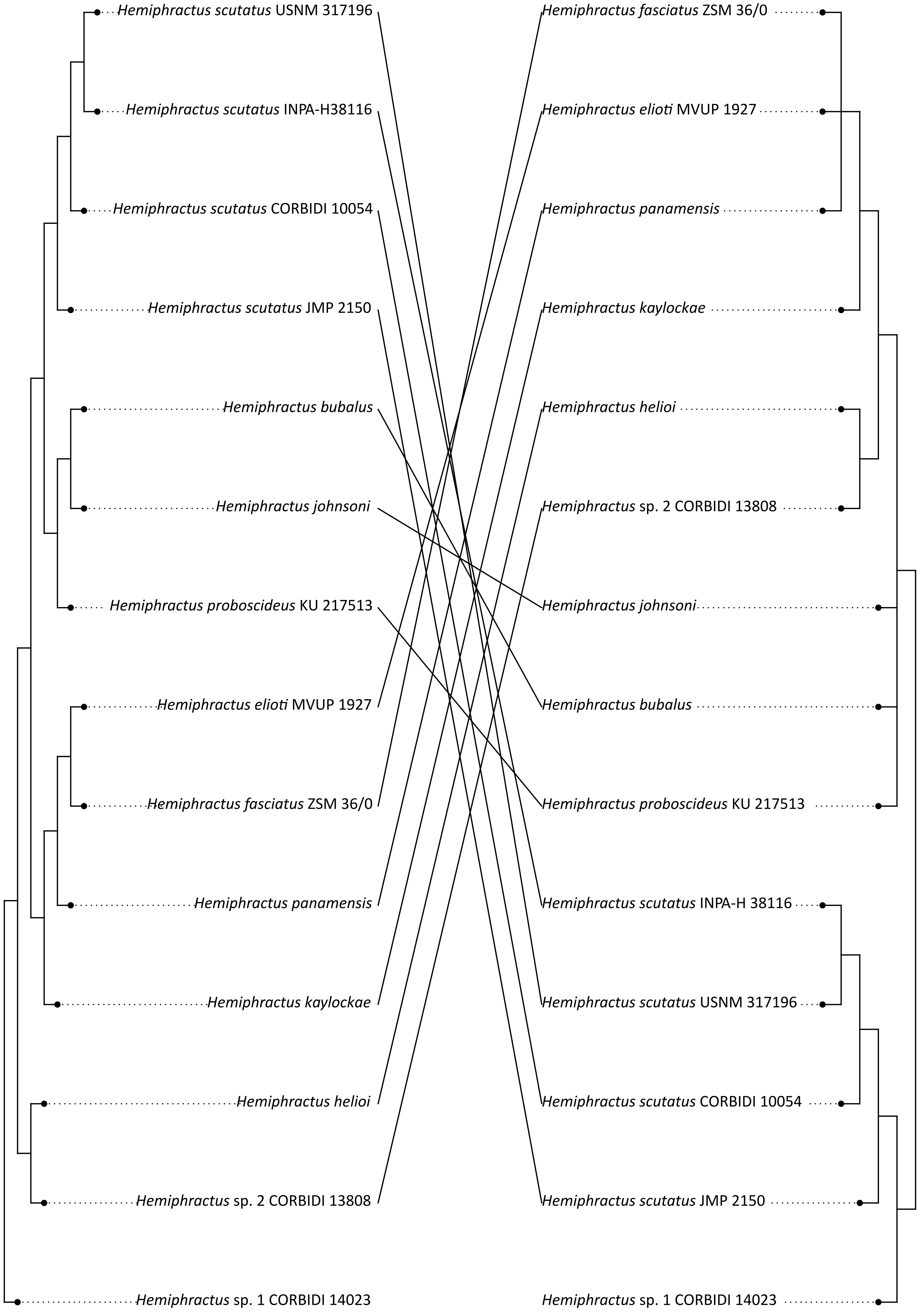


Figure S18 (Continuation). Comparison between SALm (left) and SAPg (right) within *Stefania*.

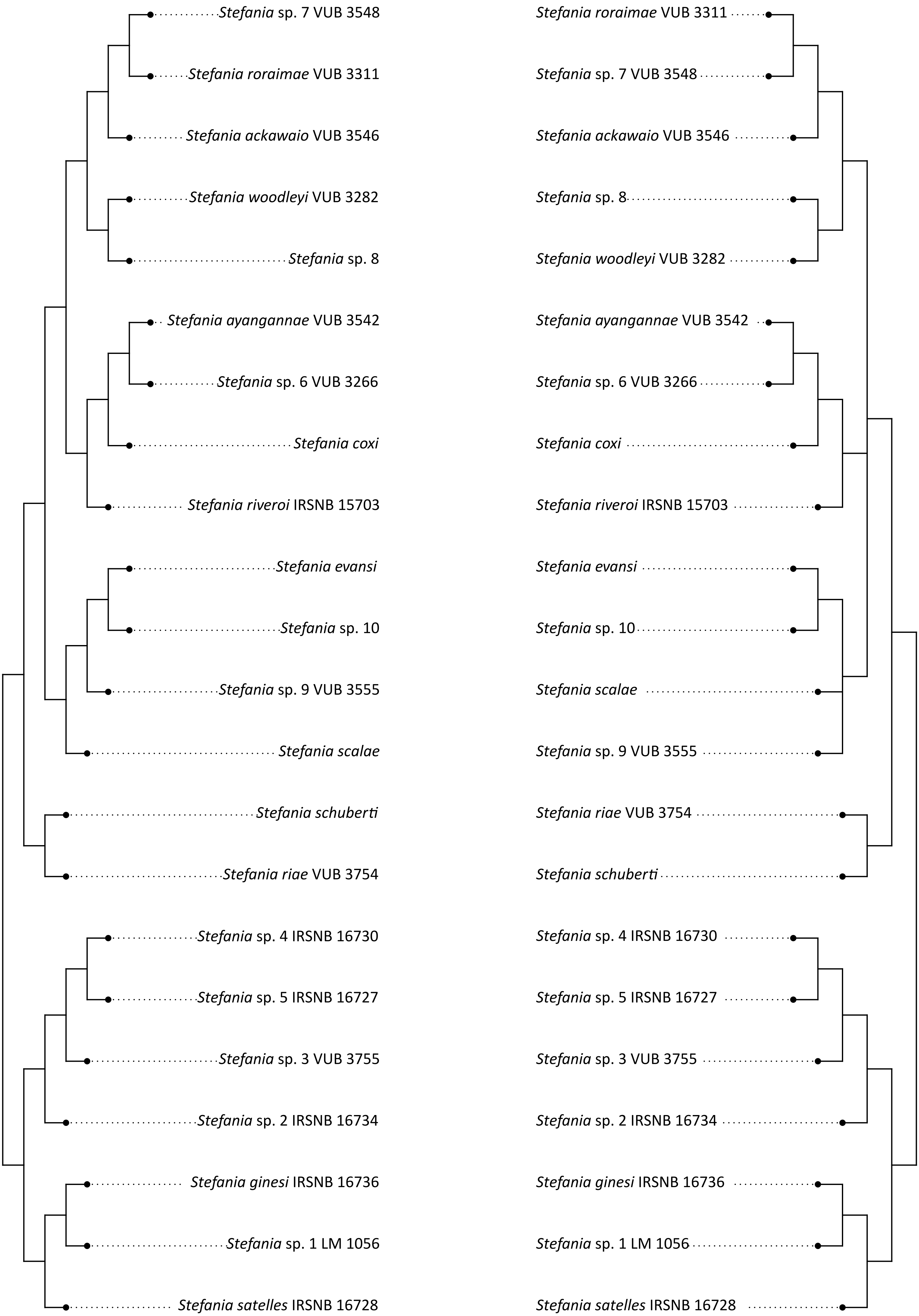


Figure S19. Comparison between SALg (left) and SAPm (right) within *Fritziana*.

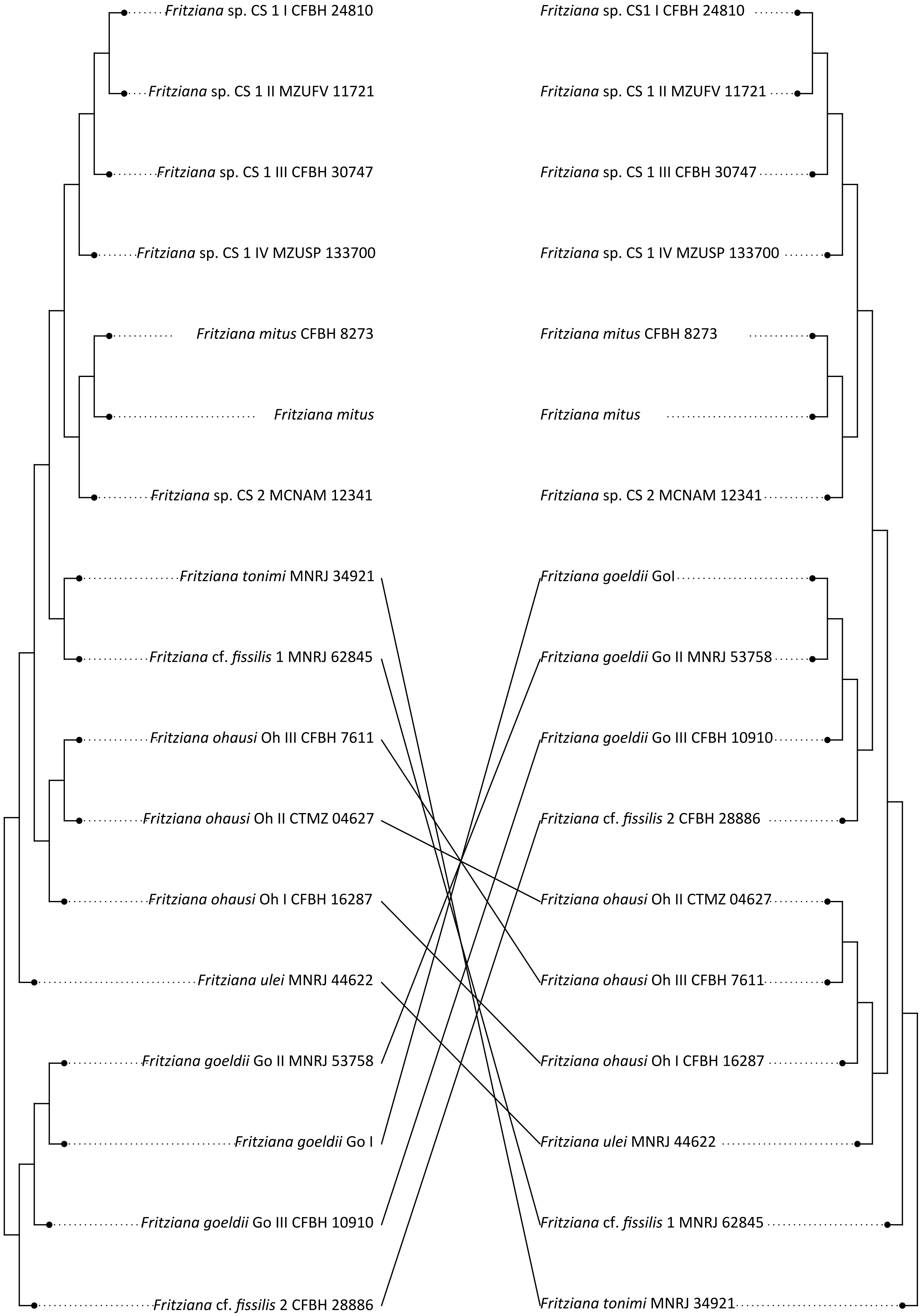


Figure S19 (Continuation). Comparison between SALg (left) and SAPm (right) within *Gastrotheca*.

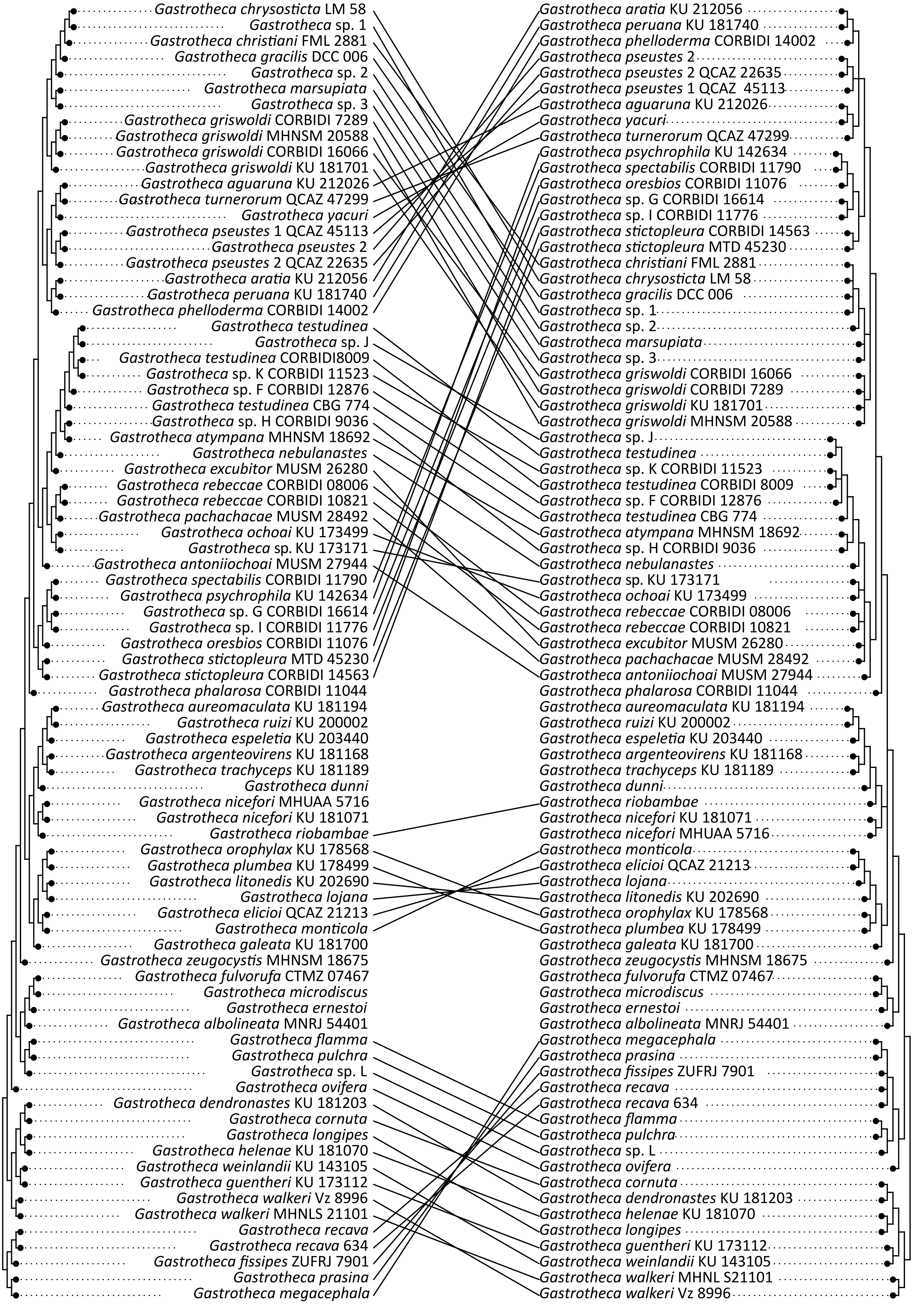


Figure S19 (Continuation). Comparison between SALg (left) and SAPm (right) within *Hemiphractus*.

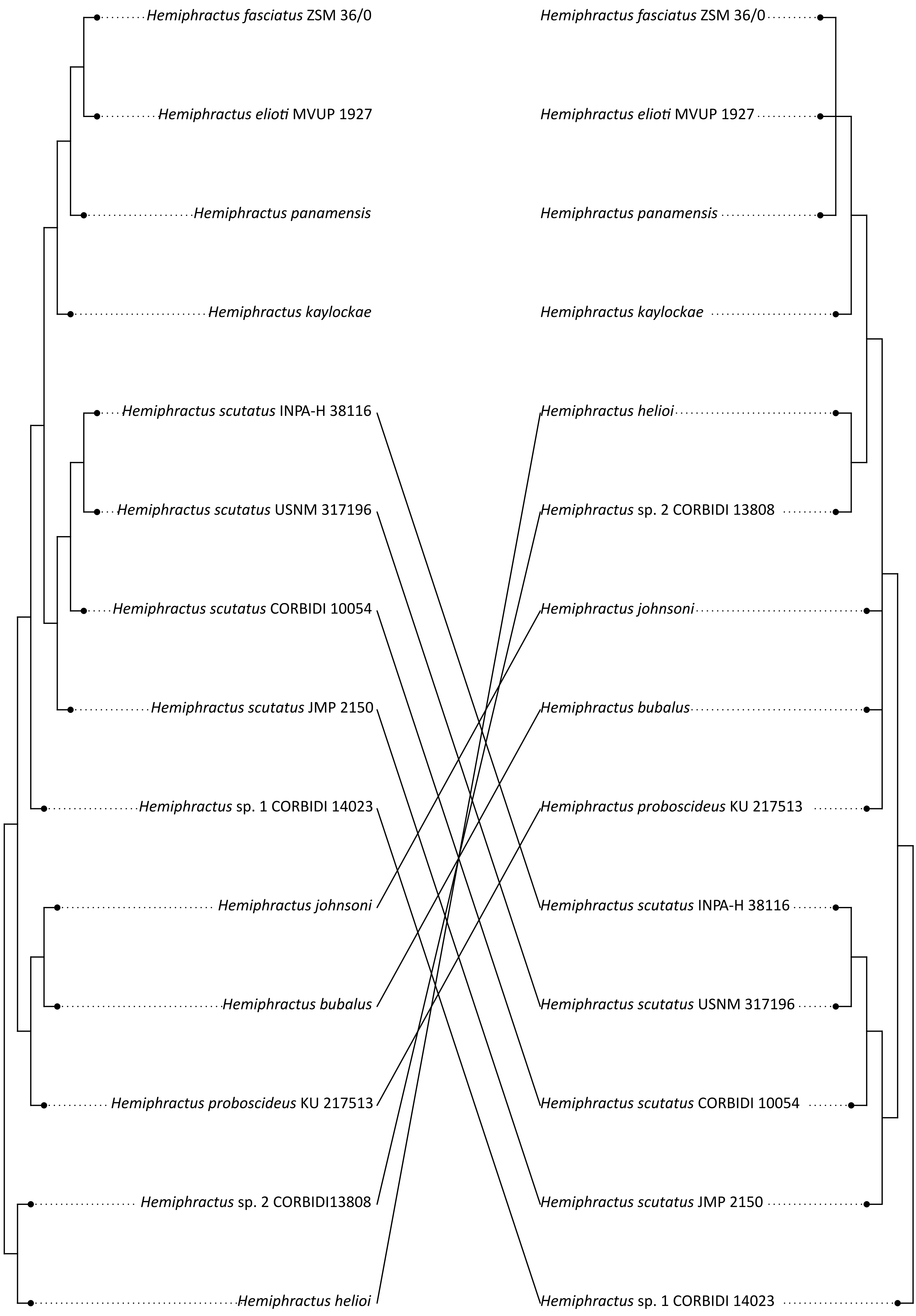


Figure S19 (Continuation). Comparison between SALg (left) and SAPm (right) within *Stefania*.

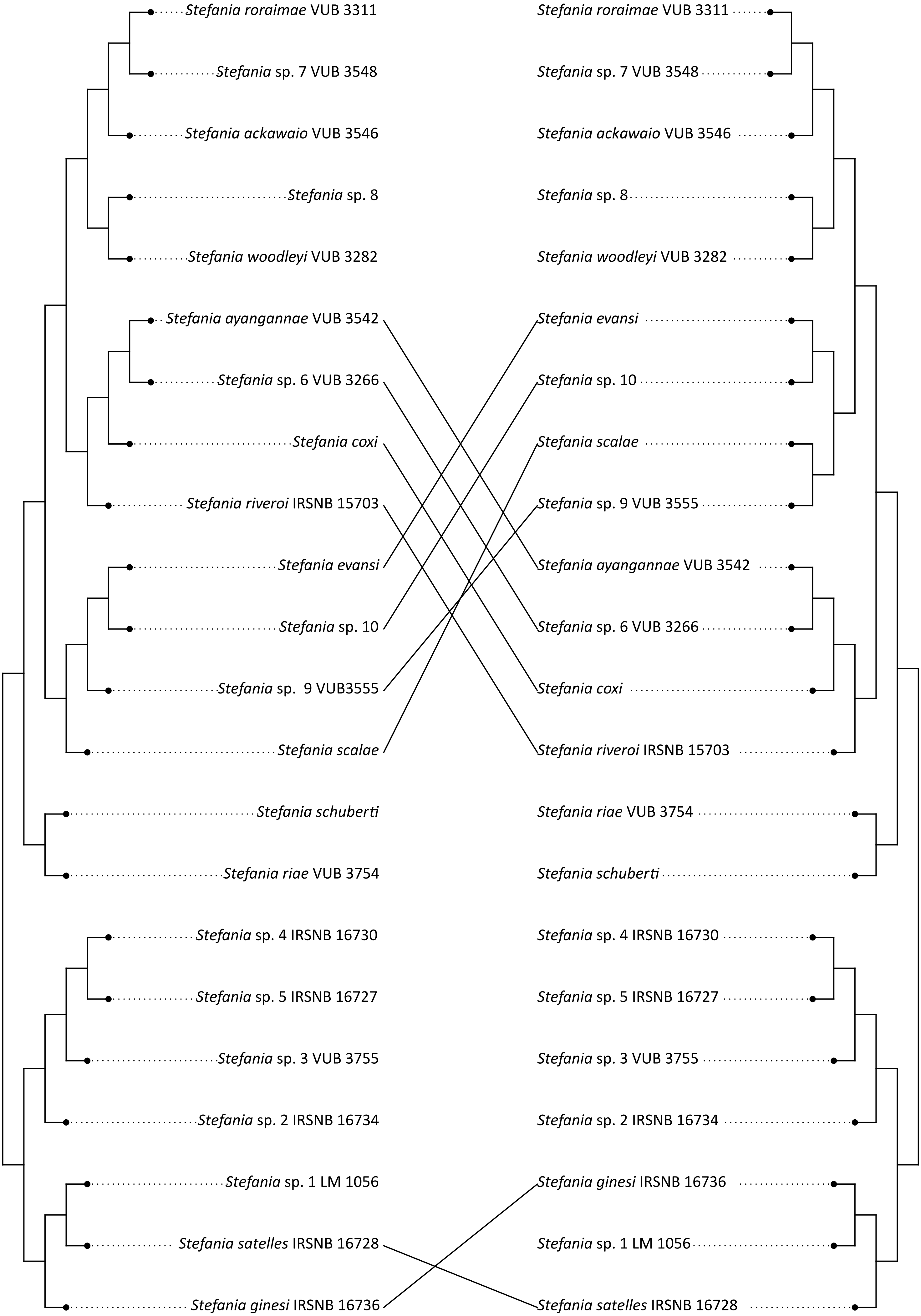


Figure S20. Comparison between SALm (left) and SAPm (right) within *Fritziana*.

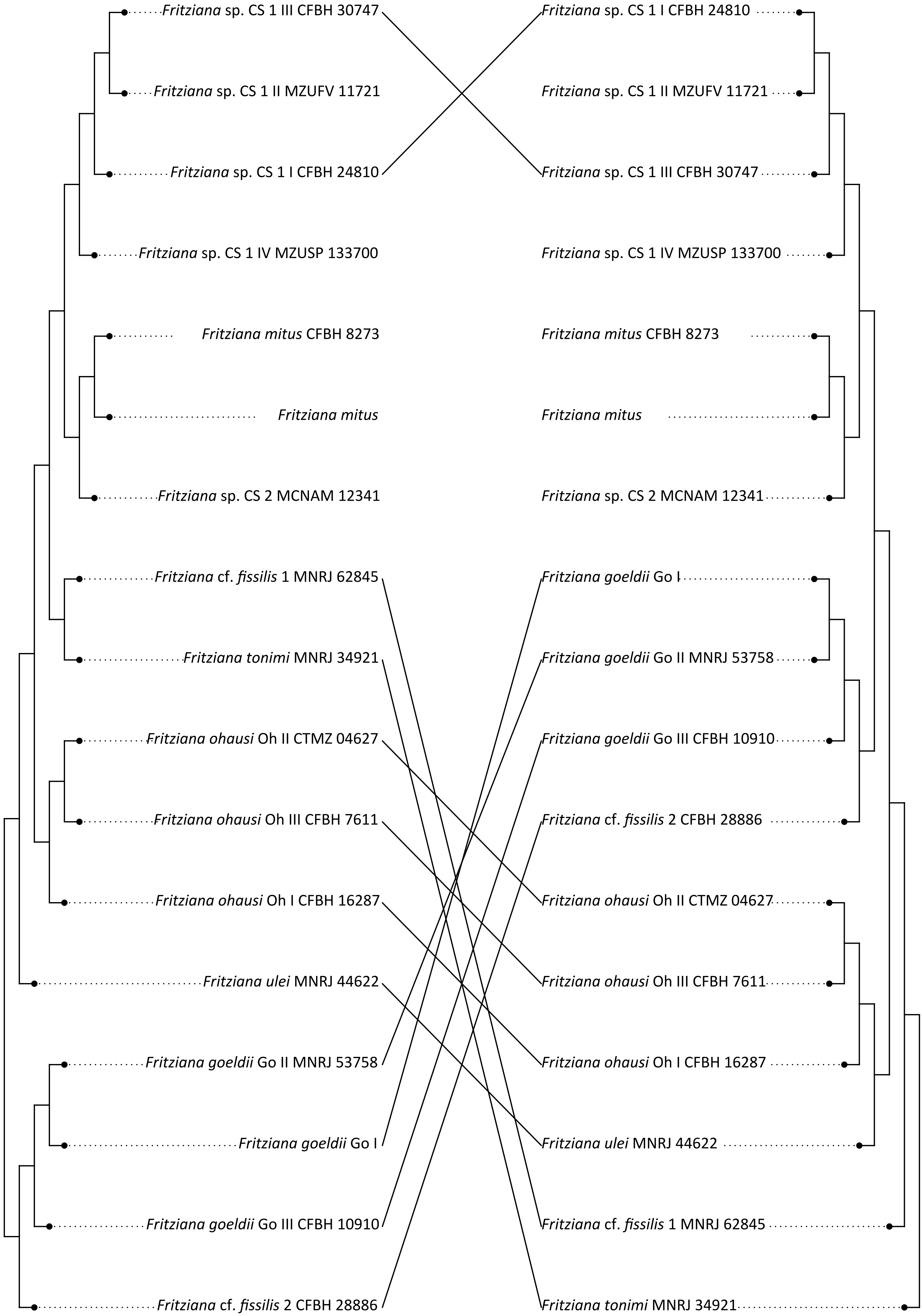


Figure S20 (Continuation). Comparison between SALm (left) and SAPm (right) within *Gastrotheca*.

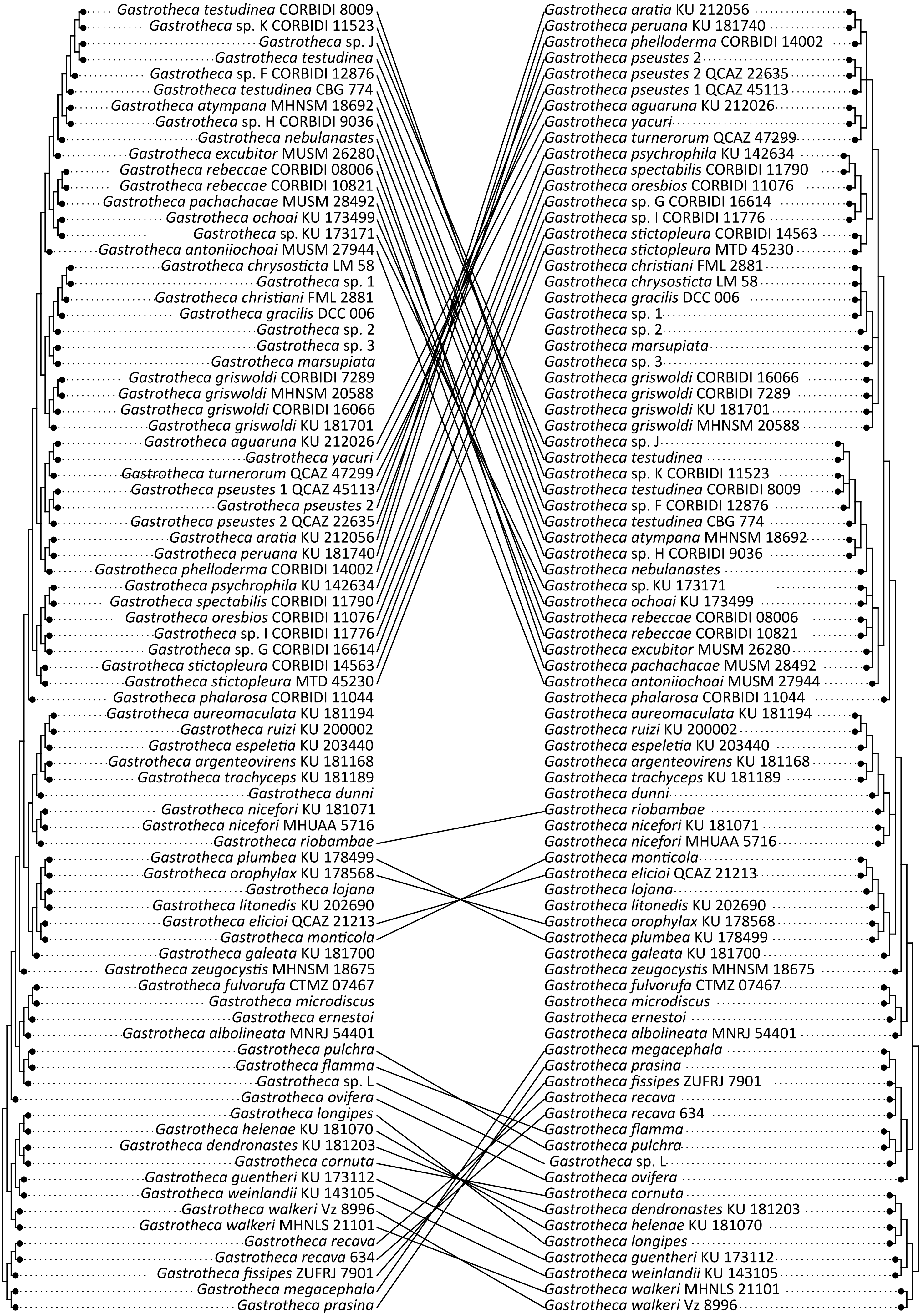


Figure S20 (Continuation). Comparison between SALm (left) and SAPm (right) within *Hemiphractus*.

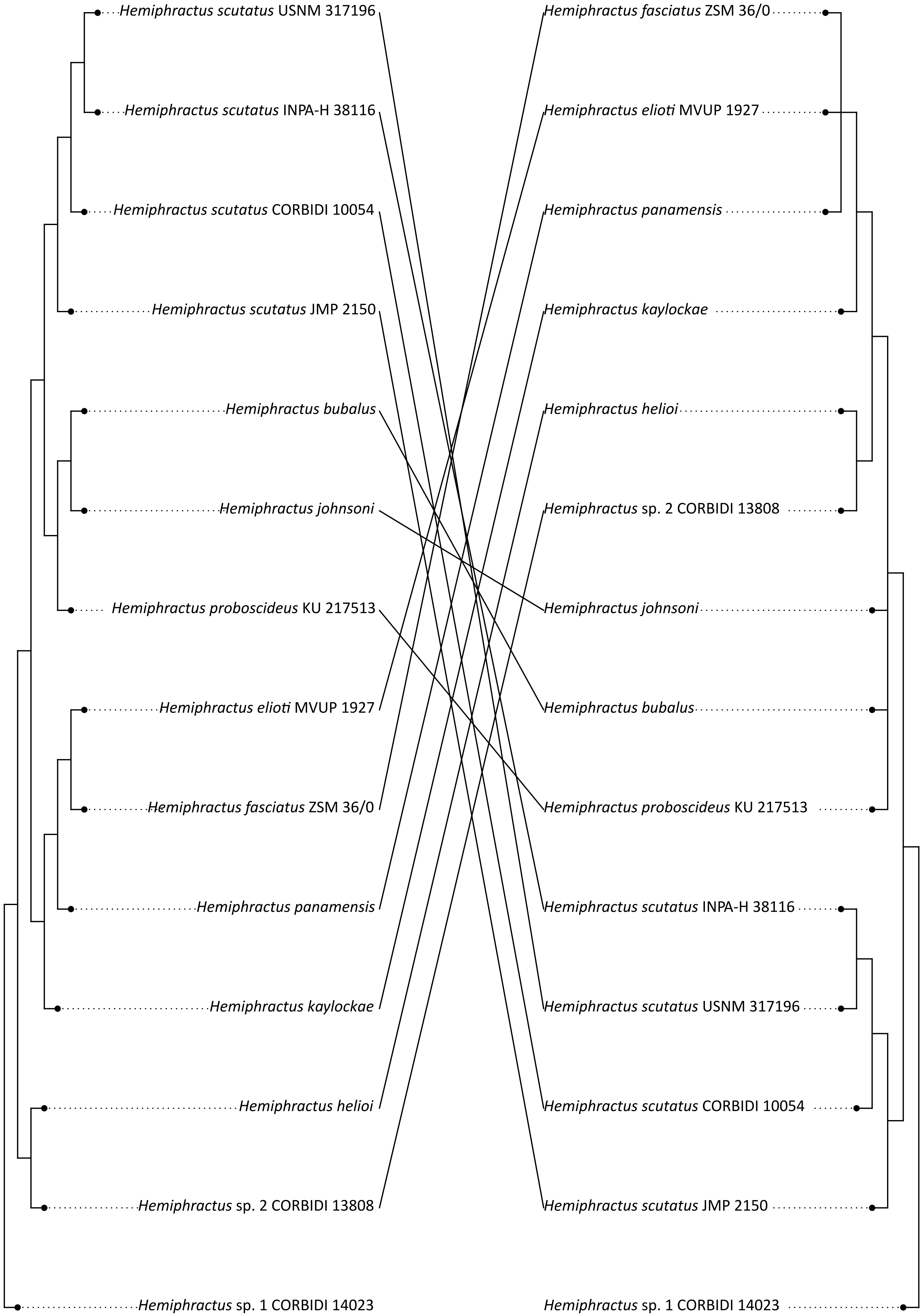


Figure S20 (Continuation). Comparison between SALm (left) and SAPm (right) within *Stefania*.

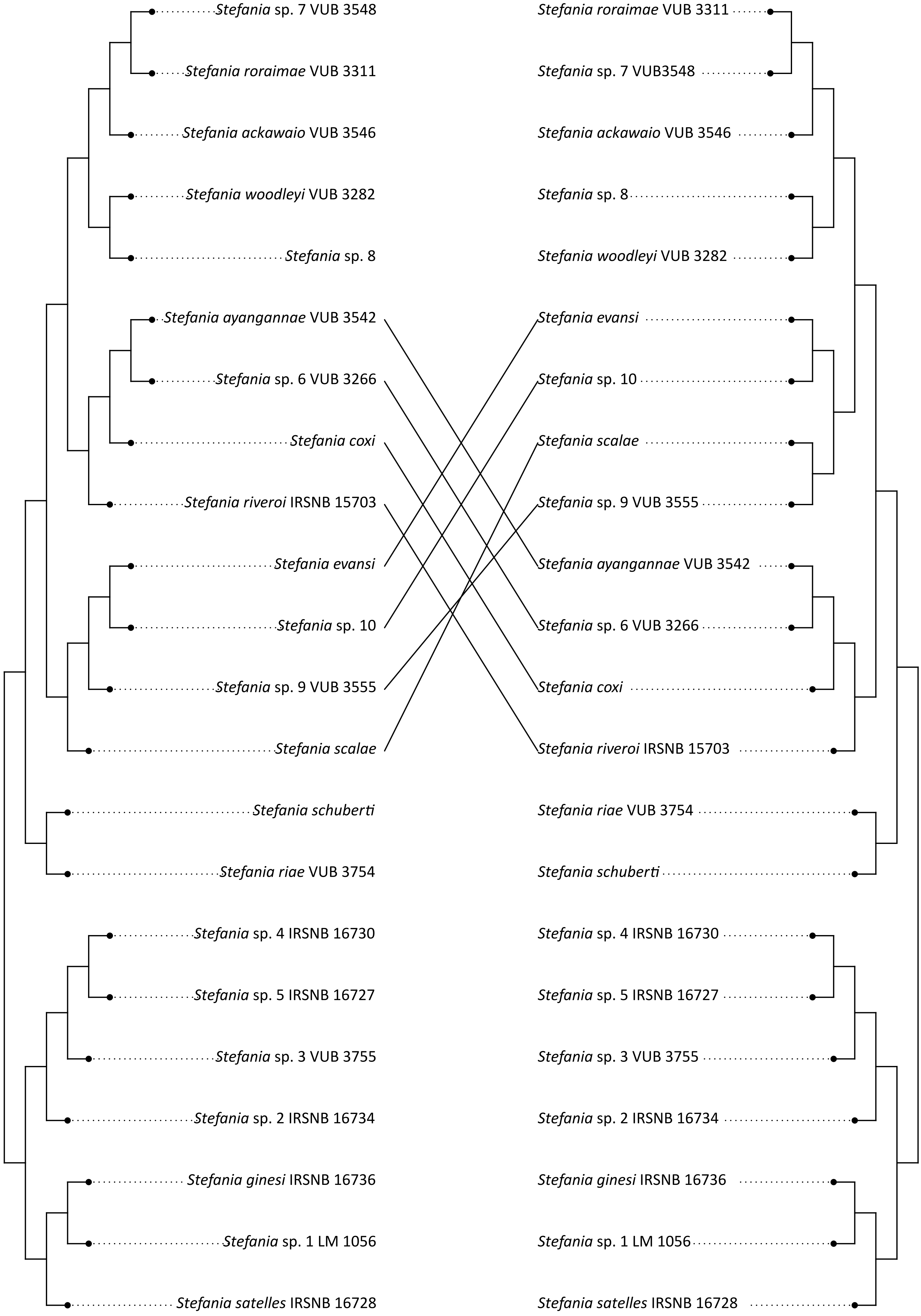


Figure S21. Comparisons between SALg (left) and SALm (right) optimal trees within *Cryptobatrachus*.

Figure S21 (Continuation). Comparisons between SALg (left) and SALm (right) optimal trees within *Fritziana.*

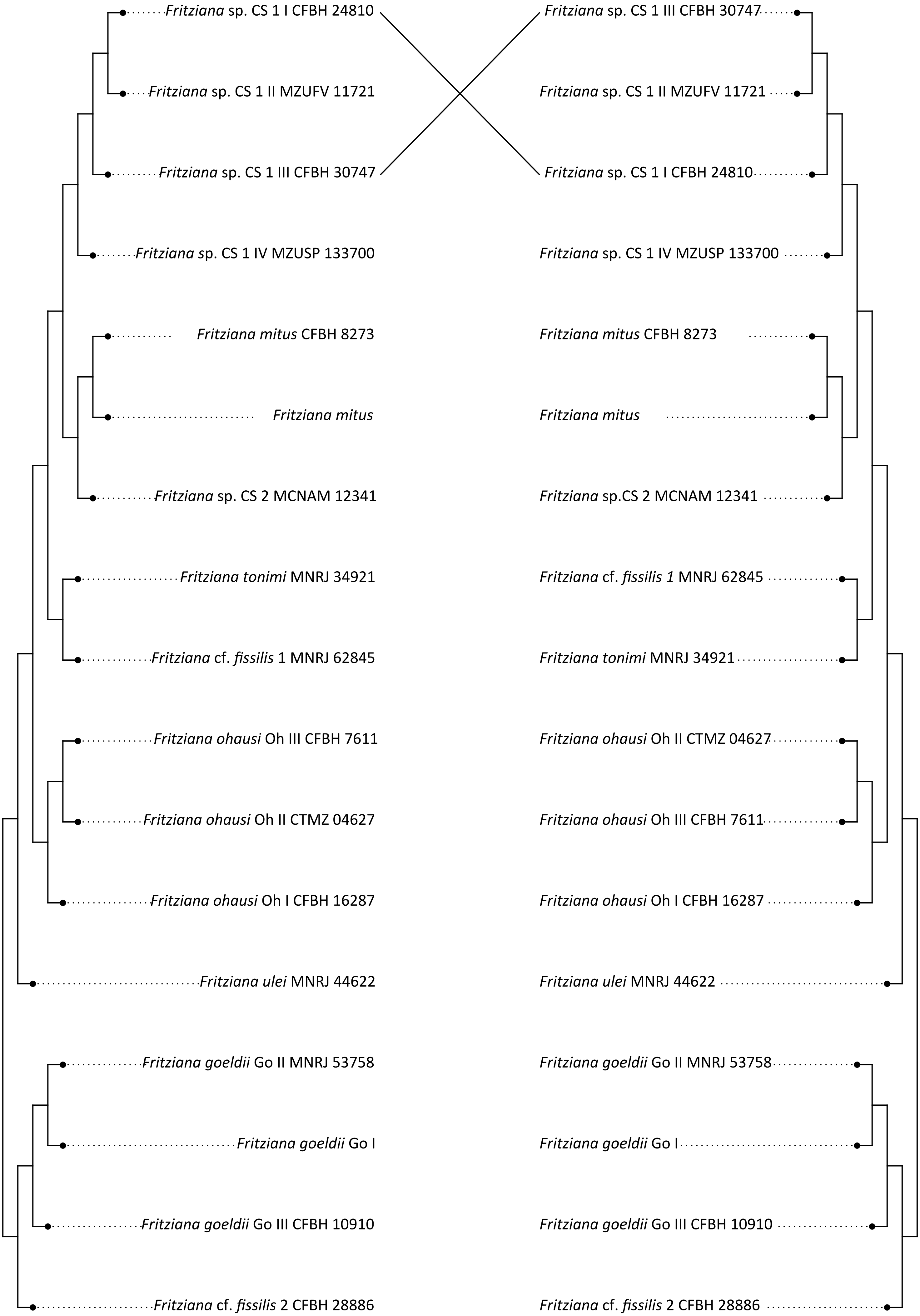


Figure S21 (Continuation). Comparisons between SALg (left) and SALm (right) optimal trees within *Gastrotheca.*

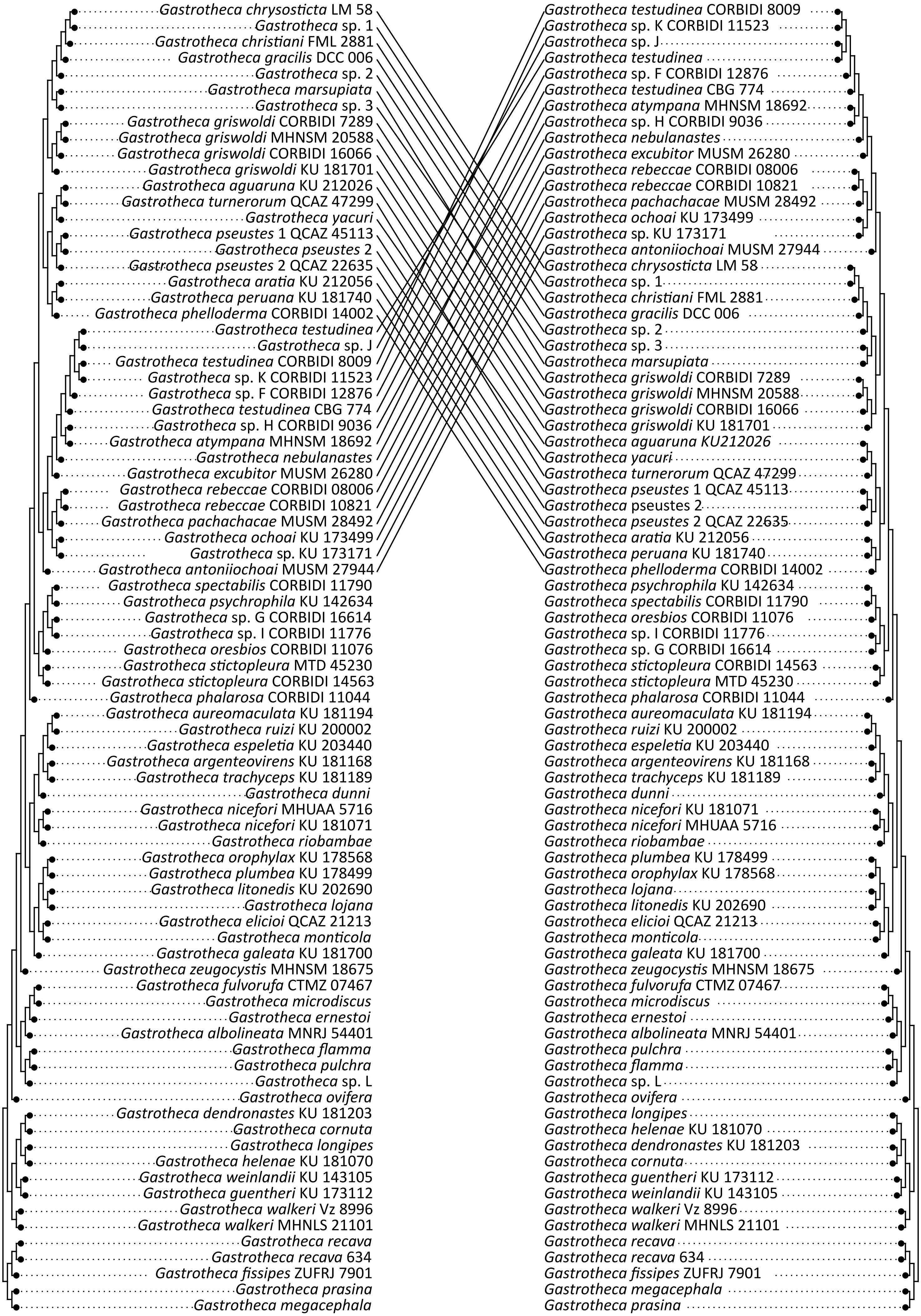


Figure S21 (Continuation). Comparisons between SALg (left) and SALm (right) optimal trees within *Hemiphractus.*

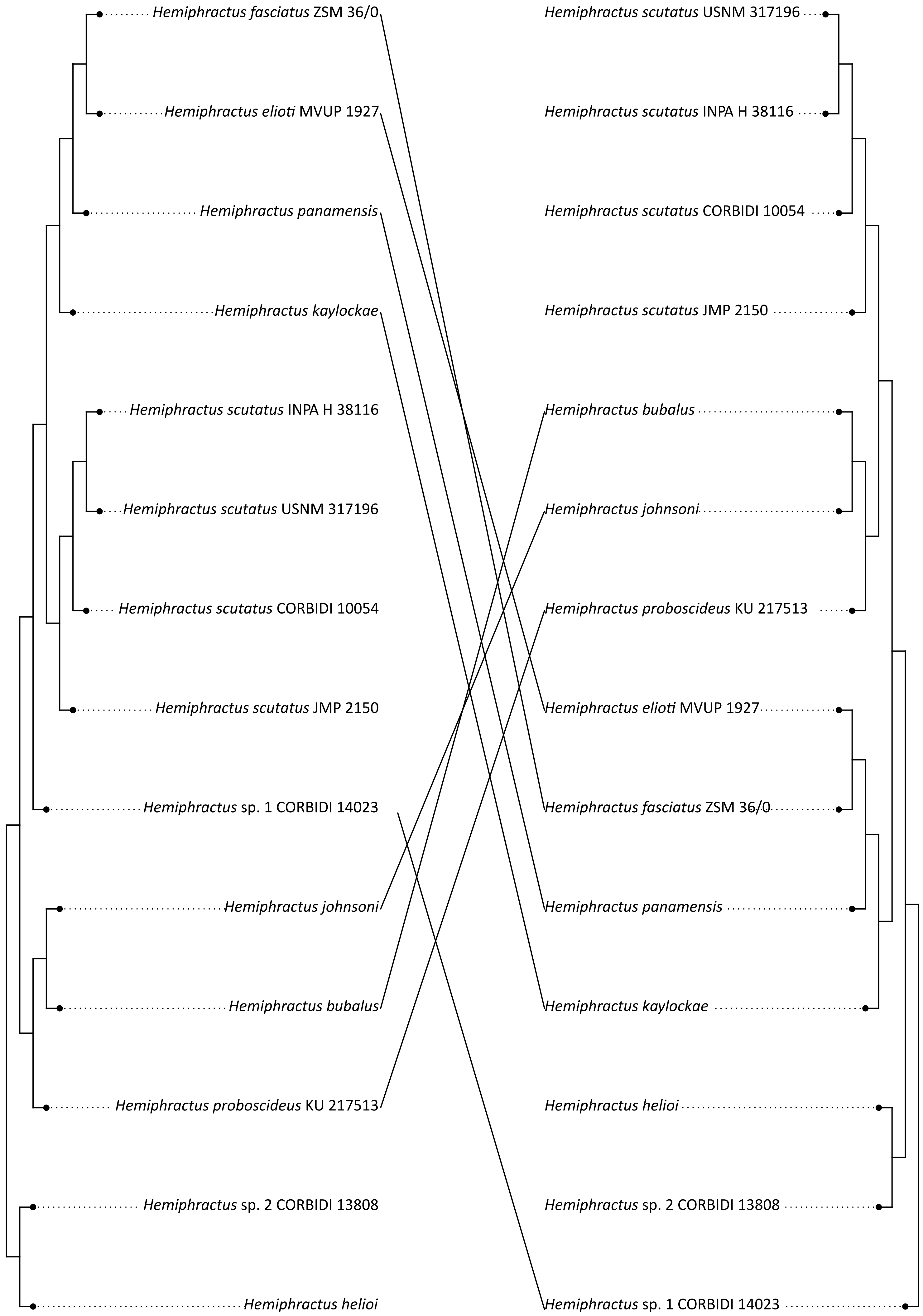


Figure S21 (Continuation). Comparisons between SALg (left) and SALm (right) optimal trees within *Stefania.*

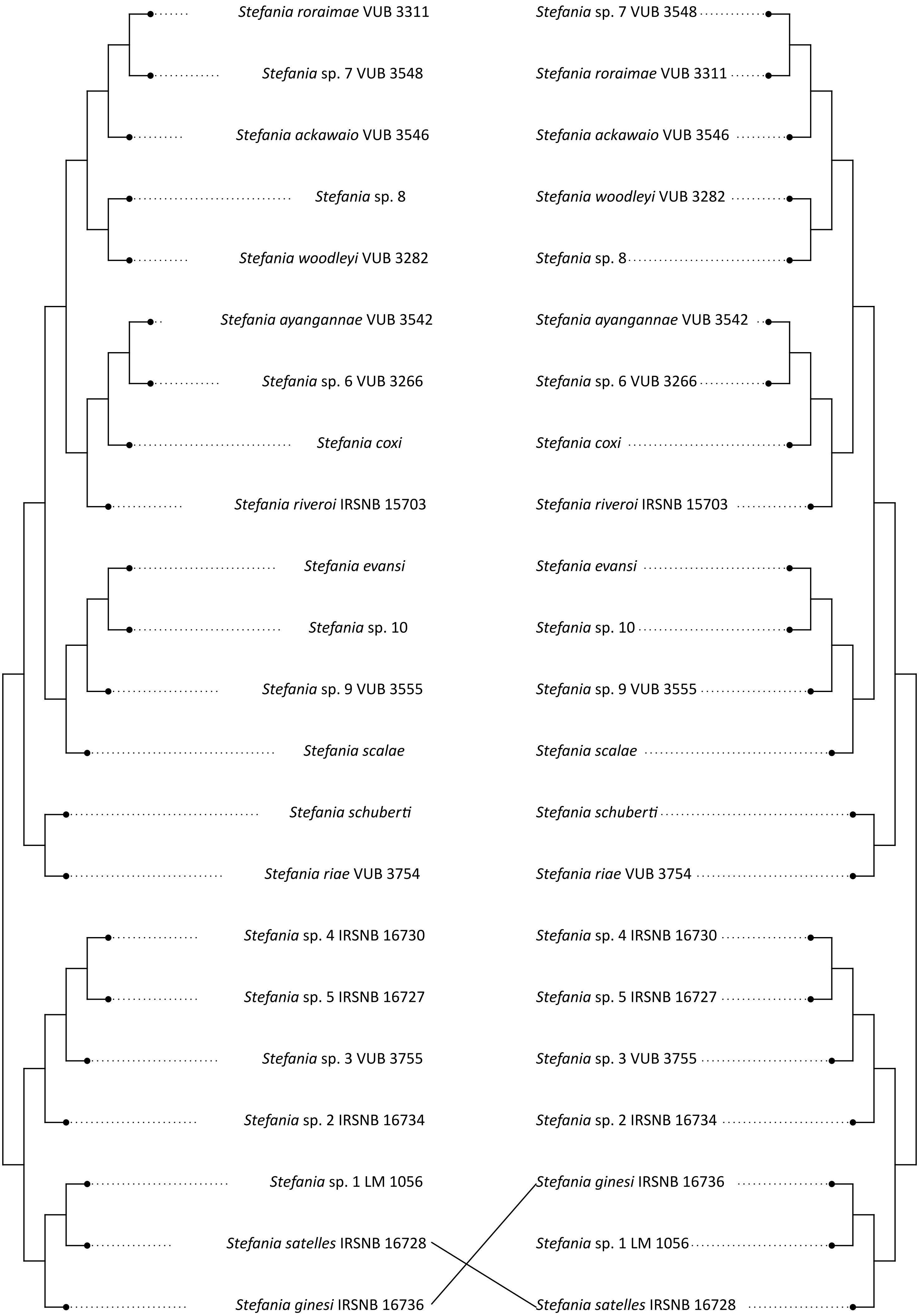


Table S1. Terminals ordered from minimum to maximum average match split distances (MSD) among TAP optimal trees. Smaller average MSD values indicate a greater potential for a terminal to behave as wildcard.

Teminal name	Average distances	Teminal name	Average distances
Hemiphractus johnsoni	14.72	*Barycholos ternetzi*	19.21
Cryptobatrachus sp.	15.40	*Batrachyla leptopus*	19.21
Gastrotheca testudinea CBG 774	15.49	*Batrachyla taeniata*	19.21
Cryptobatrachus boulengeri LM	16.66	*Boana boans*	19.21
Gastrotheca sp. 1	17.21	*Boana lanciformis*	19.21
Gastrotheca christiani FML 2881	17.36	*Brachycephalus alipioi*	19.21
Cryptobatrachus fuhrmanni MHUAA 5160	17.37	*Brachycephalus ephippium*	19.21
Gastrotheca chrysosticta LM 58	17.45	*Bryophryne cophites*	19.21
Gastrotheca gracilis DCC 006	17.53	*Bufo japonicus*	19.21
Cryptobatrachus fuhrmanni	17.72	*Calyptocephalella gayi*	19.21
Cryptobatrachus fuhrmanni JDL 14865	17.72	*Ceratophrys ornata*	19.21
Gastrotheca fissipes ZUFRJ 7901	17.87	*Ceuthomantis smaragdinus*	19.21
Gastrotheca recava	17.87	*Chacophrys pierottii*	19.21
Gastrotheca recava 634	17.87	*Colostethus pratti*	19.21
Hemiphractus bubalus	18.04	*Craugastor augusti*	19.21
Hemiphractus proboscideus KU 217513	18.04	*Craugastor podiciferus*	19.21
Cryptobatrachus remotus	18.15	*Craugastor punctariolus*	19.21
Hemiphractus elioti MVUP 1927	18.19	*Craugastor ranoides*	19.21
Hemiphractus fasciatus ZSM 36/0	18.19	*Craugastor rhodopis*	19.21
Hemiphractus panamensis	18.19	*Crossodactylodes sp.*	19.21
Gastrotheca atympana MHNSM 18692	18.72	*Crossodactylus schmidti*	19.21
Gastrotheca sp. H CORBIDI 9036	18.72	*Cycloramphus acangatan*	19.21
Gastrotheca sp. K CORBIDI 11523	18.72	*Cycloramphus boraceiensis*	19.21
Gastrotheca testudinea CORBIDI 8009	18.72	*Dendrobates auratus*	19.21
Hemiphractus helioi	18.80	*Dendropsophus nanus*	19.21
Hemiphractus kaylockae	18.80	*Diasporus aff. diastema*	19.21
Hemiphractus sp. 1 CORBIDI 14023	18.80	*Diasporus diastema*	19.21
Hemiphractus sp. 2 CORBIDI 13808	18.80	*Dryophytes arenicolor*	19.21
Gastrotheca sp. F CORBIDI 12876	18.87	*Dryophytes cinereus*	19.21
Gastrotheca sp. J	18.87	*Duttaphrynus melanostictus*	19.21
Gastrotheca testudinea	18.87	*Edalorhina perezi*	19.21
Flectonotus fitzgeraldi	18.88	*Eleutherodactylus cooki*	19.21
Flectonotus pygmaeus	18.88	*Eleutherodactylus coqui*	19.21
Acris crepitans	19.21	*Eleutherodactylus marnockii*	19.21
Adelophryne patamona	19.21	*Eleutherodactylus nitidus*	19.21
Adenomera andreae	19.21	*Eleutherodactylus planirostris*	19.21
Adenomera hylaedactyla	19.21	*Eleutherodactylus thorectes*	19.21
Agalychnis annae	19.21	*Eleutherodactylus tonyi*	19.21
Agalychnis callidryas	19.21	*Engystomops petersi*	19.21
Allobates femoralis	19.21	*Engystomops pustulosus*	19.21
Allophryne ruthveni	19.21	*Espadarana prosoblepon*	19.21
Alsodes neuquensis	19.21	*Eupsophus roseus*	19.21
Amazophrynella minuta	19.21	*Fejervarya limnocharis*	19.21
Atelognathus patagonicus	19.21	*Fritziana* cf. *fissilis* 1 MNRJ 62845	19.21
Barycholos pulcher	19.21	*Fritziana* cf. *fissilis* 2 CFBH 28886	19.21

Teminal name	**Average distances**	**Teminal name**	**Average distances**
Fritziana goeldii Go I	19.21	*Gastrotheca monticola*	19.21
Fritziana goeldii Go III CFBH 10910	19.21	*Gastrotheca nebulanastes*	19.21
Fritziana goeldii Go II MNRJ 537588	19.21	*Gastrotheca nicefori* KU 181071	19.21
Fritziana ohausi Oh III CFBH 7611	19.21	*Gastrotheca nicefori* MHUAA 5716	19.21
Fritziana ohausi Oh II CTMZ 04627	19.21	*Gastrotheca ochoai* KU 173499	19.21
Fritziana ohausi Oh I CFBH 16287	19.21	*Gastrotheca oresbios* CORBIDI 11076	19.21
Fritziana sp. CS 1 III CFBH 30747	19.21	*Gastrotheca orophylax* KU 178568	19.21
Fritziana sp. CS 1 II MZUFV 11721	19.21	*Gastrotheca ovifera*	19.21
Fritziana sp. CS 1 IV MZUSP 133700	19.21	*Gastrotheca pachachacae* MUSM 28492	19.21
Fritziana sp. CS 1 I CFBH 24810	19.21	*Gastrotheca peruana* KU 181740	19.21
Fritziana sp. CS 2 MCNAM 12341	19.21	*Gastrotheca phalarosa* CORBIDI 11044	19.21
Fritziana mitus	19.21	*Gastrotheca phelloderma* CORBIDI 14002	19.21
Fritziana mitus CFBH 8273	19.21	*Gastrotheca plumbea* KU 178499	19.21
Fritziana tonimi MNRJ 34921	19.21	*Gastrotheca prasina*	19.21
Fritziana ulei MNRJ 44622	19.21	*Gastrotheca pseustes* 2	19.21
Gastrotheca aguaruna KU 212026	19.21	*Gastrotheca psychrophila* KU 142634	19.21
Gastrotheca albolineata MNRJ 54401	19.21	*Gastrotheca pulchra*	19.21
Gastrotheca antoniiochoai MUSM 27944	19.21	*Gastrotheca rebeccae* CORBIDI 08006	19.21
Gastrotheca aratia KU 212056	19.21	*Gastrotheca rebeccae* CORBIDI 10821	19.21
Gastrotheca argenteovirens KU 181168	19.21	*Gastrotheca riobambae*	19.21
Gastrotheca aureomaculata KU 181194	19.21	*Gastrotheca ruizi* KU 200002	19.21
Gastrotheca cornuta	19.21	*Gastrotheca* sp. 2	19.21
Gastrotheca dendronastes KU 181203	19.21	*Gastrotheca* sp. 3	19.21
Gastrotheca dunni	19.21	*Gastrotheca pseustes* 2 QCAZ 22635	19.21
Gastrotheca ernestoi	19.21	*Gastrotheca yacuri*	19.21
Gastrotheca espeletia KU 203440	19.21	*Gastrotheca turnerorum* QCAZ 47299	19.21
Gastrotheca excubitor KU 173171	19.21	*Gastrotheca elicioi* QCAZ 21213	19.21
Gastrotheca excubitor MUSM 26280	19.21	Gastrotheca sp. G CORBIDI 16614	19.21
Gastrotheca flamma	19.21	*Gastrotheca* sp. I CORBIDI 11776	19.21
Gastrotheca fulvorufa CTMZ 07467	19.21	*Gastrotheca* sp. L	19.21
Gastrotheca galeata KU 181700	19.21	*Gastrotheca spectabilis* CORBIDI 11790	19.21
Gastrotheca griswoldi CORBIDI 16066	19.21	*Gastrotheca stictopleura* CORBIDI 14563	19.21
Gastrotheca griswoldi CORBIDI 7289	19.21	*Gastrotheca stictopleura* MTD 45230	19.21
Gastrotheca griswoldi KU 181701	19.21	*Gastrotheca trachyceps* KU 181189	19.21
Gastrotheca griswoldi MHNSM 20588	19.21	*Gastrotheca walkeri* MHNLS 21101	19.21
Gastrotheca guentheri KU 173112	19.21	*Gastrotheca walkeri* Vz 8996	19.21
Gastrotheca helenae KU 181070	19.21	*Gastrotheca weinlandii* KU 143105	19.21
Gastrotheca pseustes 1 QCAZ 45113	19.21	*Gastrotheca zeugocystis* MHNSM 18675	19.21
Gastrotheca litonedis KU 202690	19.21	*Haddadus binotatus*	19.21
Gastrotheca lojana	19.21	*Heleophryne purcelli*	19.21
Gastrotheca longipes	19.21	*Hemiphractus scutatus* CORBIDI 10054	19.21
Gastrotheca marsupiata	19.21	*Hemiphractus scutatus* INPA-H38116	19.21
Gastrotheca megacephala	19.21	*Hemiphractus scutatus* JMP 2150	19.21
Gastrotheca microdiscus	19.21	*Hemiphractus scutatus* USNM 317196	19.21

Teminal name	Average distances	Teminal name	Average distances
Holoaden bradei	19.21	*Proceratophrys avelinoi*	19.21
Holoaden luederwaldti	19.21	*Pseudis minuta*	19.21
Hydrolaetare caparu	19.21	*Pseudopaludicola falcipes*	19.21
Hyla chinensis	19.21	*Psychrophrynella guillei*	19.21
Hylodes phyllodes	19.21	*Psychrophrynella wettsteini*	19.21
Hylorina sylvatica	19.21	*Ranoidea caerulea*	19.21
Hyloxalus chlorocraspedus	19.21	*Rheobates palmatus*	19.21
Hypodactylus brunneus	19.21	*Rhinella arenarum*	19.21
Hypodactylus dolops	19.21	*Rhinoderma darwinii*	19.21
Insuetophrynus acarpicus	19.21	*Rupirana cardosoi*	19.21
Ischnocnema guentheri	19.21	*Scinax staufferi*	19.21
Ischnocnema juipoca	19.21	*Sclerophrys brauni*	19.21
Lepidobatrachus laevis	19.21	*Scythrophrys sawayae*	19.21
Leptodactylus latrans	19.21	*Smilisca baudinii*	19.21
Leptodactylus melanonotus	19.21	*Sooglossus thomasseti*	19.21
Limnodynastes salmini	19.21	*Stefania ackawaio* VUB 3546	19.21
Limnomedusa macroglossa	19.21	*Stefania ayangannae* VUB 3542	19.21
Lithodytes lineatus	19.21	*Stefania coxi*	19.21
Lynchius nebulanastes	19.21	*Stefania evansi*	19.21
Lynchius parkeri	19.21	*Stefania ginesi* IRSNB 16736	19.21
Macrogenioglottus alipioi	19.21	*Stefania riae* VUB 3754	19.21
Mannophryne trinitatis	19.21	*Stefania riveroi* IRSNB 15703	19.21
Megaelosia goeldii	19.21	*Stefania roraimae* VUB 3311	19.21
Melanophryniscus klappenbachi	19.21	*Stefania satelles* IRSNB 16728	19.21
Myersiohyla kanaima	19.21	*Stefania scalae*	19.21
Noblella lochites	19.21	*Stefania schuberti*	19.21
Noblella sp. MTD 45180	19.21	*Stefania* sp. 10	19.21
Nyctimystes infrafrenatus	19.21	*Stefania* sp. 1 LM 1056	19.21
Nymphargus bejaranoi	19.21	*Stefania* sp. 2 IRSNB 16734	19.21
Odontophrynus achalensis	19.21	*Stefania* sp. 3 VUB 3755	19.21
Oreobates cruralis	19.21	*Stefania* sp. 4 IRSNB 16730	19.21
Oreobates saxatilis	19.21	*Stefania* sp. 5 IRSNB 16727	19.21
Paratelmatobius cardosoi	19.21	*Stefania* sp. 6 VUB 3266	19.21
Phrynopus auriculatus	19.21	*Stefania* sp. 7 VUB 3548	19.21
Phrynopus bracki	19.21	*Stefania* sp. 8	19.21
Phyllodytes luteolus	19.21	*Stefania* sp. 9 VUB 3555	19.21
Physalaemus cuvieri	19.21	*Stefania woodleyi* VUB 3282	19.21
Physalaemus gracilis	19.21	*Strabomantis biporcatus*	19.21
Phyzelaphryne miriamae	19.21	*Strabomantis bufoniformis*	19.21
Pleurodema brachyops	19.21	*Telmatobius truebae*	19.21
Pleurodema diplolister	19.21	*Thoropa miliaris*	19.21
Pristimantis cruentus	19.21	*Trachycephalus typhonius*	19.21
Pristimantis curtipes	19.21	*Uperoleia laevigata*	19.21
Pristimantis gaigeae	19.21	*Yunganastes ashkapara*	19.21
Pristimantis ridens	19.21	*Yunganastes fraudator*	19.21
Pristimantis simonsii	19.21	*Zachaenus parvulus*	19.21

Table S2. Terminals ordered from minimum to maximum average match split distances (MSD) among SAP5th optimal trees. Smaller average MSD values indicate a greater potential for a terminal to behave as wildcard.

Teminal name	Average distances	Teminal name	Average distances
Cryptobatrachus sp.	16.22	*Batrachyla taeniata*	19.41
Gastrotheca marsupiata	16.60	*Boana boans*	19.41
Gastrotheca sp. 3	16.60	*Boana lanciformis*	19.41
Cryptobatrachus boulengeri LM	16.67	*Brachycephalus alipioi*	19.41
Gastrotheca fissipes ZUFRJ 7901	17.04	*Brachycephalus ephippium*	19.41
Gastrotheca sp. 1	17.06	*Bryophryne cophites*	19.41
Gastrotheca griswoldi CORBIDI 7289	17.17	*Bufo japonicus*	19.41
Cryptobatrachus fuhrmanni MHUAA 5160	17.31	*Calyptocephalella gayi*	19.41
Gastrotheca christiani FML 2881	17.43	*Ceratophrys ornata*	19.41
Gastrotheca chrysosticta LM 58	17.45	*Ceuthomantis smaragdinus*	19.41
Gastrotheca gracilis DCC 006	17.50	*Chacophrys pierottii*	19.41
Stefania scalae	17.73	*Colostethus pratti*	19.41
Stefania sp. 9 VUB 3555	17.73	*Craugastor augusti*	19.41
Cryptobatrachus fuhrmanni	17.89	*Craugastor podiciferus*	19.41
Cryptobatrachus fuhrmanni JDL 14865	17.89	*Craugastor punctariolus*	19.41
Cryptobatrachus remotus	18.02	*Craugastor ranoides*	19.41
Gastrotheca griswoldi CORBIDI 16066	18.24	*Craugastor rhodopis*	19.41
Gastrotheca griswoldi MHNSM 20588	18.24	*Crossodactylodes sp*	19.41
Gastrotheca griswoldi KU 181701	18.33	*Crossodactylus schmidti*	19.41
Hemiphractus elioti MVUP 1927	18.40	*Cycloramphus acangatan*	19.41
Hemiphractus fasciatus ZSM 36/0	18.40	*Cycloramphus boraceiensis*	19.41
Hemiphractus panamensis	18.40	*Dendrobates auratus*	19.41
Hemiphractus scutatus CORBIDI 10054	18.46	*Dendropsophus nanus*	19.41
Hemiphractus scutatus INPA-H38116	18.46	*Diasporus aff diastema*	19.41
Hemiphractus scutatus USNM 317196	18.46	*Diasporus diastema*	19.41
Gastrotheca recava	18.54	*Dryophytes arenicolor*	19.41
Gastrotheca recava 634	18.54	*Dryophytes cinereus*	19.41
Gastrotheca megacephala	18.91	*Duttaphrynus melanostictus*	19.41
Gastrotheca prasina	18.91	*Edalorhina perezi*	19.41
Gastrotheca sp. 2	18.91	*Eleutherodactylus cooki*	19.41
Stefania evansi	18.95	*Eleutherodactylus coqui*	19.41
Stefania sp. 10	18.95	*Eleutherodactylus marnockii*	19.41
Acris crepitans	19.41	*Eleutherodactylus nitidus*	19.41
Adelophryne patamona	19.41	*Eleutherodactylus planirostris*	19.41
Adenomera andreae	19.41	*Eleutherodactylus thorectes*	19.41
Adenomera hylaedactyla	19.41	*Eleutherodactylus tonyi*	19.41
Agalychnis annae	19.41	*Engystomops petersi*	19.41
Agalychnis callidryas	19.41	*Engystomops pustulosus*	19.41
Allobates femoralis	19.41	*Espadarana prosoblepon*	19.41
Allophryne ruthveni	19.41	*Eupsophus roseus*	19.41
Alsodes neuquensis	19.41	*Fejervarya limnocharis*	19.41
Amazophrynella minuta	19.41	*Flectonotus fitzgeraldi*	19.41
Atelognathus patagonicus	19.41	*Flectonotus pygmaeus*	19.41
Barycholos pulcher	19.41	*Fritziana* cf. *fissilis* 1 MNRJ 62845	19.41
Barycholos ternetzi	19.41	*Fritziana* cf. *fissilis* 2 CFBH 28886	19.41
Batrachyla leptopus	19.41	*Fritziana goeldii* Go I	19.41

Teminal name	Average distances	Teminal name	Average distances
Fritziana goeldii Go III CFBH 10910	19.41	*Gastrotheca peruana* KU 181740	19.41
Fritziana goeldii Go II MNRJ 53758	19.41	*Gastrotheca phalarosa* CORBIDI 11044	19.41
Fritziana ohausi Oh III CFBH 7611	19.41	*Gastrotheca phelloderma* CORBIDI 14002	19.41
Fritziana ohausi Oh II CTMZ 04627	19.41	*Gastrotheca plumbea* KU 178499	19.41
Fritziana ohausi Oh I CFBH 16287	19.41	*Gastrotheca pseustes* 2	19.41
Fritziana sp. CS 1 III CFBH 30747	19.41	*Gastrotheca psychrophila* KU 142634	19.41
Fritziana sp. CS 1 II MZUFV 11721	19.41	*Gastrotheca pulchra*	19.41
Fritziana sp. CS 1 IV MZUSP 133700	19.41	*Gastrotheca rebeccae* CORBIDI 08006	19.41
Fritziana sp. CS 1 I CFBH 24810	19.41	*Gastrotheca rebeccae* CORBIDI 10821	19.41
Fritziana sp. CS 2 MCNAM 12341	19.41	*Gastrotheca riobambae*	19.41
Fritziana mitus	19.41	*Gastrotheca ruizi* KU 200002	19.41
Fritziana mitus CFBH 8273	19.41	*Gastrotheca pseustes* 2 QCAZ 22635	19.41
Fritziana tonimi MNRJ 34921	19.41	*Gastrotheca yacuri*	19.41
Fritziana ulei MNRJ 44622	19.41	Gastrotheca turnerorum QCAZ 47299	19.41
Gastrotheca aguaruna KU 212026	19.41	*Gastrotheca elicioi* QCAZ 21213	19.41
Gastrotheca albolineata MNRJ 54401	19.41	*Gastrotheca* sp. F CORBIDI 12876	19.41
Gastrotheca antoniiochoai MUSM 27944	19.41	*Gastrotheca* sp. G CORBIDI 16614	19.41
Gastrotheca aratia KU 212056	19.41	*Gastrotheca* sp. H CORBIDI 9036	19.41
Gastrotheca argenteovirens KU 181168	19.41	*Gastrotheca* sp. I CORBIDI 11776	19.41
Gastrotheca atympana MHNSM 18692	19.41	*Gastrotheca* sp. J	19.41
Gastrotheca aureomaculata KU 181194	19.41	*Gastrotheca* sp. K CORBIDI 11523	19.41
Gastrotheca cornuta	19.41	*Gastrotheca* sp. L	19.41
Gastrotheca dendronastes KU 181203	19.41	*Gastrotheca spectabilis* CORBIDI 11790	19.41
Gastrotheca dunni	19.41	*Gastrotheca stictopleura* CORBIDI 14563	19.41
Gastrotheca ernestoi	19.41	*Gastrotheca stictopleura* MTD 45230	19.41
Gastrotheca espeletia KU 203440	19.41	*Gastrotheca testudinea*	19.41
Gastrotheca sp. KU 173171	19.41	*Gastrotheca testudinea* CBG 774	19.41
Gastrotheca excubitor MUSM 26280	19.41	*Gastrotheca testudinea* CORBIDI 8009	19.41
Gastrotheca flamma	19.41	*Gastrotheca trachyceps* KU 181189	19.41
Gastrotheca fulvorufa CTMZ 07467	19.41	*Gastrotheca walkeri* MHNLS 21101	19.41
Gastrotheca galeata KU 181700	19.41	*Gastrotheca walkeri* Vz 8996	19.41
Gastrotheca guentheri KU 173112	19.41	*Gastrotheca weinlandii* KU 143105	19.41
Gastrotheca helenae KU 181070	19.41	*Gastrotheca zeugocystis* MHNSM 18675	19.41
Gastrotheca pseustes 1 QCAZ 45113	19.41	*Haddadus binotatus*	19.41
Gastrotheca litonedis KU 202690	19.41	*Heleophryne purcelli*	19.41
Gastrotheca lojana	19.41	*Hemiphractus bubalus*	19.41
Gastrotheca longipes	19.41	*Hemiphractus helioi*	19.41
Gastrotheca microdiscus	19.41	*Hemiphractus johnsoni*	19.41
Gastrotheca monticola	19.41	*Hemiphractus kaylockae*	19.41
Gastrotheca nebulanastes	19.41	*Hemiphractus proboscideus* KU 217513	19.41
Gastrotheca nicefori KU 181071	19.41	*Hemiphractus scutatus* JMP 2150	19.41
Gastrotheca nicefori MHUAA 5716	19.41	*Hemiphractus* sp. 1 CORBIDI 14023	19.41
Gastrotheca ochoai KU 173499	19.41	*Hemiphractus* sp. 2 CORBIDI 13808	19.41
Gastrotheca oresbios CORBIDI 11076	19.41	*Holoaden bradei*	19.41
Gastrotheca orophylax KU 178568	19.41	*Holoaden luederwaldti*	19.41
Gastrotheca ovifera	19.41	*Hydrolaetare caparu*	19.41
Gastrotheca pachachacae MUSM 28492	19.41	*Hyla chinensis*	19.41

Teminal name	Average distances	Teminal name	Average distances
Hylodes phyllodes	19.41	*Ranoidea caerulea*	19.41
Hylorina sylvatica	19.41	*Rheobates palmatus*	19.41
Hyloxalus chlorocraspedus	19.41	*Rhinella arenarum*	19.41
Hypodactylus brunneus	19.41	*Rhinoderma darwinii*	19.41
Hypodactylus dolops	19.41	*Rupirana cardosoi*	19.41
Insuetophrynus acarpicus	19.41	*Scinax staufferi*	19.41
Ischnocnema guentheri	19.41	*Sclerophrys brauni*	19.41
Ischnocnema juipoca	19.41	*Scythrophrys sawayae*	19.41
Lepidobatrachus laevis	19.41	*Smilisca baudinii*	19.41
Leptodactylus latrans	19.41	*Sooglossus thomasseti*	19.41
Leptodactylus melanonotus	19.41	*Stefania ackawaio* VUB 3546	19.41
Limnodynastes salmini	19.41	*Stefania ayangannae* VUB 3542	19.41
Limnomedusa macroglossa	19.41	*Stefania coxi*	19.41
Lithodytes lineatus	19.41	*Stefania ginesi* IRSNB 16736	19.41
Lynchius nebulanastes	19.41	*Stefania riae* VUB 3754	19.41
Lynchius parkeri	19.41	*Stefania riveroi* IRSNB 15703	19.41
Macrogenioglottus alipioi	19.41	*Stefania roraimae* VUB 3311	19.41
Mannophryne trinitatis	19.41	*Stefania satelles* IRSNB 16728	19.41
Megaelosia goeldii	19.41	*Stefania schuberti*	19.41
Melanophryniscus klappenbachi	19.41	*Stefania* sp. 1 LM 1056	19.41
Myersiohyla kanaima	19.41	*Stefania* sp. 2 IRSNB 16734	19.41
Noblella lochites	19.41	*Stefania* sp. 3 VUB 3755	19.41
Noblella sp. MTD45180	19.41	*Stefania* sp. 4 IRSNB 16730	19.41
Nyctimystes infrafrenatus	19.41	*Stefania* sp. 5 IRSNB 16727	19.41
Nymphargus bejaranoi	19.41	*Stefania* sp. 6 VUB 3266	19.41
Odontophrynus achalensis	19.41	*Stefania* sp. 7 VUB 3548	19.41
Oreobates cruralis	19.41	*Stefania* sp. 8	19.41
Oreobates saxatilis	19.41	*Stefania woodleyi* VUB 3282	19.41
Paratelmatobius cardosoi	19.41	*Strabomantis biporcatus*	19.41
Phrynopus auriculatus	19.41	*Strabomantis bufoniformis*	19.41
Phrynopus bracki	19.41	*Telmatobius truebae*	19.41
Phyllodytes luteolus	19.41	*Thoropa miliaris*	19.41
Physalaemus cuvieri	19.41	*Trachycephalus typhonius*	19.41
Physalaemus gracilis	19.41	*Uperoleia laevigata*	19.41
Phyzelaphryne miriamae	19.41	*Yunganastes ashkapara*	19.41
Pleurodema brachyops	19.41	*Yunganastes fraudator*	19.41
Pleurodema diplolister	19.41	*Zachaenus parvulus*	19.41
Pristimantis cruentus	19.41		
Pristimantis curtipes	19.41		
Pristimantis gaigeae	19.41		
Pristimantis ridens	19.41		
Pristimantis simonsii	19.41		
Proceratophrys avelinoi	19.41		
Pseudis minuta	19.41		
Pseudopaludicola falcipes	19.41		
Psychrophrynella guillei	19.41		
Psychrophrynella wettsteini	19.41		

Table S3. Terminals ordered from minimum to maximum average match split distances (MSD) among SAPg optimal trees. Smaller average MSD values indicate a greater potential for a terminal to behave as wildcard.

Teminal name	**Average distances**	**Teminal name**	**Average distances**
Gastrotheca excubitor MUSM 26280	92.48	*Hemiphractus bubalus*	96.74
Hemiphractus johnsoni	93.46	*Hemiphractus proboscideus* KU 217513	96.74
Thoropa miliaris	93.61	*Cryptobatrachus remotus*	96.74
Cryptobatrachus sp.	94.05	*Gastrotheca griswoldi* CORBIDI 16066	96.85
Gastrotheca marsupiata	94.96	*Gastrotheca griswoldi* MHNSM 2058	96.85
Gastrotheca sp. 3	94.96	*Physalaemus cuvieri*	96.95
Cycloramphus acangatan	95.04	*Physalaemus gracilis*	96.95
Cycloramphus boraceiensis	95.04	*Ceratophrys ornata*	96.95
Zachaenus parvulus	95.04	*Chacophrys pierottii*	96.95
Gastrotheca sp. 1	95.21	*Lepidobatrachus laevis*	96.95
Cryptobatrachus boulengeri LM	95.24	*Telmatobius truebae*	96.95
Pristimantis gaigeae	95.41	*Hemiphractus elioti* MVUP 1927	97.00
Colostethus pratti	95.52	*Hemiphractus fasciatus* ZSM 36/0	97.00
Dendrobates auratus	95.52	*Hemiphractus panamensis*	97.00
Hyloxalus chlorocraspedus	95.52	*Gastrotheca* sp. KU 173171	97.02
Insuetophrynus acarpicus	95.52	*Gastrotheca ochoai* KU 173499	97.02
Rhinoderma darwinii	95.52	*Stefania evansi*	97.04
Gastrotheca griswoldi CORBIDI 7289	95.67	*Stefania* sp. 10	97.04
Cryptobatrachus fuhrmanni MHUAA 5160	95.98	*Gastrotheca* sp. 2	97.30
Edalorhina perezi	95.99	*Gastrotheca aratia* KU 212056	97.40
Agalychnis annae	96.00	*Gastrotheca peruana* KU 181740	97.40
Agalychnis callidryas	96.00	*Gastrotheca phelloderma* CORBIDI 14002	97.40
Nyctimystes infrafrenatus	96.00	*Gastrotheca pseustes* 1 QCAZ 45113	97.40
Ranoidea caerulea	96.00	*Gastrotheca pseustes* 2	97.40
Stefania scalae	96.11	*Gastrotheca pseustes* 2 QCAZ 22635	97.40
Stefania sp. 9VUB 3555	96.11	*Pristimantis curtipes*	97.41
Gastrotheca pachachacae MUSM 28492	96.15	*Pristimantis simonsii*	97.41
Gastrotheca gracilis DCC 006	96.27	*Yunganastes ashkapara*	97.41
Gastrotheca christiani FML 2881	96.31	*Yunganastes fraudator*	97.41
Gastrotheca chrysosticta LM 58	96.31	*Acris crepitans*	97.43
Cryptobatrachus fuhrmanni	96.45	*Bufo japonicus*	97.43
Cryptobatrachus fuhrmanni JDL 14865	96.45	*Dendropsophus nanus*	97.43
Rhinella arenarum	96.47	*Dryophytes arenicolor*	97.43
Sclerophrys brauni	96.47	*Dryophytes cinereus*	97.43
Allobates femoralis	96.48	*Duttaphrynus melanostictus*	97.43
Mannophryne trinitatis	96.48	*Engystomops petersi*	97.43
Rheobates palmatus	96.48	*Engystomops pustulosus*	97.43
Gastrotheca griswoldi KU 181701	96.57	*Hyla chinensis*	97.43
Gastrotheca fissipes ZUFRJ 7901	96.58	*Pseudis minuta*	97.43
Gastrotheca recava	96.59	*Smilisca baudinii*	97.43
Gastrotheca recava 634	96.59	*Adenomera andreae*	97.43

Teminal name	**Average distances**	**Teminal name**	**Average distances**
Adenomera hylaedactyla	97.43	*Gastrotheca rebeccae* CORBIDI 10821	97.47
Alsodes neuquensis	97.43	*Stefania ayangannae* VUB 3542	97.51
Atelognathus patagonicus	97.43	*Stefania coxi*	97.51
Batrachyla leptopus	97.43	*Stefania riveroi* IRSNB 15703	97.51
Batrachyla taeniata	97.43	*Stefania* sp. 6 VUB 3266	97.51
Boana boans	97.43	*Gastrotheca phalarosa* CORBIDI 11044	97.57
Boana lanciformis	97.43	*Flectonotus fitzgeraldi*	97.64
Crossodactylus schmidti	97.43	*Flectonotus pygmaeus*	97.64
Eupsophus roseus	97.43	*Gastrotheca aguaruna* KU 212026	97.90
Hydrolaetare caparu	97.43	*Gastrotheca yacuri*	97.90
Hylodes phyllodes	97.43	*Gastrotheca turnerorum* QCAZ 47299	97.90
Hylorina sylvatica	97.43	*Gastrotheca oresbios* CORBIDI 11076	97.90
Leptodactylus latrans	97.43	*Gastrotheca psychrophila* KU 142634	97.90
Leptodactylus melanonotus	97.43	*Gastrotheca* sp. G CORBIDI 16614	97.90
Limnomedusa macroglossa	97.43	*Gastrotheca* sp. I CORBIDI 11776	97.90
Lithodytes lineatus	97.43	*Gastrotheca spectabilis* CORBIDI 11790	97.90
Megaelosia goeldii	97.43	*Gastrotheca stictopleura* CORBIDI 14563	97.90
Myersiohyla kanaima	97.43	*Gastrotheca stictopleura* MTD 45230	97.90
Scinax staufferi	97.43	*Crossodactylodes* sp.	97.91
Allophryne ruthveni	97.43	*Paratelmatobius cardosoi*	97.91
Amazophrynella minuta	97.43	*Phyllodytes luteolus*	97.91
Espadarana prosoblepon	97.43	*Rupirana cardosoi*	97.91
Macrogenioglottus alipioi	97.43	*Scythrophrys sawayae*	97.91
Melanophryniscus klappenbachi	97.43	*Trachycephalus typhonius*	97.91
Nymphargus bejaranoi	97.43	*Gastrotheca antoniiochoai* MUSM 27944	97.91
Odontophrynus achalensis	97.43	*Gastrotheca megacephala*	97.91
Pleurodema brachyops	97.43	*Gastrotheca prasina*	97.91
Pleurodema diplolister	97.43	*Pristimantis cruentus*	97.91
Proceratophrys avelinoi	97.43	*Pristimantis ridens*	97.91
Pseudopaludicola falcipes	97.43	*Stefania ackawaio* VUB 3546	97.91
Gastrotheca atympana MHNSM 18692	97.46	*Stefania roraimae* VUB 3311	97.91
Gastrotheca nebulanastes	97.46	*Stefania* sp. 7 VUB 3548	97.91
Gastrotheca sp. F CORBIDI 12876	97.46	*Stefania* sp. 8	97.91
Gastrotheca sp. H CORBIDI 9036	97.46	*Stefania woodleyi* VUB 3282	97.91
Gastrotheca sp. J	97.46	*Adelophryne patamona*	97.91
Gastrotheca sp. K CORBIDI 11523	97.46	*Barycholos pulcher*	97.91
Gastrotheca testudinea	97.46	*Barycholos ternetzi*	97.91
Gastrotheca testudinea CBG 774	97.46	*Brachycephalus alipioi*	97.91
Gastrotheca testudinea CORBIDI 8009	97.46	*Brachycephalus ephippium*	97.91
Hemiphractus helioi	97.46	*Bryophryne cophites*	97.91
Hemiphractus kaylockae	97.46	*Calyptocephalella gayi*	97.91
Hemiphractus sp. 2 CORBIDI 13808	97.46	*Ceuthomantis smaragdinus*	97.91
Gastrotheca rebeccae CORBIDI 08006	97.47	*Craugastor augusti*	97.91

Teminal name	Average distances	Teminal name	Average distances
Craugastor podiciferus	97.91	*Gastrotheca helenae* KU 181070	97.91
Craugastor punctariolus	97.91	*Gastrotheca litonedis* KU 202690	97.91
Craugastor ranoides	97.91	*Gastrotheca lojana*	97.91
Craugastor rhodopis	97.91	*Gastrotheca longipes*	97.91
Diasporus aff. diastema	97.91	*Gastrotheca microdiscus*	97.91
Diasporus diastema	97.91	*Gastrotheca monticola*	97.91
Eleutherodactylus cooki	97.91	*Gastrotheca nicefori* KU 181071	97.91
Eleutherodactylus coqui	97.91	*Gastrotheca nicefori* MHUAA 5716	97.91
Eleutherodactylus marnockii	97.91	*Gastrotheca orophylax* KU 178568	97.91
Eleutherodactylus nitidus	97.91	*Gastrotheca ovifera*	97.91
Eleutherodactylus planirostris	97.91	*Gastrotheca plumbea* KU 178499	97.91
Eleutherodactylus thorectes	97.91	*Gastrotheca pulchra*	97.91
Eleutherodactylus tonyi	97.91	*Gastrotheca riobambae*	97.91
Fejervarya limnocharis	97.91	*Gastrotheca ruizi* KU 200002	97.91
Fritziana cf. *fissilis* 1 MNRJ 62845	97.91	*Gastrotheca elicioi* QCAZ 21213	97.91
Fritziana cf. *fissilis* 2 CFBH 28886	97.91	*Gastrotheca* sp. L	97.91
Fritziana goeldii Go I	97.91	*Gastrotheca trachyceps* KU 181189	97.91
Fritziana goeldii Go III CFBH 10910	97.91	*Gastrotheca walkeri* MHNLS 21101	97.91
Fritziana goeldii Go II MNRJ 5375	97.91	*Gastrotheca walkeri* Vz 8996	97.91
Fritziana ohausi Oh III CFBH 7611	97.91	*Gastrotheca weinlandii* KU 143105	97.91
Fritziana ohausi Oh II CTMZ 04627	97.91	*Gastrotheca zeugocystis* MHNSM 18675	97.91
Fritziana ohausi Oh I CFBH 16287	97.91	*Haddadus binotatus*	97.91
Fritziana sp. CS 1 III CFBH 30747	97.91	*Heleophryne purcelli*	97.91
Fritziana sp. CS 1 II MZUFV 11721	97.91	*Hemiphractus scutatus* CORBIDI 10054	97.91
Fritziana sp. CS 1 IV MZUSP 133700	97.91	*Hemiphractus scutatus* INPA-H38116	97.91
Fritziana sp. CS 1 I CFBH 24810	97.91	*Hemiphractus scutatus* JMP 2150	97.91
Fritziana sp. CS 2 MCNAM 12341	97.91	*Hemiphractus scutatus* USNM 317196	97.91
Fritziana mitus	97.91	*Hemiphractus* sp. 1 CORBIDI 14023	97.91
Fritziana mitus CFBH 8273	97.91	*Holoaden bradei*	97.91
Fritziana tonimi MNRJ 34921	97.91	*Holoaden luederwaldti*	97.91
Fritziana ulei MNRJ 44622	97.91	*Hypodactylus brunneus*	97.91
Gastrotheca albolineata MNRJ 54401	97.91	*Hypodactylus dolops*	97.91
Gastrotheca argenteovirens KU 181168	97.91	*Ischnocnema guentheri*	97.91
Gastrotheca aureomaculata KU 181194	97.91	*Ischnocnema juipoca*	97.91
Gastrotheca cornuta	97.91	*Limnodynastes salmini*	97.91
Gastrotheca dendronastes KU 181203	97.91	*Lynchius nebulanastes*	97.91
Gastrotheca dunni	97.91	*Lynchius parkeri*	97.91
Gastrotheca ernestoi	97.91	*Noblella lochites*	97.91
Gastrotheca espeletia KU 203440	97.91	*Noblella* sp. MTD 45180	97.91
Gastrotheca flamma	97.91	*Oreobates cruralis*	97.91
Gastrotheca fulvorufa CTMZ 07467	97.91	*Oreobates saxatilis*	97.91
Gastrotheca galeata KU 181700	97.91	*Phrynopus auriculatus*	97.91
Gastrotheca guentheri KU 173112	97.91	*Phrynopus bracki*	97.91

Teminal name	Average distances
Phyzelaphryne miriamae	97.91
Psychrophrynella guillei	97.91
Psychrophrynella wettsteini	97.91
Sooglossus thomasseti	97.91
Stefania ginesi IRSNB 16736	97.91
Stefania riae VUB 3754	97.91
Stefania satelles IRSNB 16728	97.91
Stefania schuberti	97.91
Stefania sp. 1 LM 1056	97.91
Stefania sp. 2 IRSNB 16734	97.91
Stefania sp. 3 VUB 3755	97.91
Stefania sp. 4 IRSNB 16730	97.91
Stefania sp. 5 IRSNB 16727	97.91
Strabomantis biporcatus	97.91
Strabomantis bufoniformis	97.91
Uperoleia laevigata	97.91

Table S4. Terminals ordered from minimum to maximum average match split distances (MSD) among SAPm optimal trees. Smaller average MSD values indicate a greater potential for a terminal to behave as wildcard.

Teminal name	**Average distances**	**Teminal name**	**Average distances**
Gastrotheca excubitor MUSM 26280	48.56	*Gastrotheca nicefori* KU 181071	53.21
Hemiphractus johnsoni	49.22	*Gastrotheca nicefori* MHUAA 5716	53.21
Cryptobatrachus sp.	50.22	*Gastrotheca riobambae*	53.21
Gastrotheca sp. 1	51.03	*Gastrotheca ruizi* KU 200002	53.21
Gastrotheca marsupiata	51.19	*Gastrotheca trachyceps* KU 181189	53.21
Gastrotheca sp. 3	51.19	*Hemiphractus helioi*	53.51
Cryptobatrachus boulengeri LM	51.3	*Hemiphractus kaylockae*	53.51
Gastrotheca griswoldi CORBIDI 7289	51.5	*Hemiphractus* sp. 2 CORBIDI 13808	53.51
Gastrotheca fissipes ZUFRJ 7901	51.71	*Gastrotheca aratia* KU 212056	53.59
Gastrotheca chrysosticta LM 58	51.96	*Gastrotheca peruana* KU 181740	53.59
Gastrotheca christiani FML 2881	51.96	*Gastrotheca phelloderma* CORBIDI 14002	53.59
Cryptobatrachus fuhrmanni MHUAA 5160	52.03	*Gastrotheca pseustes* 1 QCAZ 45113	53.59
Gastrotheca gracilis DCC 006	52.06	*Gastrotheca pseustes* 2	53.59
Gastrotheca sp. F CORBIDI 12876	52.11	*Gastrotheca pseustes* 2 QCAZ 22635	53.59
Gastrotheca pachachacae MUSM 28492	52.13	*Gastrotheca galeata* KU 181700	53.61
Gastrotheca griswoldi KU 181701	52.45	*Gastrotheca litonedis* KU 202690	53.61
Cryptobatrachus fuhrmanni	52.51	*Gastrotheca lojana*	53.61
Cryptobatrachus fuhrmanni JDL 14865	52.51	*Gastrotheca monticola*	53.61
Gastrotheca griswoldi CORBIDI 16066	52.63	*Gastrotheca orophylax* KU 178568	53.61
Gastrotheca griswoldi MHNSM 20588	52.63	*Gastrotheca plumbea* KU 178499	53.61
Gastrotheca sp. K CORBIDI 11523	52.89	*Gastrotheca elicioi* QCAZ 21213	53.61
Gastrotheca testudinea CORBIDI 8009	52.89	Gastrotheca antoniiochoai MUSM 27944	53.61
Gastrotheca sp. 2	52.9	*Gastrotheca megacephala*	53.61
Gastrotheca recava	52.9	*Gastrotheca phalarosa* CORBIDI 11044	53.61
Gastrotheca recava 634	52.9	*Gastrotheca prasina*	53.61
Hemiphractus bubalus	52.91	*Flectonotus fitzgeraldi*	53.76
Hemiphractus proboscideus KU 217513	52.91	*Flectonotus pygmaeus*	53.76
Gastrotheca sp. KU173171	52.94	*Gastrotheca aguaruna* KU 212026	54.01
Gastrotheca ochoai KU 173499	52.94	*Gastrotheca yacuri*	54.01
Cryptobatrachus remotus	53.0	*Gastrotheca turnerorum* QCAZ 47299	54.01
Hemiphractus elioti MVUP 1927	53.06	*Gastrotheca oresbios* CORBIDI 11076	54.01
Hemiphractus fasciatus ZSM 36/0	53.06	*Gastrotheca psychrophila* KU 142634	54.01
Hemiphractus panamensis	53.06	*Gastrotheca* sp. G CORBIDI 16614	54.01
Gastrotheca sp. J	53.15	*Gastrotheca* sp. I CORBIDI 11776	54.01
Gastrotheca testudinea	53.15	*Gastrotheca spectabilis* CORBIDI 11790	54.01
Gastrotheca atympana MHNSM 18692	53.15	*Gastrotheca stictopleura* CORBIDI 14563	54.01
Gastrotheca nebulanastes	53.15	*Gastrotheca stictopleura* MTD 45230	54.01
Gastrotheca sp. H CORBIDI 9036	53.15	*Acris crepitans*	54.01
Gastrotheca testudinea CBG 774	53.15	*Adelophryne patamona*	54.01
Gastrotheca rebeccae CORBIDI 08006	53.19	*Adenomera andreae*	54.01
Gastrotheca rebeccae CORBIDI 10821	53.19	*Adenomera hylaedactyla*	54.01
Gastrotheca argenteovirens KU 181168	53.21	*Agalychnis annae*	54.01
Gastrotheca aureomaculata KU 181194	53.21	*Agalychnis callidryas*	54.01
Gastrotheca dunni	53.21	*Allobates femoralis*	54.01
Gastrotheca espeletia KU 203440	53.21	*Allophryne ruthveni*	54.01

Teminal name	Average distances	Teminal name	Average distances
Alsodes neuquensis	54.01	*Fritziana* cf. *fissilis* 1 MNRJ 62845	54.01
Amazophrynella minuta	54.01	*Fritziana* cf. *fissilis* 2 CFBH 28886	54.01
Atelognathus patagonicus	54.01	*Fritziana goeldii* Go I	54.01
Barycholos pulcher	54.01	*Fritziana goeldii* Go III CFBH 10910	54.01
Barycholos ternetzi	54.01	*Fritziana goeldii* GoII MNRJ 53758	54.01
Batrachyla leptopus	54.01	*Fritziana ohausi* Oh III CFBH 7611	54.01
Batrachyla taeniata	54.01	*Fritziana ohausi* Oh II CTMZ 04627	54.01
Boana boans	54.01	*Fritziana ohausi* Oh I CFBH 16287	54.01
Boana lanciformis	54.01	*Fritziana* sp. CS 1 III CFBH 30747	54.01
Brachycephalus alipioi	54.01	*Fritziana* sp. CS 1 II MZUFV 11721	54.01
Brachycephalus ephippium	54.01	*Fritziana* sp. CS 1 IV MZUSP 133700	54.01
Bryophryne cophites	54.01	*Fritziana* sp. CS 1 I CFBH 24810	54.01
Bufo japonicus	54.01	*Fritziana* sp. CS 2 MCNAM 12341	54.01
Calyptocephalella gayi	54.01	*Fritziana mitus*	54.01
Ceratophrys ornata	54.01	*Fritziana mitus* CFBH 8273	54.01
Ceuthomantis smaragdinus	54.01	*Fritziana tonimi* MNRJ 34921	54.01
Chacophrys pierottii	54.01	*Fritziana ulei* MNRJ 44622	54.01
Colostethus pratti	54.01	*Gastrotheca albolineata* MNRJ 54401	54.01
Craugastor augusti	54.01	*Gastrotheca cornuta*	54.01
Craugastor podiciferus	54.01	*Gastrotheca dendronaste* s KU 181203	54.01
Craugastor punctariolus	54.01	*Gastrotheca ernestoi*	54.01
Craugastor ranoides	54.01	*Gastrotheca flamma*	54.01
Craugastor rhodopis	54.01	*Gastrotheca fulvorufa* CTMZ 07467	54.01
Crossodactylodes sp	54.01	*Gastrotheca guentheri* KU 173112	54.01
Crossodactylus schmidti	54.01	*Gastrotheca helenae* KU 181070	54.01
Cycloramphus acangatan	54.01	*Gastrotheca longipes*	54.01
Cycloramphus boraceiensis	54.01	*Gastrotheca microdiscus*	54.01
Dendrobates auratus	54.01	*Gastrotheca ovifera*	54.01
Dendropsophus nanus	54.01	*Gastrotheca pulchra*	54.01
Diasporus aff diastema	54.01	*Gastrotheca* sp. L	54.01
Diasporus diastema	54.01	*Gastrotheca walkeri* MHNLS 21101	54.01
Dryophytes arenicolor	54.01	*Gastrotheca walkeri* Vz 8996	54.01
Dryophytes cinereus	54.01	*Gastrotheca weinlandii* KU 143105	54.01
Duttaphrynus melanostictus	54.01	*Gastrotheca zeugocystis* MHNSM 18675	54.01
Edalorhina perezi	54.01	*Haddadus binotatus*	54.01
Eleutherodactylus cooki	54.01	*Heleophryne purcelli*	54.01
Eleutherodactylus coqui	54.01	*Hemiphractus scutatus* CORBIDI 10054	54.01
Eleutherodactylus marnockii	54.01	*Hemiphractus scutatus* INPA-H38116	54.01
Eleutherodactylus nitidus	54.01	*Hemiphractus scutatus* JMP 2150	54.01
Eleutherodactylus planirostris	54.01	*Hemiphractus scutatus* USNM 317196	54.01
Eleutherodactylus thorectes	54.01	*Hemiphractus* sp. 1 CORBIDI 14023	54.01
Eleutherodactylus tonyi	54.01	*Holoaden bradei*	54.01
Engystomops petersi	54.01	*Holoaden luederwaldti*	54.01
Engystomops pustulosus	54.01	*Hydrolaetare caparu*	54.01
Espadarana prosoblepon	54.01	*Hyla chinensis*	54.01
Eupsophus roseus	54.01	*Hylodes phyllodes*	54.01
Fejervarya limnocharis	54.01	*Hylorina sylvatica*	54.01

Teminal name	Average distances	Teminal name	Average distances
Hyloxalus chlorocraspedus	54.01	*Psychrophrynella wettsteini*	54.01
Hypodactylus brunneus	54.01	*Ranoidea caerulea*	54.01
Hypodactylus dolops	54.01	*Rheobates palmatus*	54.01
Insuetophrynus acarpicus	54.01	*Rhinella arenarum*	54.01
Ischnocnema guentheri	54.01	*Rhinoderma darwinii*	54.01
Ischnocnema juipoca	54.01	*Rupirana cardosoi*	54.01
Lepidobatrachus laevis	54.01	*Scinax staufferi*	54.01
Leptodactylus latrans	54.01	*Sclerophrys brauni*	54.01
Leptodactylus melanonotus	54.01	*Scythrophrys sawayae*	54.01
Limnodynastes salmini	54.01	*Smilisca baudinii*	54.01
Limnomedusa macroglossa	54.01	*Sooglossus thomasseti*	54.01
Lithodytes lineatus	54.01	*Stefania ackawaio* VUB 3546	54.01
Lynchius nebulanastes	54.01	*Stefania ayangannae* VUB 3542	54.01
Lynchius parkeri	54.01	*Stefania coxi*	54.01
Macrogenioglottus alipioi	54.01	*Stefania evansi*	54.01
Mannophryne trinitatis	54.01	*Stefania ginesi* IRSNB 16736	54.01
Megaelosia goeldii	54.01	*Stefania riae* VUB 3754	54.01
Melanophryniscus klappenbachi	54.01	*Stefania riveroi* IRSNB 15703	54.01
Myersiohyla kanaima	54.01	*Stefania roraimae* VUB 3311	54.01
Noblella lochites	54.01	*Stefania satelles* IRSNB 16728	54.01
Noblella sp. MTD 45180	54.01	*Stefania scalae*	54.01
Nyctimystes infrafrenatus	54.01	*Stefania schuberti*	54.01
Nymphargus bejaranoi	54.01	*Stefania* sp. 10	54.01
Odontophrynus achalensis	54.01	*Stefania* sp. 1 LM 1056	54.01
Oreobates cruralis	54.01	*Stefania* sp. 2 IRSNB 16734	54.01
Oreobates saxatilis	54.01	*Stefania* sp. 3 VUB 3755	54.01
Paratelmatobius cardosoi	54.01	*Stefania* sp. 4 IRSNB 16730	54.01
Phrynopus auriculatus	54.01	*Stefania* sp. 5 IRSNB 16727	54.01
Phrynopus bracki	54.01	*Stefania* sp. 6 VUB 3266	54.01
Phyllodytes luteolus	54.01	*Stefania* sp. 7 VUB 3548	54.01
Physalaemus cuvieri	54.01	*Stefania* sp. 8	54.01
Physalaemus gracilis	54.01	*Stefania* sp. 9 VUB 3555	54.01
Phyzelaphryne miriamae	54.01	*Stefania woodleyi* VUB 3282	54.01
Pleurodema brachyops	54.01	*Strabomantis biporcatus*	54.01
Pleurodema diplolister	54.01	*Strabomantis bufoniformis*	54.01
Pristimantis cruentus	54.01	*Telmatobius truebae*	54.01
Pristimantis curtipes	54.01	*Thoropa miliaris*	54.01
Pristimantis gaigeae	54.01	*Trachycephalus typhonius*	54.01
Pristimantis ridens	54.01	*Uperoleia laevigata*	54.01
Pristimantis simonsii	54.01	*Yunganastes ashkapara*	54.01
Proceratophrys avelinoi	54.01	*Yunganastes fraudator*	54.01
Pseudis minuta	54.01	*Zachaenus parvulus*	54.01
Pseudopaludicola falcipes	54.01		
Psychrophrynella guillei	54.01		

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