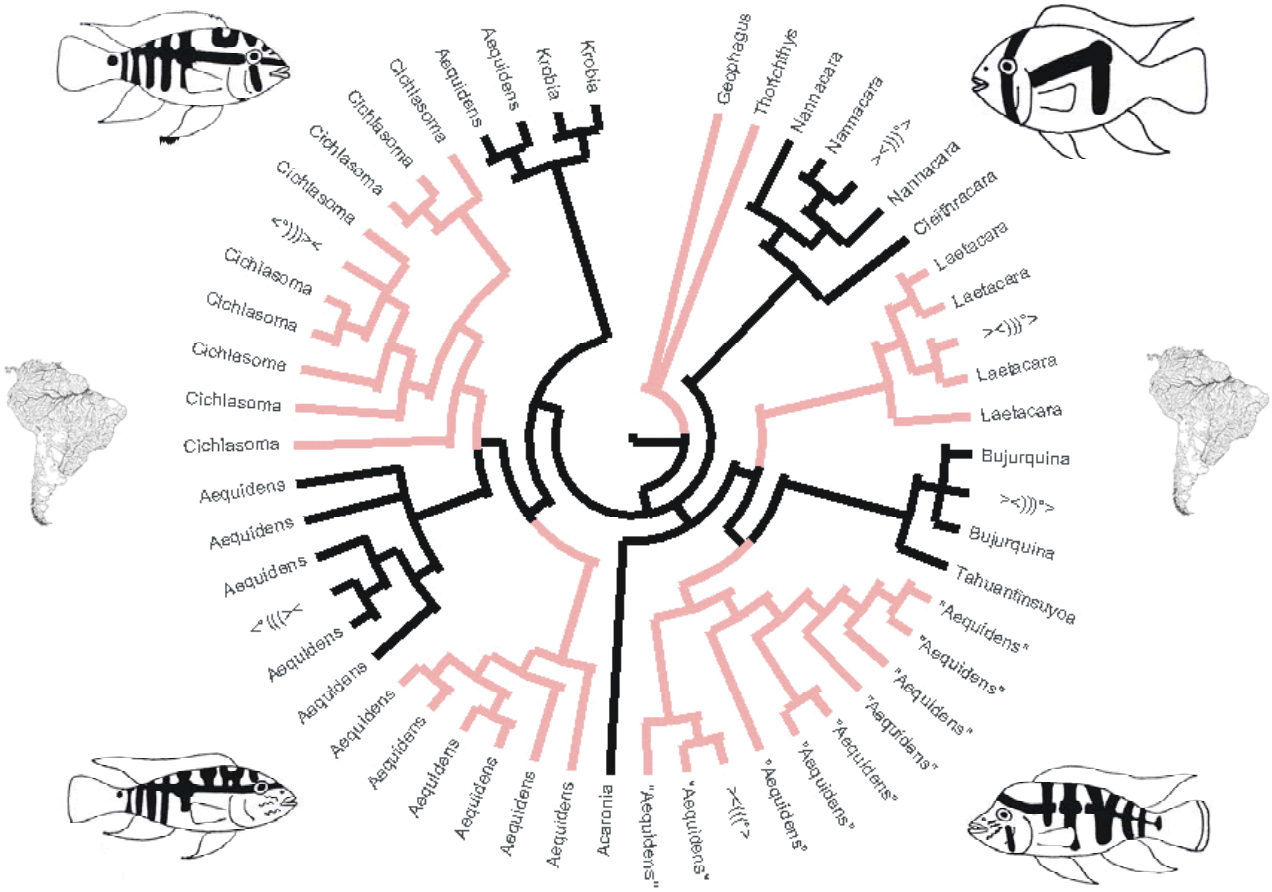
Molecular phylogeny of Neotropical cichlids of the tribe Cichlasomatini (Perciformes: Cichlidae: Cichlasomatinae) with biogeographic implications

Fylogeneze a biogeografie Neotropických cichlid tribu Cichlasomatini (Perciformes: Cichlidae: Cichlasomatinae)



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Abstract:

The tribe of the cichlid fishes, Cichlasomatini Kullander, 1998, comprises to date 69 valid species living in South America. This Neotropical assemblage contains genera *Aequidens, Cichlasoma (sensu stricto), Krobia, Bujurquina, Tahuantinsuyoa, Cleithracara, Laetacara, Nannacara* and *Acaronia* and the *´Aequidens´* species groups at present.

The presented thesis represents the first molecular study dealing with more extensive sampling of species of the tribe Cichlasomatini. Sixty-three representatives of all nine described genera and taxonomically unresolved species group are included. Herein both mitochondrial (16S rRNA, cytochrome b), and nuclear (first intron in S7 ribosomal gene) markers were used for study of the phylogenetic relations.

The methods of Maximum Parsimony, Maximum Likelihood and Bayesian methodsm of phylogenetic analysis were based on sequences of total length 1542 bp.

The influence of every gene signal to each node of the concatenated tree was explored using the Partitioned Bremer Support. The test of concatenation of genes and the test of other alternative hypothesis was performed by Approximately Unbiased Test.

The method of Molecular Clock was applied to estimate the divergence time within *´Aequidens´* and *Cichlasoma* lineages.

Main conlusions of this thesis:

1) Both *´Aequidens´* species groups always form the well supported monophylum closely related to the genera *Bujurquina* and *Tahuantinsuyoa*.

2) *Aequidens*, *Cichlasoma* and *Krobia* form <u>together</u> other monophyletic clade.

3) *Aequidens* as a genus is <u>not</u> monophyletic, species of the genus *Cichlasoma* create its inner group.

4) The species of *Aequidens potaroensis* does not belong to *Aequidens* genus, it rather appears to be the species of *Krobia* genus.

5) *Nannacara* genus forms the sister group to *Cleithracara*.

6) The position of *Acaronia* and *Laetacara* genera remains unresolved.

7) The hypothetic ancestor of the tribe Cichlasomatini originated in the Guyana rivers or in Amazonia.

Abstrakt:

Neotropický tribus cichlid, Cichlasomatini Kullander (1998), zahrnuje dnes 69 platných druhů. Tato skupina obsahuje rody *Aequidens, Cichlasoma (sensu stricto), Krobia, Bujurquina, Tahuantinsuyoa, Cleithracara, Nannacara, Laetacara* a *Acaronia* a skupinu druhů označovaných jako *´Aequidens´* (dosud u nich nebyla provedena taxonomická revize a nebylo pro ně stanoveno rodové jméno).

Předkládaná práce, zaměřená výhradně na tribus Cichlasomatini, je první molekulární studií zkoumající rozsáhlejší počet taxonů. V práci je zahrnuto 63 zástupců všech devíti popsaných rodů a taxonomicky nejasné skupiny druhů. Ke studiu fylogenetických vztahů byly použity jak mitochondriální (16S rRNA, cytochrom b) tak i jaderné markery (intron v ribosomálním genu S7).

Metoda maximální parsimonie, maximální věrohodnosti a Bayesovské metody byly použity při fylogenetických analýzách sekvenčních dat o délce 1542 nukleotidů.

Vliv každého genu na jednotlivé uzly ve výsledném stromu byl testován za použití metody Partitioned Bremer Support. Možnost konkatenace genů a další alternativní hypotézy byly testovány pomocí Approximately Unbiased testu.

Metoda Molecular Clock byla použita při odhadu času divergence linií v rámci skupiny *´Aequidens´* a v rámci rodu *Cichlasoma*.

Hlavní výsledky této práce:

1) Skupiny druhů *´Aequidens´ pulcher* a *´Aequidens´ rivulatus* jsou sesterské a tvoří monofylum spolu s rody *Bujurquina* a *Tahuantinsuyoa*.

2) *Aequidens*, *Cichlasoma* a *Krobia* tvoří společně další monofyletickou skupinu.

3) Rod *Aequidens* není monofyletický a rod *Cichlasoma* je jeho vnitřní skupinou.

4) Druh *Aequidens potaroensis* nepatří do rodu *Aequidens*, ale náleží pravděpodobně do rodu *Krobia*.

5) Rod *Nannacara* je sesterským taxonem k rodu *Cleithracara*.

6) Pozice rodů *Acaronia* a *Laetacara* zůstává stále nevyřešena.

7) Hypotetický předek tribu Cichlasomatini pocházel z Amazonské či Guyanské oblasti.

Resumo (PT)

A tribo de ciclídeos, **Cichlasomatini** Kullander (1998), contem 69 espécies válidas, existentes na América do Sul. Esta reunião Neotrópica inclui os géneros *Aequidens, Cichlasoma (sensu stricto), Krobia, Bujurquina, Tahuantinsuyoa, Cleithracara, Nannacara, Laetacara*, *Acaronia* e o grupo *´Aequidens´* conjunto de espécies sem designação genérica válida. Esta tese é o primeiro estudo molecular mais abrangente com um número de amostras extensivo na tribo Cichlasomatini, 63 representantes dos nove géneros descritos e dos grupos de espécies sistemáticamente indefenidos.

Neste trabalho foram usados dois marcadores mitocondriais (16S rRNA, citocroma b) e dois marcadores nucleares (intron em S7 ribosomal géne) para determinar as relações filogenéticas. Os metódos filogenéticos de Maximum Parsimony, Maximum Likelihood e os metódos Bayesianos foram baseados em dados de DNA de 1542 nucleótidos. A influência de todos os genes aos vínculos da melhor árvore parsimonial foi obtido através do metódo de Partitioned Bremer Support. A possibilidade de concatenação dos genes e as hipóteses alternativas das árvores foram testadas usando o metódo de Approximately Unbiased Test. O metódo de Molecular Clock foi usado para estimar o tempo da divergência dentro das clades de grupo *´Aequidens´* e do género *Cichlasoma*.

As maiores conclusões deste trabalho:

1) As espécies *´Aequidens´ pulcher* e *´Aequidens´ rivulatus* são clades irmãs, e criam sempre um grupo monofilético bem apoiado pelos géneros *Bujurquina* e *Tahuantinsuyoa*.

2) *Aequidens*, *Cichlasoma* e *Krobia* formam uma clade monofilética.

3) O género *Aequidens* não é monofilético contendo um grupo interno do género *Cichlasoma*.

4) *Aequidens potaroensis* não pertenece ao género *Aequidens* sendo mais provável a sua proximidade ao género *Krobia*.

5) O género *Nannacara* está mais relacionado com o género *Cleithracara*.

6) A posição dos grupos *Acaronia* e *Laetacara* permanece ainda por esclarecer sendo a sua posição indeterminada.

Abstracto (ES)

La tribu de cíclidos, **Cichlasomatini** Kullander (1998) contiene 69 especies que viven en América del Sur. Esta agrupación Neotropical comprende géneros *Aequidens, Cichlasoma (sensu stricto), Krobia, Bujurquina, Tahuantinsuyoa, Cleithracara, Nannacara, Laetacara* y *Acaronia,* y el *´Aequidens´* grupos de especies (hasta hoy sin nombre genérico válido).

Esta tesis es el primer estudio molecular con el número de muestras más extenso en Cichlasomatini. Los representantes de los nueve géneros descritos y los dos grupos de especies taxonómicamente sin resolver están incluidos.

En este trabajo, los dos marcadores mitocondriales (16S rRNA, citocromo b) y nucleares (intron en S7 ribosomal gen) fueron utilizados para explorar las relaciones filogenéticas.

Los métodos filogenéticos de Maximum Parsimony, Maximum Likelihood y los métodos Bayesianos se basaron en los datos de DNA de 1542 nucleótidos.

La influencia de todos los genes en los nodos del mejor árbol parsimonial fue estudiada aplicando el método de Partitioned Bremer Support. La posibilidad de concatenación de genes y las hipótesis alternativas de topología de árbol se examinaron usando el método de Approximately Unbiased Test.

El método de Molecular Clock fue usado para estimar el tiempo de la divergencia dentro de los clados del grupo *´Aequidens´* y del género *Cichlasoma*.

Principales conclusiones de este trabajo:

1) Los dos grupos de especies *´Aequidens´ pulcher* y *´Aequidens´ rivulatus* son clados hermanos, y siempre generan el monofilo tolerado por los géneros de *Bujurquina* y *Tahuantinsuyoa*.

2) *Aequidens*, *Cichlasoma* y *Krobia* se encuentran juntos en otro clado monofilético.

3) *Aequidens* como género, no es monofilo, pero tiene el grupo interno de género *Cichlasoma*.

4) La especie *Aequidens potaroensis* no pertenece al género *Aequidens*, parece más probable que corresponda al género *Krobia*.

5) El género *Nannacara* es el más aproximado al *Cleithracara*.

6) La posición de los géneros *Acaronia* y *Laetacara* permanece sin resolver.

Contains

1 Introduction

1.1 The concepts of cichlid taxonomy and phylogeny

The family Cichlidae with the geographic distribution covering recent South and Central America, West Indies, Africa, Levant, Syria, Iran, India, Sri Lanka, and Madagascar (STIASSNY, 1991, NELSON, 1994, MURRAY, 2001) is composed of more than 1500 valid species up to date (FROESE & PAULY, 2006). Despite the geographic range is often considered to be Gondwanan distribution (SPARKS & SMITH, 2004), there is neither recent nor fossil evidence from the Australia continent (MURRAY, 2001).

The subfamily Etroplinae containing the Indian and the part of Madagascan taxa forms the basal clade of cichlids (SPARKS & SMITH, 2004, FARIAS ET AL., 2001). The rest of cichlids being composed of the basal subfamily Ptychochrominae (Madagascar; SPARKS & SMITH, 2004), one of two sister clades, Neotropical Cichlids, the subfamily Cichlinae sensu SPARKS & SMITH (2004), the other of African Cichlids, the subfamily Pseudocrenilabrinae (FARIAS ET AL., 2000, 2001). This basic topology is based on the molecular or both morphological and molecular data. There is another different topology from KULLANDER (1998), in which African, Madagascan and Indian taxa are organized as two sister clades, forming a monophylum relative to the Netropical clade. In SPARKS & SMITH (2004) however, there is the best molecular report about the cichlid phylogeny, where the monophyly of major geographic clades was very well supported.

The monophyly of Neotropical cichlid was not, however, well supported. There is one African genus, *Heterochromis*, included inside the Neotropical clade by KULLANDER, (1998) and also by FARIAS ET AL. (1999) but with the questionable support of clades only.

KULLANDER (1998) established the group Cichlasomatini as a tribe and revised especially the phylogeny of Neotropical Cichlids. In his work, based on the morphological characters, KULLANDER (1998) established four new subfamilies and five new tribes. According to KULLANDER (1998) the subfamily Cichlasomatinae contained three tribes – Acaroniini, Heroini and Cichlasomatini. The separation of the genus *Acaronia* as independent clade was negated later (FARIAS ET AL., 1999, 2000, 2001, SPARKS & SMITH, 2004), but none of these works was focused on the Cichlasomatini tribe in more detail.

The intraclade phylogeny of the Neotropical cichlids was studied on the basis of morphological (STIASSNY, 1991, KULLANDER, 1998), and molecular or combined datasets (FARIAS, 1999, 2000, 2001, SPARKS & SMITH, 2004, LÓPEZ-FERNÁNDEZ, 2005).

KULLANDER (1998) established the systematics according to his morphology-based results (see topology in Fig. 1).

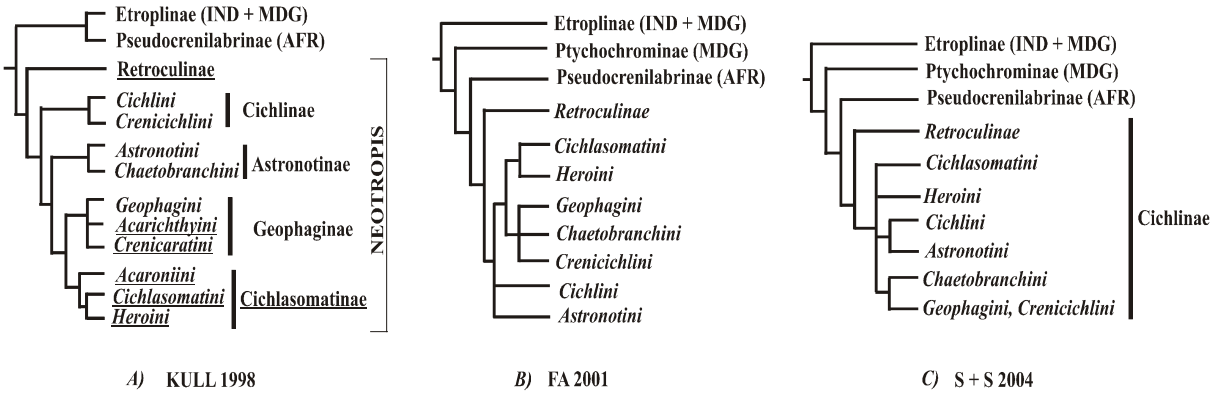


Fig. 1: Phylogenetic tree of the family Cichlidae, A) according to KULLANDER (1998); based on the morphological data, underlined taxa were newly established in KULLANDER (1998); B) according to FARIAS ET AL. (2001); based on the cytochrome b gene sequences; C) following SPARKS & SMITH (2004); based on the molecular data, four genes used, two mitochondrial and two nuclear. See also the incongruence between the subfamily Cichlinae sensu KULLANDER (1998) and sensu SPARKS & SMITH (2004). Modified.

Several points of the studies of FARIAS ET AL. (1999, 2000, 2001), LOPÉZ-FERNANDÉZ ET AL. (2005), and SPARKS & SMITH (2004) comparing with the KULLANDER´S (1998) taxonomical organisation:

- The *Retroculus* clade is always basal taxon of the Neotropical Cichlids.

- The monophyly of the subfamily Cichlinae (sensu KULLANDER, 1998, see Fig.1) was not supported, the tribe Crenicichlini is more related to the subfamily Geophaginae (LOPÉZ-FERNANDÉZ ET AL., 2005, FARIAS, 1999) whereas the tribe Cichlini appears to be more basal

- The monophyly of the subfamily Astronotinae (sensu KULLANDER, 1998) was also not supported, the tribe Chaetobranchini is more related to the tribe Geophagini (FARIAS 2000, 2001, SPARKS AND SMITH, 2004), whereas the tribe Astronotini are considered like basal taxa (FARIAS, 2001)

- The monophyly of the subfamily Cichlasomatinae (sensu KULLANDER, 1998) is supported (FARIAS ET AL., 1999, 2000, 2001) or the trees are unresolved with regard to this question (SPARKS & SMITH, 2004).

1.2 The formation of the tribe Cichlasomatini

KULLANDER (1983a) revised the *Cichlasoma* group *sensu stricto,* which contained four species at that time (*C. bimaculatum, C. taenia, C. dimerus* and *C. portalegrense*), and he newly described eight more species from South America (see the Appendix 3).

This revision more or less devided the species sorted to date to the catch-all genus *Cichlasoma* (*Cichlasoma* sensu lato according to REGAN (1905b) with more than 100 species). KULLANDER (1983a) separated the *Cichlasoma* (*sensu stricto)* species from the rest, which are assumed like *´Cichlasoma´ sensu lato* or already have established own generic name (e.g. ŘÍČAN & KULLANDER, 2006). All of the *´Cichlasoma´* species belong now to the sister tribe Heroini.

Three years later, KULLANDER (1986) established three new genera within Cichlasomatini, i.e. *Bujurquina, Laetacara* and *Tahuantinsuyoa*, which were separated from the former genus *Aequidens (sensu lato)*. In 1989, the same author newly established two genera, *Krobia* and *Cleithracara*, also separating the species from the group *Aequidens (sensu lato)* (KULLANDER, 1989).

The genus *Nannacara* was established by REGAN (1905a).

STIASSNY (1991) separated the recent Cichlasomatinae species into two groups called Cichlasomines Group A and Cichlasomines Group B. These Groups A and B were later renamed as Heroines and Cichlasomines, respectively (KULLANDER, 1996). Later in KULLANDER (1998), these groups got the status of the tribes Heroini and Cichlasomatini. The only difference was in the genus *Acaronia* – STIASSNY (1991) put *Acaronia* to the Group A (recent Heroines), whereas KULLANDER (1998) established an independent tribe Acaroniini, which forms sister group to both Cichlasomatini and Heroini tribes (see Fig. 1).

1.3 Diagnosis of the tribe Cichlasomatini

The large scales and four dentary lateralis foramina are the synapomorphic characters of Cichlasomatini (KULLANDER, 1983a, 1998). Cichlasomatini have strictly triserial or uniserial predorsal squamation pattern whereas other cichlids have more stochastic patterns (STIASSNY, 1991). Cichlasomatini also differ in behaviour, they lay the eggs in the compact circular plaque whereas other substrate-spawning cichlids lay eggs in loose or non-circular more open plaques (STIASSNY, 1991).

Complete names of taxa (with author(s) and year of description) are mentioned in the Appendix 3.

1.4 Short characteristics of genera belonging to the tribe Cichlasomatini

The following text contains the short characteristics of all genera currently placed in the tribe Cichlasomatini. I listed selected chracters only, for more osteological characters see KULLANDER (1983a, 1986,1989).

Aequidens Eigenmann & Bray, 1894

The type species *Aequidens tetramerus* (as *Acara tetramerus* Heckel, 1840).

Aequidens was a catch-all group, which was revised by KULLANDER (1983, 1986 and 1989), and five new genera were established here (see above). Up to the present we have 16 species of the genus *Aequidens* (FROESE & PAULY, 2006).

The rest of the *Aequidens (sensu lato)* group at that time, which have not yet got new genus name remains as a *´Aequidens´* species groups (see below).

In the same manner, *Guianacara* (recent Geophaginae subfamily) was classified as the member of *Aequidens (sensu lato)* group.

The genus *Aequidens* has the Amazonian, Guyanan, Orinoco and upper Paraguayan geographical distribution (see the map in the Appendix 4.2).

Selected morphological character: The genus *Aequidens* differs from the genus *Cichlasoma* by naked dorsal and anal fins (*Cichlasoma* has scaled fins). *Aequidens* also has the caudal peduncle with 2-3 complete vertebrae (*Cichlasoma* none; KULLANDER, 1983a). *Aequidens* has three spins in the anal fin (like *Krobia*), whereas *Cichlasoma* has four anal fin spins (KULLANDER 1989). *Aequidens* has naked preoperculum (KULLANDER 1983a) in contrast to *Cichlasoma* where it is scaled. Predorsal squamation has triserial pattern. Standard length that *Aequidens* species reach is about 100 – 120 mm (exceptionally 160 mm in *Aequidens tetramerus*).

Cichlasoma Swainson, 1839

The type species *Cichlasoma bimaculatum* (originally *Labrus bimaculatus* Linnaeus, 1758).

Cichlasoma was the large catch-all group with more than 100 species which was revised by KULLANDER (1983a). The *Cichlasoma (sensu stricto)* group contains 12 species up to present (KULLANDER, 1983a, FROESE & PAULY, 2006).

The *Cichlasoma* genus has the large geographic distribution including Orinoco, Amazonian, Guyanan, Paraná river basins and basins of the Eastern Brazil rivers. See the map in the Appendix 4.5.

Selected morphological characters: *Cichlasoma* species have usually scaled dorsal and anal fins, which divides them from *Aequidens, Bujurquina, Tahuantinsuyoa* and *Nannacara*. *Cichlasoma* also has

five bars between the midlateral spot and the spot in the caudal fin base. Other genera have only four (ŘÍČAN ET AL, 2005, KULLANDER, 1989) or even less bars (KULLANDER, 1983a). Predorsal squamation has the triserial pattern except for two species (KULLANDER, 1989). *Cichlasoma* has 13 abdominal + 13 caudal vertabrae in contrast to *Laetacara, Cleithracara* and *Nannacara*, that have fewer – 12 + 12, 11 + 13 respectively (KULLANDER, 1983a, see more in other genera). *Cichlasoma* has scaled preoperculum (KULLANDER, 1983a) like *Laetacara* and *Nannacara,* and in contrast to *Aequidens*, which has it naked. *Cichlasoma* can reach the 100 mm SL.

Bujurquina Kullander, 1986

The type species *Bujurquina moriorum* Kullander, 1986.

Until 1986, *Bujurquina*-species were referred as *´Aequidens´ syspilus* group (KULLANDER, 1983a). The genus Bujurquina contains up to date 17 valid species (FROESE & PAULY, 2006). The species are very similar one to each other and it is difficult to identify them (KULLANDER, 1986). *Bujurquina* species are larvophile mouth-brooders, they keep their newly hatched juveniles (not eggs) in the mouth. (KULLANDER, 1986). The geographic distribution covers the upper and middle Amazon river with the tributaries, Orinoco and Paraná rivers. See the map in the Appendix 4.4.

Selected morphological characters: *Bujurquina* has uniserial predorsal squamation pattern (KULLANDER, 1986), shared with *Tahuantinsuyoa, Krobia, Nannacara, ´Aequidens´* and two species of *Cichlasoma*. The lateral band is oblique, like in *Krobia* and *Tahuantinsuyoa*. The anal and dorsal fins are naked (like *Aequidens, Bujurquina, Tahuantinsuyoa, Laetacara* and *Nannacara*). Bujurquina has 13 + 13 vertebrae (like *Cichlasoma, Aequidens* and *Krobia*)

Tahuantinsuyoa Kullander, 1986

The type species *Tahuantinsuyoa macantzatza* Kullander, 1986. To date there are two valid species in this genus (FROESE & PAULY, 2006, KULLANDER, 1991).

This genus is closely related to *Bujurquina*. *Tahuantinsuyoa* also represents the larvophile mouthbrooder as *Bujurquina* (KULLANDER, 1986). Both species have geographic range in the upper Ucayali basin in Peru (Amazon). See the map in the Appendix 4.10.

Selected morphological characters: *Tahuantinsuyoa* has the uniserial predorsal squamation pattern (KULLANDER, 1986), shared with *Bujurquina.* The differences are in the orbital stripe (the lateral stripe continues above the orbita towards the nostrils in *Bujurquina*, or there is oblique caudodorsaly going stripe in *Tahuantinsuyoa*, respectively). *Tahuantinsuyoa* has one caudal vertebra more than *Bujurquina*. (KULLANDER, 1986).

***Laetacara* Kullander, 1986**

The type species *Laetacara flavilabris* (as *Acara flavilabris* Cope, 1870). Until 1986 referred as the *´Aequidens´ dorsiger* group (in KULLANDER, 1983a). Up to date, there are four species and several undescribed forms (FROESE & PAULY, 2006, Neodat II database, STAWIKOWSKI & WERNER, 1998). Geographic distribution comprises the Amazon river, upper Orinoco river and Paraná river basins. See the map in the Appendix 4.8.

Selected morphological characters: *Laetacara* has scaly preoperculum (similar to *Nannacara, Cichlasoma*). Also in osteological characters *Laetacara* is most similar to *Cichlasoma* and *Nannacara* (see KULLANDER, 1986). *Laetacara* has the triserial predorsal squamation (like *Cichlasoma, Aequidens* and *Cleithracara*) (KULLANDER, 1986). There is absence of the caudal spot like in *Cleithracara* and *Nannacara*; one species (*L. thayeri*) has scaled anal and dorsal fins (like *Cichlasoma* and *Cleithracara*) (KULLANDER, 1983a). *Laetacara* has the number of vertebrae: 12 abdominal + 12 caudal (exceptionally 12 + 11), like *Cleithracara* (KULLANDER, 1983a, 1986).

***Krobia* Kullander, 1989**

The type species *Krobia guianensis* (as *Acara guianensis* Regan, 1905a). There are only two species (*K. guianensis, K. itanyi*; FROESE & PAULY, 2006) but at least two additional undescribed forms more (KULLANDER, 1989).

Until 1989 *Krobia* species were referred as the *´Aequidens´ guianensis* group (in KULLANDER, 1983a).

The geographic distribution includes the Guyanan rivers (see the map in the Appendix 4.7), and one of the undescibed forms, *Krobia* sp. „Xingu“, lives in the Xingu river drainage (Amazon).

Selected morphological characters: The *Krobia*-species have uniserial predorsal squamation, the lateral band is oblique, going from head towards the end of the dorsal fin. This character is shared with *Bujurquina* and *Tahuantinsuyoa* (KULLANDER, 1989). *Krobia*-species have scaled anal and dorsal fins (like Cichlasoma) (KULLANDER, 1989). They have 3-4 vertebrae in the caudal peduncle (like *Aequidens*), and three anal fin spins (like *Aequidens*) (KULLANDER, 1989).

***Cleithracara* Kullander, 1989**

The type and the only species *Cleithracara maronii* (as *Acara maronii*, Steindachner, 1882). Until 1989 known as *´Aequidens´ maronii* (in KULLANDER, 1983a).

Cleithracara has Guyanan geographic distribution with the rare records from Trinidad (KULLANDER, 1983a). See the map in the Appendix 4.6.

Selected morphological characters: *Cleithracara* has triserial predorsal squamation, extensively scaled fins (shared with *Cichlasoma*), scaled praeoperculum (shared with *Nannacara, Laetacara*) (KULLANDER, 1989). Cleithracara has the number of vertebrae: 12 abdominal + 12 caudal (exceptionally 12 + 11) (KULLANDER, 1983a, 1989). *Cleithracara* can reach the maximum size of 71 mm SL (KULLANDER, 1989).

Nannacara Regan, 1905

The type species *Nannacara anomala* Regan, 1905. Currently, there are six valid species in this genus. (FROESE & PAULY, 2006).

Geographic range includes the Guyanan rivers and the downstream of the Orinoco river (see the map in the Appendix 4.9).

Selected morphological characters: *Nannacara* has 7 + 7 principal caudal fin rays, instead of 8 + 8 as all other cichlids (KULLANDER, 1989). *Nannacara* has the uniserial predorsal squamation, which, however, differs from the other genera with the uniserial pattern *Krobia, Bujurquina* and *Tahuantinsuyoa* (KULLANDER, 1989). *Nannacara*-species have the caudal peduncle without vertebrae (like *Cichlasoma*), and have the number of total vertebrae 11 abdominal + 13 caudal (which differs from all genera in the tribe except *Acaronia* (KULLANDER, 1989)). *Nannacara* has naked anal and dorsal fin (like *Aequidens*, *Bujurquina* and *Tahuantinsuyoa*).

Acaronia Myers, 1940

The type species *Acaronia nassa* (as *Acara nassa* Heckel, 1840). The genus comprises two species at present (KULLANDER, 1989, FROESE & PAULY, 2006).

The geographic distribution contains Guyanan rivers, Orinoco and Amazon basins (see the map in the Appendix 4.1). This genus has not been traditionaly classified in the tribe Cichlasomatini (KULLANDER, 1983b, 1998, STIASSNY, 1991) but recent molecular studies suppose the possible validity of this hypothesis (FARIAS ET AL., 1999, 2000, 2001, SPARKS & SMITH, 2004, see also text above).

Selected morphological characters: *Acaronia* has extremely large mouth and the structure of gill aparatus is very different from the other genera, possibly with respect to the piscivory (KULLANDER, 1983b). Probably this was the reason for considering *Acaronia* as outstanding taxa like in KULLANDER (1998). The anal and dorsal fins are naked (like *Aequidens*, *Bujurquina, Tahuantinsuyoa* and *Nannacara).Acaronia* has only 12 + 12 or 11 + 13 vertebrae (like *Nannacara*, *Cleithracara* and *Laetacara*)

´Aequidens´ species groups

Up to date, six species are members of one monophyletic clade and one additional species (*´Aequidens´ hoehnei*) which is probably not monophyletic with the others, but remains still unclassified (KULLANDER, 1983a, STAWIKOWSKI & WERNER, 1998).

KULLANDER (1983a) considered these species as *´Aequdiens´ pulcher* species group with six species. Later, KULLANDER (1998) established two groups: *´Aequidens´ pulcher* group with three species and *´Aequidens´ rivulatus* group with remaining three species. The geographic distribution covers the Orinoco river, the Maracaibo rivers, the Magdalena river, the Pacific slope of South America and Panaman and Costa Rican rivers. The species *´Aequidens´ hoehnei* lives in the upper Araguaia river (Amazon). See the map in the Appendix 4.3.

Selected morphological characters: *´Aequidens´* has four bars between the midlateral spot and the spot in the caudal fin base (the similar coloration pattern to the genera *Aequidens, Bujurquina, Tahuantinsuyoa, Krobia, Acaronia* and *Laetacara*) *´Aequidens´* has the blue reflecting colour pattern on the head and sides (shared characters with several *Aequidens*-species, *Bujurquina* and *Tahuantinsuyoa*).

1.5 Suggested phylogenetic relationships within the tribe Cichlasomatini

Up to date, there has not yet been published any study focused exclusively on the phylogenetic relations within the Cichlasomatini tribe. Several studies solved the phylogenetic relationships within the whole Neotropical cichlid clade (KULLANDER, 1998,

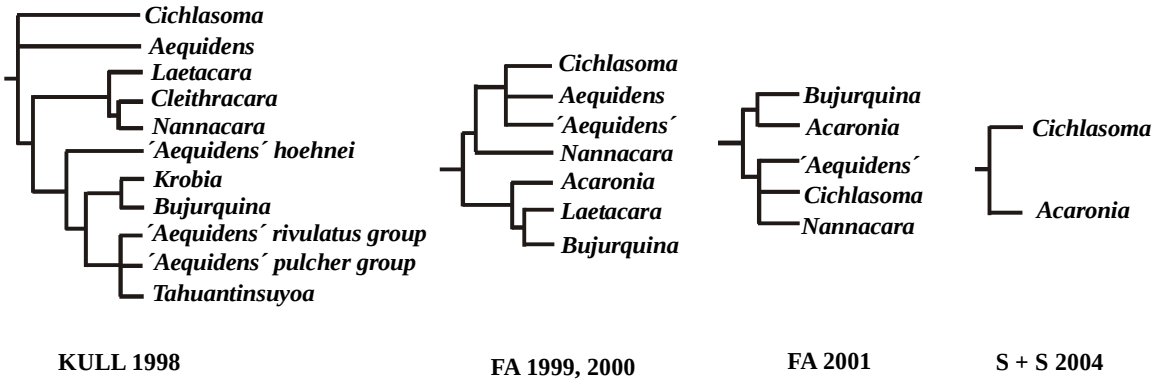


Fig. 2: Results of published studies on Neotropical cichlids, none of them focused on Cichlasomatini exclusively, topologies are parts of larger trees concerning about Cichlasomatini from following papers: KULL 1998 = KULLANDER (1998), tree based on the morphological characters, FA 1999, 2000 = topology from FARIAS ET AL. (1999), based on molecular data of cytochrome b gene and on Total evidence method data (FARIAS ET AL. 2000). FA 2001 = FARIAS ET AL. (2001) topology based on cytochrome b gene molecular data. Modified.

FARIAS, 1999, 2000, 2001) or even within the whole Cichlidae family (SPARKS & SMITH, 2004). See the topologies in Figs. 1 - 2.

There are several conflicts in the results of the studies, shown in Fig. 2.

Firstly, *Acaronia* resulted into the tribe Cichlasomatini in all molecular and Total evidence analysis (FARIAS ET AL., 1999, 2000, 2001, SPARKS & SMITH, 2004) whereas in KULANDER´s (1998) morphological study, *Acaronia* stands outside of the tribe Cichlasomatini, being classified in the tribe Acaroniini (not shown in Fig. 2).

Except of KULLANDER (1998) there have never been included species of all described genera of the tribe in the study of phylogenetic relations.

The relations of the genus *Krobia* to the other genera has not yet been studied by molecular dates, we only know the result from the morphological study (KULLANDER 1998). This suggests that *Krobia* is sister taxon to *Bujurquina*.

Genera *Bujurquina* and *Tahuantinsuyoa*, considered as sister genera by KULLANDER (1989), were, however, separated into two clades in KULLANDER (1998; see Fig. 2) clustering with other genera.

The genera *Cichlasoma* and *Aequidens* appear to be the close relatives, according to the morphological (KULLANDER, 1998) and molecular data (FARIAS, 1999, 2000).

The positions of the genera *Nannacara* and *Laetacara* that are both included in FARIAS (1999, 2000, 2001) also differs from the morphology-based study (KULLANDER, 1998).

1.6 Geographical distribution and historical biogeography of cichlids

The origin of cichlid is considered to be in Madagascar, from where two lineages diverged, one to India and one to the African continent (MURRAY, 2001). The common ancestor of the Neotropical cichlids separated from the African cichlids (FARIAS, 1999, 2000, 2001, SPARKS & SMITH, 2004, KULLANDER, 1998, STIASSNY, 1991).

There are two alternative hypotheses of the estimation of the time when Neotropical and African cichlids separated. First, there is the hypothesis of the continental distribution considering that the separation had to occur before the African and South American continent separation (i.e. ~100 Mya; FARIAS, ET AL., 1999, SPARKS & SMITH, 2004). The alternative hypothesis counts with the over-ocean distribution after the continent separation, in ~ 55 Mya. The ancestor reached the South American continent due to the ocean streams and along the zone of ocean islands (MURRAY, 2001). Because of the lack of fossil material from the period of 50 – 100 Mya and because of the high level

of cichlid tolerance to the salt water, the second hypothesis appears to be more probable (MURRAY, 2001).

1.6.1 Provinces of fish distribution in South America

In South America there are ten fish faunal provinces and Cichlasomatini live in nine o them. There is the province of Patagonian rivers in the South of the continent, where no representatives of Cichlasomatini live (After STAWIKOWSKI & WERNER, 1998, LUNDBERG ET AL., 1998). See Fig. 18.

- ⇨ Amazon river basin
- ⇨ Orinoco river basin
- ⇨ Guyanan rivers
- ⇨ Paraná river basin
- ⇨ Magdalena river basin
- ⇨ Maracaibo rivers
- ⇨ Pacific slope of South America
- ⇨ Uruguay river basin
- ⇨ Eastern Brazil rivers

Species of the tribe Cichlasomatini live in the rivers of South America, and one species (*´Aequidens´ coeruleopunctatus*) extends to Central America up to the Southern Costa Rica (Neodat II database, STAWIKOWSKI & WERNER, 1998). The southern border of geographic distribution of the tribe Cichlasomatini is the Paraná river and its tributaries. Several *Cichlasoma* species, one *Laetacara* and one *Bujurquina* species can be found in the Paraná river (Neodat II database, FROESE & PAULY, 2006, KULLANDER, 1986; see the map in the Appendix 4.4, 4.5 and 4.8). Cichlasomatini have the cis- and trans-Andean distribution, there are three trans-Andean species (from the *´Aequidens´ rivulatus* species group) spread in the rivers of the whole Pacific slope of Andes down to Lima city in Peru (Neodat II database, STAWIKOWSKI & WERNER 1998).

The most of Cichlasomatini-species live in the Amazon and Orinoco river basins, and there are also various species living in the Guyanan rivers, which flow down from the Guyana Shield. (Neodat II database, FROESE & PAULY, 2006). Only few species of this tribe are encountered in the rivers of Eastern Brazil, which are not connected with the Amazon river (several *Cichlasoma* and one *Aequidens* species, see the map in the Appendix 4.2 and 4.5).

There are three species with very large geographic distribution, *Cichlasoma bimaculatum* and *Aequidens tetramerus*, living in the Orinoco, Amazon, Guyana, Eastern Brazil and the Northern Paraná rivers, and there is also *Acaronia nassa* with the Amazonian, Orinoco and Guyanan geographic distribution (Neodat II database, FROESE & PAULY, 2006, STAWIKOWSKI & WERNER, 1998; see the map in the Appendix 4.1, 4.2 and 4.5).

The geological history of the Neotropical area was very complicated and it was ifluenced mainly by the uplift of Andes and by the sea level oscillations during the geological periods (LUNDBERG ET AL., 1998).

South America formed the part of the former Gondwana continent. In the middle Cretaceous (130 – 120 Mya = million years ago) the separation of the South American and African continents initiated (SPARKS & SMITH, 2004) and about 105 Mya only the continental bridge persisted between the recent Eastern Brasil (South America) and the coast of recent Guinea gulf (Africa). The uplift of Andes started at about 90 Mya in Late Cretaceous (LUNDBERG ET AL., 1998).

Firstly, in South America, there was long major river going in the direction from the South to the North practically cross the whole continent along the forming Andes (from recent middle Chile to recent Colombia) and the rivers from the western middle part of the continent had flown into its basin. Later, because of the creation of Andes, the rivers flew more in the west-eastern direction. The recent Amazon river was separated into two basins till the Late Miocene (8 Mya; LUNDBERG ET AL., 1998).One river was flowing eastward (recent lower Amazon) and the other (recent upper and middle Amazon) flew westward and it was forming the tributary of the major river, which flew along Andes. The similar separation subsisted also in the recent Orinoco river.

The Cordillera Oriental, Cordillera Occidental and Cordillera Central and Mérida Andes take place in recent Colombia and Venezuela, respectively, and they are very important geographic barrier in the expansion of species in the west-east direction (LUNDBERG ET AL., 1998) in the Northern part of the South American continent. The Mérida Andes and Cordillera Oriental are found in the northwestern part of the continent and separate the recent Orinoco basin from the rest of the northwestern rivers, the Cordillera Occidental and Central then separate the valley of the Magdalena river from the Atrato river valley.

Several lakes were created during the geological history of the continent, but paradoxically, it appears that they did not have such influence to the fish speciation

or radiation, as was thought in several other studies (LUNDBERG ET AL., 1998, STAWIKOWSKI & WERNER, 1998).

1.6.2 Important events in geological history in context of the fish biogeography

- 83 – 67 Mya (Late Cretaceous) the sea persisted in the continent eastward to the iniciation of Andes, reaching from the northern part of the continent down to the recent southern Bolivia. The other sea gulf also persisted in the today´s Paraná river valley. There was the river Eastern Amazon (recent lower Amazon) flowing eastward.
- 67 – 61 Mya (Cretaceous/Tertiary) – the sea level decreased and there occurred long major river with the source in recent Argentina and the estuary in recent Colombia (LUNDBERG ET AL., 1998). The river more or less copied the western border of the continent. Also the Paraná river had already appeared.
- 61 – 60 Mya (Early Tertiary) – reforming of the sea eastward to forming Andes because of again higher sea level (LUNDBERG ET AL., 1998)
- 60 – 43 Mya (Late Paleocene – Middle Eocene) - sea level decreased, the major river had again occurred, with the tributaries of recent upper and middle Amazon and the upper parts of recent Amazon tributaries in present Peru, and the rivers of today´s western Orinoco. There are another two rivers, Eastern Orinoco (recent lower Orinoco) and the persisting Eastern Amazon.
- 43 – 20 Mya (middle Eocen – early Miocene) – the river organisation more or less persisted, there was small sea gulf in the estuary of the major Andean river and the „Lago Pozo" lake was created in lowlands of recent upper Amazon, (LUNDBERG 1998). From 30 Mya, there was also noticable the effect of the formation of Mérida Andes and Cordillera Oriental (however, had already begun in Paleocene). „Lago Petaca" lake in Paraná river basin had appeared (LUNDBERG ET AL., 1998). In this period, the geological activity of the forming of Andes was most evident.
- 20 – 11,8 Mya (early – middle Miocene) - the sea from the northwest of the continent had again appeared and reached down to the today´s upper Amazon. In this part, there was created the „Lago Pebas" lake, connected with this sea (LUNDBERG ET AL., 1998).
- 11,8 – 10 Mya (late Miocene) – the sea and the lake (as mentioned above) expanded, there was also another sea gulf in the present Paraná river valley (LUNDBERG ET AL., 1998).
- 10 Mya (Late Miocene) occurred the final separation of recent Paraná and recent Amazon basins (MONTOYA-BURGOS, 2003).
- 10 - 8 Mya (Late Miocene) – the sea from both areas desappeared (LUNDBERG ET AL., 1998)
- 8 Mya (Late Miocene) – the connection between Eastern Amazon and the western rivers, the connection of Eastern Orinoco with their western tributaries (LUNDBERG ET AL., 1998)
- 8 – 5 Mya - there was the separation of the Orinoco river basin from the Amazon river basin (HUBERT & RENNO, 2006).
- 5 – 4,2 Mya - increasing of sea level, the marine incoursions from the Guyanan coast and inner Amazonia occurred (HUBERT & RENNO, 2006).

1.6.3 Selected events and „change points“ of fish fauna (HUBERT & RENNO, 2006)

1) Separation of Orinoco from Amazon occurred in 8 – 5 Mya (LUNDBERG ET AL., 1998) by Vaupes arch in the west (recent Colombia). Up to date, the connection between the Orinoco and Amazon basins (Rio Casiquiare, Southern Venezuela) persists (LUNDBERG ET AL., 1998).

2) Probably, connection of the Guyanan rivers with the Amazon and Orinoco. During last high sea level (5 – 4,2 Mya) the eastern part of the Guyana Shield with Guyanan rivers remained separated from the western part, where the rivers flew to Orinoco (HUBERT & RENNO, 2006), but there was the connection of marine water from the Guyana coast and the inner Amazonia (HUBERT & RENNO, 2006).

There were the change points of fish fauna between these basins (HUBERT & RENNO, 2006). There were two considerable Amazon-Guyanan points of contact, one in the upper Trombetas river (Amazon) with the upper Essequibo river (Guyana) and then the upper Branco (Amazon) river with the middle Essequibo river. The Orinoco-Guyanan connection was in the upper Cuyuní river (Essequibo) with the right-side Orinoco tributaries.

3) Separation of recent Paraná and recent Amazon basins in 10 Mya (MONTOYA-BURGOS, 2003) by the Michicola arch (HUBERT & RENNO, 2006). The most recent contact points were in the upper Tapajós river (Amazon) with the upper Paraguay river (Paraná) and the upper Mamoré river (Amazon) with the upper Paraguay river (Paraná).

4) Uplift of Mérida Andes and Cordillera Occidental, Central and Oriental in 30 – 11,8 Mya as a barrier in dispersion of species from cis-Andean part to the trans-Andean part.

1.6.4 The events with the faunal distribution context

There are several points in the Cichlasomatini distribution patterns, which could be considered in context of the geological events via estimation of the lineage separation time. The mutation rate of used genes has already been suggested in literature, therefore, the Molecular Clock method (e.g. STURMBAUER & MEYER, 1993), could be used for tree constructing. The estimation of the time of the separation of studied clades could be done using the suggested values of the mutation rate found in literature. The suggested interval of possible time of clade separation is then tested if could be caused by the geological event or not. The eventual overlapping of estimated time of clade separation and the time of the geological event is searched.

1.6.5 Two separation events tested in the Molecular Clock tree

1) The separation time between two clades of ´*Aequidens*´ geoup, the trans-Andean ´*Aequidens*´ *rivulatus* and cis-Andean ´*Aequidens*´ *pulcher* lineages.

   Geological hypothesis background: the separation of the Pacific and Atlantic basins probably occurred in the interval of 30 - 11,8 Mya by the forming of Andes as the geographic barrier in the North-western part of the continent (LUNDBERG ET AL., 1998). If the separation of two lineages was caused by this geological event, the estimated time of separation of two lineages has to be in interval overlapping the interval of 30 – 11,8 Mya.

2) The separation between two lineages of *Cichlasoma* genus. The species *C. dimerus, C. pussilum* and *C. portalegrense* as the Paraná-drainage-taxa and the *C. orinocense, C. amazonarum, C. bimaculatum* and *C. araguaiense* as the Orinoco and Amazonian taxa.

   Geological hypothesis background: the definitive separation of the Paraná and Amazon basins occured in 10 Mya (MONTOYA-BURGOS, 2003). If the separation of two lineages was caused by this geological event, the estimated time of separation has to overlap the time of this event (i.e. 10 Mya).

2 Main goals of the present study

- To resolve the phylogenetic relations within the tribe Cichlasomatini based on the molecular data of two mitochondrial and one nuclear gene.
- To test the monophyly of the genera included in the molecular phylogenetic analyses.
- To compare the morphological taxonomic concept (KULLANDER, 1998) with the results based on the molecular data.
- To suggest the hypothetic scenario of the biogeographic history of the tribe Cichlasomatini.
- To estimate the time of separation in two selected lineages in context of their geographic distribution and the geological events.

3 Material and Methods

3.1 Materials

I analysed 63 specimens belonging to 34 species or undescribed forms of the tribe Cichlasomatini (see the Appendix 1–2). As outgroups I used three species, one from the sister tribe Heroini (*Thorichthys meeki*), and two from the tribe Geophagini (*Geophagus brasiliensis, Geophagus steindachneri*).

The fish used in this thesis originated from the localities in South America and 1) were imported by the specialized company to Germany or 2) were collected directly in localities (Venezuela, Argentina). 3) Fish from aquarium stocks were used as the alternative source of samples. 4) Several nucleotide sequences were eventually downloaded from the GeneBank (available on www.ncbi.nlm.nih.gov).

The list of analysed fish samples and species togehther with their original localities is given in the Appendix 2. There are also the access numbers of GeneBank sequences.

3.2 The list of markers

Three markers, two mitochondrial and one nuclear were used for phylogenetic analysis:

Mitochondrial: 16S rRNA – The whole locus coding the 16S rRNA has about 1800 bp (BURK ET AL., 2002). The fragment of 612 bp was used in this study. Because of the secondary structure, there are parts in the molecule referred to as „stems“, which have the complementary fragments in the other part of the same molecule and „loops“, which do not. The rate of sequence evolution differs between stems and loops since the former structure is more conservative.

Mitochondrial: cytochrome b – the fragment of the 420 bp of length was amplified, the whole gene has about 1140 bp (RUBER ET AL., 2004). This is the coding gene. Different models of evolution were used for every position of triplet or whole triplets were considered to be characters, respectively.

Nuclear: first intron in S7 ribosomal protein – nuclear marker, this is the non-coding fragment sequence. There are several deletions in the sequences, that also have phylogenetic signal.

3.3 Molecular Methods

3.3.1 Preparation and isolation of DNA

Small piece of fish fin was carefully collected from live specimens and fixed in pure 96% ethanol and stored in –18 °C.

Isolation of DNA was done using the comercially available isolation kit (Dneasy® Tissue Kit, QuiGen). The pieces of tissue were dried to get rid of the fixation medium (ethanol) and the total genomic DNA was extracted following the manufacturer´s protocol.

3.3.2 Amplification and sequencing of DNA

The fragments of mitochondrial coding gene of cytochrome b, mitochondrial gene for RNA (16S rRNA) and nuclear intron in ribosomal S7 gene were amplified using method Polymerase Chain Reaction (PCR).

Three sets of primers were used, one of them was degenerated (i.e. it represents a mixture of molecules with different bases at the given position). The list of primers is given in the Table 1.

gene	name of primer	F/R	Length of fragment after alignment	sequence of primer	citation of source
16S	mtD-32	forward	612	5´-CCGGTCTGAACTAGATCACGT-3´	Marescalchi, 2004
	mtD-34	reverse		5´-CGCCTGTTTAACAAAAACAT-3´	Marescalchi, 2004
cyt b parcial	GluDG-L	forward	420	5´-TGACTTGAARAACCAYCGTTG-3´	Martin & Bermingham, 1998; from Palumbi, 1991
	H 15149	reverse		5´-AAACTGCAGCCCCTCAGAATGATA-3´	Kocher et al., 1989
S7	S7RPEX1F	forward	508	5'-TGGCCTCTTCCTTGGCCGTC-3´	Chow & Hazama, 1998
	S7RPEX2R	reverse		5'-AACTCGTCTGGCTTTTCGCC-3´	Chow & Hazama, 1998

Table 1: The primers used for amplification of three genes with the literature source.

The amplifications were performed on the thermo-cycler PTC-200 (MJ Research) and iCyclerTM Thermal Cycler (BIO-RAD) under the following protocols:

The reaction mix of 25ul contains:

12,5 μl of PPP Master Mix (75mM Tris-HCl, 20mM $(NH_4)_2SO_4$, 200uM dATP, dCTP, dGTP, dTTP, 2,5 U Taq DNA polymerase, stabilisators)

2 – 3 μl of 25mM $MgCl_2$

6,5 – 7,5 μl of PCR H_2O

1 μl of forward primer

1 μl of reverse primer

1 μl of isolated DNA

The temperatures and times of amplification steps are given in the Table 2.

Programme for 16S			
step	repeats	temperature	time
1	1x	94°C	2min
2	31x	94°C	30s
		48°C	30s
		72°C	30s
3	1x	72°C	8min
4	1x	4°C	5min
5		END	

Programme for Cyt-b			
step	repeats	temperature	time
1	1x	93°C	2min
2	40x	93°C	1min
		46°C	1min
		72°C	1min
3	1x	72°C	10min
4	1x	4°C	5min
5		END	

Programme for S7			
step	repeats	temperature	time
1	1x	94°C	2min
2	35x	94°C	30s
		60°C	1min
		72°C	1min
3	1x	72°C	8min
4	1x	4°C	5min
5		END	

Table 2: The PCR programmes used for amplification of three genes, 16S rRNA, cytochrome b and the first intron in S7 ribosomal gene.

The PCR products were controlled by horizontal electrophoresis applying of 4 μl of the mix on the agarose gel (0,4g agarose in 50ml of 0,5M TBE buffer with 1,5ul EtBr), and with the reference standard (GeneRulerTM 100bp DNA Ladder Plus) for 30 minutes with the voltage of 110V. This also provided to check the intensity of amplified DNA.

The comercially available kit QIAquick® PCR Purification Kit (QiaGen) was used for purification of PCR products. Alternatively, when the obtained product was weak, the whole amount of product was applied into the gel electroforesis and was extracted from the gel (the piece of gel with the product was cut) and then were purified by the QIAquick® Gel Extraction Kit (QiaGen).

The PCR products were sequenced using the same primers and the sequencing kit ABI PRISM® BigDye™ Terminator v3.1 Ready Reaction Cycle Sequencing Kit (Applied Biosystems). Because of the limited financial support the quarter-reaction was used, see the following protocol:

The reaction mix of 20 ul contains:

6,68 - 9,68 µl of H_2O

6 µl of the 5x Sequencing Buffer

2 µl of the Cycle Sequencing Mix

0,32 µl of primer

2-5 µl of the purified PCR product

After the sequencing PCR reaction the product was precipitated by ethanol, the protocol follows:

1) Mix the product of PCR with 2µl of 3M NaAc and with 50 µl of pure 96 % ethanol
2) Let the microtube stand in the room temperature for 10 minutes.
3) Centrifuge in 13000 rpm for 30 minutes,
4) Carefully remove the ethanol, the product appears as a small pellet on the bottom of microtube.
5) Add 50 µl of 70% ethanol.
6) Centrifuge in 13000 rpm for 10 minutes.
7) Remove carefully the ethanol.
8) Let dry the microtubes in 37 °C for 40 minutes.
9) Add 25ul of the formamide, let it stand for 20 minutes to resuspend the pellet.
10) Heat the product on 94 °C for 2 minutes, after this heating, cool it rapidly (on ice).
11) Apply to the automatic sequencer.

The capillary electrophoresis and fragment scoring was performed on an automatic sequencer 3130 Genetic Analyser (from Applied Biosystems) in the Institute of Animal Physiology and Genetics, Czech Academy of Science, Liběchov.

3.3.3 Alignment of the sequences

The amplified fragments were sequenced in both forward and reverse senses. After the control of sequences and connection of reverse and forward in CHROMAS software version 1.45 (available on http://www.technelysium.com.au/chromas.html), the sequences were aligned using the ClustalX algorithm as implemented in the software package BIOEDIT (HALL, 1999) with the default settings of penalties. After aligning, the manual control and corrections were done. The beginnings and endings parts were cut to all sequences start in the same position. The alignment was exported to the NEXUS format.

The alignment of mitochondrial **16S rRNA** gene was modified with respect to the secondary structure of the ribosomal RNA. The models already designed for mammals (BURK ET AL., 2002), and for catfish family Doradidae (MOYER ET AL., 2004) were used to homologise the stems and loops parts of used fragment of the gene.

After the alignment of mitochondrial **cytochrome b,** all sequences were translated to protein in the software MEGA, version 3.1 (KUMAR ET AL., 2004), using the mitochondrial vertebrate code to check if there are no stop-codons in the gene.

In the nuclear intron in **S7 ribosomal gene** especially the parts of indels were optimised by the manual control, because it was necessary to correct some sequences.

3.4 Datasets

Sixty-three sequences were obtained for every gene. They were analysed like large alignment (LA). This alignment contained various sequences from the same species but different specimens. Hence, the sequences of several specimens were removed and the reduced alignment (RA) was used for the following analyses. This RA contained 38 taxa (+/-3 sequences, it depended on the availability of sequences in the GeneBank (for every gene). Only the LA of the combined dataset of all three genes was analysed by Bayesian Methods (See more Fig. 11).

The RA of every gene and the combined alignments of more genes were analysed by all methods mentioned above (MP, ML, Bayesian Methods).

3.5 Phylogenetic analyses

For phylogenetic analyses I used the softwares PAUP 4b1 (SWOFFORD, 2002) and MrBayes 3.1 (RONQUIST & HUELSENBECK, 2003).They both request the NEXUS format of dataset as an input file. The algorithms of Maximum Parsimony, Maximum Likelihood and Bayesian method of Markov Chain Monte Carlo were used for the analyses.

3.5.1 Maximum parsimony

The method of Maximum Parsimony searches for the tree with the lowest number of evolutionary changes required to explain our data (SWOFFORD & SULLIVAN, 2003)

The PAUP software was used to analyse the dataset by MP method. The most parsimonious tree was searched by heuristic search algorithm with the random addition of sequences, the TBR algorithm of branch swapping (Tree Bisection/Reconnection), holding of two trees in memory between two following steps of searching and with 100 replicates (*command: Hsearch addseq=random hold=2 swap=TBR nrep=100).*

In the nuclear S7 intron, the gaps were also coded as the fifth state (*command: pset gapmode=newstate*) for including the information of deletions (only one gap for each deletion was used, the other gaps replaced by „missing data“ code). However, because the topologies were identical, just the mapping of deletions on the Bayesian tree was done (see the Table 4 for deletions and Fig. 8 where mapped on the tree in the Chapter Results)

The support of all nodes was tested by bootstraping with 1000 replicates. The swapping algorithm was the same as in the basic analysis (TBR), but the number of held trees was decreased to one, and the number of replicates during individual steps of bootstraping was decreased to 10. In the result cladogram there were kept only the nodes with the support higher than 50 % (*command: bootstrap nrep=1000 search=heuristic /multrees=no*).

3.5.2 Modeltest

For the Maximum Likelihood analysis (see below) the adequate model of evolution had to be searched for every dataset by the Modeltest software, version 3.7 (POSADA & CRANDALL, 1998). This software chooses the simplest model suitable for the dataset out of

64 evolutionary models for nucleotide dataset and test by Hierarchical Likelihood Ratio Test (hLRT), if the more complicated model significantly ameliorates the likelihood value of the tree in context to increasing of the degrees of freedom (POSADA & CRANDALL,1998).

3.5.3 Maximum Likelihood method

The likelihood analysis represents the searching of the tree topology in context with the parameters selected under given evolutionary model and branch length, which would maximize the probability of observing our dataset (VON HAESELER & STRIMMER, 2003).

The proposed model from the Modeltest software (POSADA & CRANDALL, 1998) for each dataset was used for Likelihood analysis in the PAUP software, version 4b10 (SWOFFORD, 2002).

Due to the enormous time required for the ML analysis, the searching and bootstraping (339 replicates) were done only for the concatenated dataset of reduced alignment of 38 sequences. However, the ML trees (without bootstraping) of separate genes were also used for the testing of hypotheses using Approximately Unbiased test (see below) which requires the sitelikelihood scores counted from the best likelihood tree. And the ML trees were constructed in the Molecular Clock method given below.

3.5.4 Bayesian Methods

The Bayesian method works with the algorithm of Metropolis Coupled Markov Chain Monte Carlo (MCMCMC) (YANG & RANNALA, 1997) and with the posterior probabilities (see e.g. ALTEKAR et al., 2004). The posterior probability indicates the % value of presence of the node in last trees of analysis, that were used for the constructing of the consensual tree (RONQUIST & HUELSENBECK, 2003).

The MrBayes software, version 3.1 (RONQUIST & HUELSENBECK, 2003) was used for construction of trees by the Bayesian methods. The parameters of analysis were calculated under the model GTR with gamma distribution (*command lset nst=6 rates=invgamma*), the analysis was done with number of generation of 1,000,000 and 2,500,000 respectively (dependent on the signal stability of found trees), with the sampling frequency of every 100 trees. All analysis were done with two paralell runs (except for the „codon“ model applied on cytochrome b), the consensual tree was constructed after the analysis from last 7500 – 15000 trees.

The dataset of the cytochrom b was analysed by the nucleotide model „Codon“, which counts the codons as characters. The concatenated dataset (see Figs. 11 – 14) was

analysed using the partition of the gene of 16S rRNA into loops and stems. The sequences of the cytochrom b gene were also divided into three partitions – first, second and third codon position. Six partitions were obtained – three of the codon positions in the cytochrome b gene, two of stem and loops of the 16S rRNA gene and one of the S7 gene. For each partition the own model was counted during the analysis (*command* (after the enter of partitions*): lset applyto=(1,2,3,4,5,6) nst=6 rates=invgamma;*).

3.5.5 Concatenation of genes

Two concatenations of genes were done:

1) Both mitochondrial genes (cytochrome b gene and 16S r RNA gene)
2) All three genes (the mitochondrial genes and nuclear intron in S7 gene).

Two pre-analysis and one post-analysis tests of incongruence in concatenated dataset were done. The ILD Partition Homogenity test implemented in PAUP test the lenght of concatenated tree parsimony in comparison to the lenghts of each gene separately with the random selection of characters. The Approximately Unbiased test (SHIMODAIRA, 2002) statistically compares the sitelikelihood scores of every genetree. The Partitioned Bremer Support (WAHLBERG ET AL., 2005, BREMER 1994) could find conflict nodes in the observed phylogenetic tree from the concatenated dataset.

3.5.6 Incongruence Length Difference Partition Homogenity test (FARRIS ET AL., 1995)

This test works with the parsimony lengths and compare the length of the concatenated tree with the lengths of the partitioned tree and then with the randomly selected characters from dataset. (FARRIS ET AL., 1995)

This test was run as implemented in the software PAUP 4b1 (SWOFFORD, 2002). *(command: charset genes=16S:1-612, cytb:613-1034, S7:1035-1542; hompart partition=genes;)*

3.5.7 Approximately Unbiased Test (SHIMODAIRA, 2002), AU test

This test could be used to test if two topologies are significantly different or not counting with sitelikelihood scores of every position and the total likelihood scores. This test compares the best tree based on the dataset of one gene with the tree of the same dataset but constructed under constraints of other gene. If the constrained tree has

significantly worse likelihood score, the topologies are considered to be different (SHIMODAIRA, 2002).

The AU test was done to answer the question whether is possible to concatenate datasets of three genes (two mitochondrial: cytochrome b and 16S rRNA, and one nuclear: intron in S7 gene).

The sitelikelihood scores for the best tree from every gene were counted using software PAUP. Then the same scores were counted for every gene but with two constraints (two topologies from two other genes). It means nine trees tested at all. The output file from PAUP was analyzed by AU test as implemented in the software CONSEL (SHIMODAIRA & HASEGAWA, 2001).

3.5.8 Bremer Branch Support (BREMER, 1994)

The Bremer Branch Support is the value that means how many steps longer is the best parsimonious tree, which does not contain the tested node (BREMER, 1994).

The PAUP programme was used for counting the values of the Bremer Support and the software NONA (GOLOBOFF, 1994) to check of the values, respectively. In PAUP, the method of „anticonstraint" was used (e.g. WAHLBERG ET AL., 2005).

First, the nodes were defined as constraints for analysis. Then the most parsimonious tree was constructed and the score (tree length) was counted.

Then the tested nodes were set as constraints for PAUP and were counted the lengths of trees constructed by the dataset that are NOT compatible with the tested constraint (*commands: hsearch enforce converse constraints = „nodeX"*).

The same was done for each node = constraint.

The difference between the length of the best parsimonous tree and the length of the tree with „anticonstraints" is the value of the Bremer support (BREMER, 1994).

3.5.9 Partitioned Bremer support (PBS)

This method indicates the Bremer Support for the node from every gene separately in the concatenated dataset (WAHLBERG ET AL., 2005). The values of PBS for each node were calculated in the programme PAUP (SWOFFORD, 2002) by the same method of „anticonstraints" as in the Bremer Support test (see above). The concatenated dataset of all three genes was used and the sets of characters (three genes) were defined as divided parts in the input NEXUS file (*command: charset genes=16S:1-612, cytb:613-1034, S7:1035-1543;*).

In addition to the total length of the best „anticonstrained“ tree, the distribution of characters of separated genes on this tree was counted like partition of every gene-characters in the total length. The sume of these three values from every used gene has to result the total length of the tree (for every gene: *commands: exclude all; include „name of character set“; lenfit;).*

Therefore, four values of length were obtained, one total and three of every gene. The values of the partitioned length differences between the best and the constrained tree is the Partitioned Bremer Support value of every gene for the tested node.

The values were placed on the cladogram, see Fig. 17. The test was done for all three genes using default nodes from the concatenated cladogram (Figs. 12 – 14). The higher number the more support for the node from tested gene. The negative value means that there is conflict in the node because the gene support different, more parsimonious topology (WAHLBERG ET AL., 2005).

3.5.10 Conclusion of the concatenation

Despite the reject the possibility of concatenation of all samples (see the Chapter Results 4.2.5), the tree from all genes was constructed. The tree without the conflict taxa was also constructed to check whether the topology of remaining taxa change or not by the presence of conflict taxa.

See the tree of all three genes in Figs. 12 – 14 but it is necessary to notice that this concatenation was supported only with exception of several taxa (see more in the Chapters Results 4.2.5 and Discussion 5.4).

3.6 The test of alternative phylogenetic hypotheses

The topologies of the best trees were obtained, but there still remain several alternative hypotheses that were published previously or that resulted from several analyses but not in the concatenated tree. Last but not the least, the test of possible position of unclearly resolved or unresolved clades in the tree was tested.

The method of Approximatelly Unbiased test (SHIMODAIRA, 2002; see above) was used for the test of 38 hypothetic topologies of the tree, using constraints defined by user (topologies mentioned below). The test was done in the concatenated dataset from all three genes. There are also 12 hypotheses that was unnecessary to test, because their topology was congruent with the concatenated cladogram. But I mention them here to demonstrate in one list all considerable views of the taxonomic and phylogenetic relations. These hypotheses mentioned below have the mark of asterisk (*).

For better orinetation, I decided to mark the higher clades by abbreviations. See more information about these clades in the Chapter Results 4.2.1. There are four intergeneric clades:

- ⇨ **BTA clade** – *Bujurquina* + *Tahunatinsuyoa* + ´*Aequidens*´ genera
- ⇨ **CA clade** – *Cichlasoma* + *Aequidens* (*A. potaroensis* excluded) genera
- ⇨ **KA clade** – *Krobia* genus + *A. potaroensis*
- ⇨ **NC clade** – *Nannacara* + *Cleithracara* genera

Tests of hypothesis:

The monophyly of the tribe sensu KULLANDER (1998) (*Acaronia* excluded) and sensu FARIAS ET AL. (1999, 2000, 2001).

1) Monophylum of Cichlasomatini (FARIAS ET AL., 1999, 2000, 2001).*
2) Monophylum of Cichlasomatini without *Acaronia* (KULLANDER, 1998)

The monophyly of the genera belonging to Cichlasomatini. The genus *Aequidens* was tested as considered (with all species included) and then without the species *A. potaroensis*, which appears not to be *Aequidens* (see more in Chapter Results 4.2.1)

3) Monophylum of *Aequidens* genus
4) Monophylum of *Aequidens* genus (*A. potaroensis* excluded)
5) Monophylum of *Cichlasoma* genus
6) Monophylum of *Nannacara* genus*
7) Monophylum of *Laetacara* genus*
8) Monophylum of *Krobia* genus*
9) Monophylum of ´*Aequidens*´ species group*

Several intergeneric relations, the source of hypothesis is in brackets after the topology

10) Monophylum of *Bujurquina* + *Tahuantinsuyoa* genera (KULLANDER, 1986)*
11) Monophylum of **NC clade** (KULLANDER 1989, 1998)*
12) Monophylum of *Aequidens* + ´*Aequidens*´ (part of *Aequidens* sensu lato, resulted in FARIAS ET AL. (1999, 2000), but moreover it is the last remaining group without own generic name, hence, the question of exclusion from *Aequidens* has to be tested)
13) Monophylum of **BTA clade***
14) Monophylum of *Tahuantinsuyoa* + ´*Aequidens*´ (KULLANDER, 1998)
15) Monophylum of *Aequidens* + *Cichlasoma* (KULLANDER 1983a)
16) Monophylum of **CA clade** (KULLANDER 1983a, the same as hypothesis 15 but with exclusion of *A. potaroense* due to its position outside *Aequidens*)*
17) Monophylum of **CA clade + KA clade** (for test of hypothesis that all *Aequidens* species are included to the relationship with *Cichlasoma*, the *Krobia* genus also included) *
18) Monophylum of *Bujurquina* + *Krobia* (KULLANDER, 1998)
19) Monophylum of (*Bujurquina* + *Tahuantinsuyoa*) + (*Krobia* + *A. potaroense*) (from the hypothesis *Bujurquina* + *Krobia*, in KULLANDER, 1989, 1998)

The position of the unresolved or the conflicted taxa; the positions of *Laetacara* genus in several considerable nodes of the best tree and also the test of several morphological hypotheses (if the source, is in brackets):

20) Monophylum of *Laetacara* + *Nannacara* (KULLANDER, 1986)
21) Monophylum of *Laetacara* + **NC clade** (KULLANDER, 1986, 1989, 1998)
22) Monophylum of *Laetacara* + **BTA clade**
23) Monophylum of *Laetacara* + *Krobia*
24) Monophylum of *Laetacara* + **KA clade**
25) Monophylum of *Laetacara* + **CA clade**
26) Monophylum of *Laetacara* + **CA clade** + **KA clade**

The position of other conflicted taxa, *Acaronia* genus:

27) Monophylum of *Acaronia* + **BTA clade***
28) Monophylum of *Acaronia* + *Laetacara*

29) Monophylum of *Acaronia* + *Laetacara* + **BTA clade***

30) Monophylum of *Acaronia* + **CA clade** + **KA clade**

31) Monophylum of *Acaronia* + **NC clade**

The phylogenetic relations among major clades, position of (*Nannacara* + *Cleithracara*) clade:

32) Monophylum of **NC clade** + **BTA clade**

33) Monophylum of **NC clade** + **CA clade** + **KA clade**

34) Monophylum of **NC clade** + *Laeatacara* + *Acaronia*

The sister position of three major clades:

35) Monophylum of **CA clade** + **KA clade** + **BTA clade**

Geographic hypotheses of Guyanan taxa:

36) Monophylum of *Aequidens potaroensis* and *Aequidens chimantanus* (two species living in Guyana Shield, one in the Orinoco river basin, one in Guyanan rivers)

37) Monophylum of *Aequidens potaroensis* + *A. chimantanus* + *Krobia* (the same as hypothesis 34, additionally with *Krobia,* which appears to be monophylum with *A. potaroensis)*

38) Monophylum of **NC clade** + **KA clade** (all Guyanan taxa from my sampling with the basins of Guyanan rivers, not Orinoco; resulted in concatenated mitochondrial cladogram, but not in concatenated cladogram from all three genes.)

3.7 Biogeography and testing hypotheses of distribution

The best tree from the concatenated mitochondrial and concatenated all-three-genes dataset was used for mapping of the river basins of each species. See Fig 18 and 19.

The Cladogram Area of the river provinces was constructed based on 85 characters of the presence/absence of all species (also genera and higher clades) belonging to the tribe Cichlasomatini. The list of provinces see in the Chapter Introduction 2.6.1. The tree was constructed using the Maximum Parsimony method in the software PAUP 4b1 (see the Fig. 18).

Several geographic hypotheses were also tested using the AU test. (See above the Geographic hypotheses of the Guyanan taxa and the Table 7 in the Chapter Results)

3.8 The reconstruction of eventual biogegraphic scenarios

The distribution data of all studied species were mapped on the resulted trees (See Fig. 19 in Chapter Results) using the most parsimonious criterion and then were checked using software WinClada, version 1.00.08 (NIXON, 2002). This software also maps the characters (here the distribution data) on the tree by the most parsimonious explanation of our dataset, i.e. the lowest amount of the changes in the tree.

The most parsimonious scenario was then postulated, based on the tree with the mapped distribution data. (See Fig. 19 in Chapter Results).

3.9 The Molecular Clock method

The ML tree was constructed for every gene separately in the software PAUP 4b1 with the clock algorithm enforced (*command: set clock=yes*).

Then the best tree found had to be tested, if the datas behave „clocklike". It means that there is not any sequence with the significantly different rate of evolution. The –lnL values of both the original and clock likelihood trees were tested using the Likelihood Ratio Test (according to POSADA, 2003) following the:

LRT = 2 x (-lnL original tree – -lnL clock tree).

The value is then compared with the critical value of Chi-square for the number of the degrees of freedom. The degrees of freedom were set as the number of the OTUs (Operational Taxonomic Unit) minus 2 (POSADA, 2003).

The tested null hypothesis is, that the rate of evolution is homogeneous along all branches. If not, the value of LRT will be higher than the critical value for Chi-square and the hypothesis will be rejected.

When LRT rejects the null hypothesis of homogeneity, Molecular Clock algorithm cannot be applied, and the problematic taxon has to be found and removed from dataset.

I used the Relative Rate Test to assess the taxa, which did not behaved according to the Molecular Clock hypothesis. This test counts the distances of each ingroup species from the outgroup species. I used the Mega software, version 3.1 (KUMAR ET AL., 2004). Such taxa were subsequently removed from the analysis and resulting linearized tree was further used.

Then the distances for two tested separations (*´Aequidens´ pulcher* versus *´Aequidens´ rivulatus* and the Amazon/Orinoco *Cichlasoma* species versus the Paraná *Cichlasoma* species) were counted for every gene under the model suggested by Modeltest using the MEGA software, version 3.1 (KUMAR ET AL., 2004) and PAUP 4b1.

3.9.1 The substitution rates used in this study

I used the following **mutation rates** found in litarature for every used gene separately. To my knowledge, there is no study dealing with the values of the mutation rates in Neotropical cichlids for no gene, hence, I used the rates for the most relative group.

Cytochrome b: the mutation rate of **1.5%** substitutions per My (=Million years) per lineage (= 3% per My of divergence rate) estimated in African cichlids (STURMBAUER & MEYER,1993, KOBLMULLER ET AL., 2004).

16S rRNA: the mutation rate of **0.5%** substitutions per My per lineage (= 1% per My of divergence rate) estimated in Labridae (Perciformes; Mabuchi et al., 2004).

Intron in S7 ribosomal gene: the mutation rate of **0.2 – 0.23%** substituotions per My per lineage (= 0,4 – 0,46% of divergence rate) estimated in Haemulidae (Perciformes; BERNARDI & LAPE, 2005).

3.9.2 Additional estimation

Because of the differences in the ratio of observed divergences and the mutation rates estimated by several authors in literature and due to the large intervales in suggested mutation rates, I decided to do other, alternative estimation of time of separation. This estimation should cover the interval of suggested rates published in literature.

For 16S rRNA, the extreme maximum and minimum values found in literature were used. I. e. the mutation rate of **0.14%** per My (RITCHIE ET AL., 1996; estimated in Trematomidae) and the mutation rate of **0.7%** per My (HUYSE ET AL., 2004; estimated in Gobiidae).

For cytochrome b only the extreme minimum was used (**0.38%** per My estimated in Cyprinidae; CHANG ET AL., 2006) because the value of maximum was observed in the African cichlids and was, hence, used in the first estimation. Additionaly, the value of cytochrom b mutation rate for general teleost fish was applied (**0.5%** of substitution per My per lineage; CARDENAS ET AL., 2005)

For S7 intron, the only value was found in literature, hence, this additional estimation was not done for it.

4 Results

The main goals of this study being to resolve the phylogenetic relations within the tribe Cichlasomatini and to test the monophyly of genera and higher groups as published to date. The morphology-based phylogenetic relations (KULLANDER, 1998) were tested and conflicting and congruent topologies with molecular data were found. The hypothetic biogeographic scenario was postulated based on the analysed data. There was also done the test for the coincidence of dispersal and vicariance events in context of the main geological events during the geological history.

4.1 Molecular analysis

Sixty-three sequences of 1542 bp length of all three genes were obtained. The sequence of mitochondrial 16S rRNA gene was 612 pb long and one additional sequence was downloaded from GeneBank. The other mitochondrial marker, part of the cytochrome b sequence of the lenght of 422 pb, was analysed. For the Bayesian methods, the alignment was shortened to 420 pb to homologise the codon position to start on the first position of the triplet. Three additional sequences from GeneBank were used for the cytochrome b analysis. The sequences of nuclear intron in the S7 ribosomal gene with the length of 508 pb were done.

4.1.1 Parsimonious analysis

dataset	N° of samples	total characters	variable characters	parsimony informative	length of tree	N° of trees	RI	CI
16S	43	612	176	130	497	61	0.6859	0.4849
cyt b	40	422	181	150	859	26	0.6191	0.3097
Mitoch	38	1034	351	275	1285	6	0.617	0.382
S7	38	508	237	128	341	144	0.892	0.8211
KOMBI	38	1542	589	406	1702	1	0.657	0.4542

Table 3: The values describing the parsimonious analyses. There are mentioned the number of samples in the dataset, the amounts of total / variable / parsimony informative characters in every gene dataset, the length of tree and the number of the best identically parsimonious trees. RI = retention index, CI = consistency index. The gray patches indicate the concatenated datasets.

4.1.2 Models of sequence evolution

The Modeltest software (POSADA & CRANDALL, 1998) suggested the model of TrN+I+G for the 16S rRNA dataset, for the cytochrome b the model of GTR+I+G was proposed and the HKY+G model was suggested for the nuclear intron in S7 gene. For both concatenated datasets, 1) only mitochondrial genes and 2) mitochondrial + nuclear gene, the model of GTR+I+G was suggested.

4.2 Phylogenetic relations

4.2.1 Major clades in resulting trees

For the better understanding of the mentioned results, the schema of major clades will follow:

Generally, four major clades were identified:

The Clade 1 – called hereafter **BTA clade**, included the genera *Bujurquina*, *Tahuantinsuyoa* and *´Aequidens´*.

The Clade 2 – **CA clade** - *Cichlasoma* and *Aequidens* (*A. potaroensis* excluded)

The **NC clade** - *Nannacara* + *Cleithracara* genera

The **KA clade** - *Krobia* genus (included *A. potaroensis*)

The relationships among these clades were not clearly resolved, see also the Fig. 15 and 16. There was one monophyletic group of three species (*Aequidens metae, A. diadema, A. sp.* "Atabapo") within the **CA clade**. The other species *Aequidens tetramerus* (in mitochondrial genes accompanied by *Aequidens sp.* "Jaru") were always sister to the *Cichlasoma* + *Aequidens patricki* clade. As a basal taxon to the rest, there was always *Aequidens chimantanus*, in analysis of nuclear gene it was accompanied by *Aequidens* sp. "Atabapo".

The genera *Bujurquina* and *Tahuantinsuyoa* were sister genera and formed the monophylum with *´Aequidens´* group within the **BTA clade**.

The remaining genera, *Acaronia* and *Laetacara*, were not included in any of the major clades because their position differed dependently on the gene marker analysed and their phylogenetic signal was conflicting based on tested genes.

The genus *Laeatacara* was always monophyletic, the genus *Nannacara* resulted always monophyletic and the group *´Aequidens´* also always formed well-supported monophylum. The genus *Cichlasoma* was monophyletic only in both mitochondrial genes. In the nuclear gene, the *Aequidens patricki* species clustered inside the *Cichlasoma* genus. By contrast, the monophylum of *Aequidens* genus was always rejected, and its polyphyletic status

resulted from the appartenance of *Aequidens potaroensis* into the genus *Krobia*. Even if the *A. potaroensis* is not taken into account, the *Aequidens* genus remained paraphyletic relative to its inner group of genus *Cichlasoma*.

4.2.2 The analysis of the mitochondrial 16S rRNA gene

The fragment of 612 bp was used for both MP and Bayesian analyses. The trees were constructed (See Figs. 3-4) using the reduced alignment of 42 sequences, one of them used from GeneBank, see the list of samples in the Appendix 2.

The best parsimonious tree and the Bayesian tree did not have any conflict, the MP tree (strict consensus of 61 trees) had more polytomies, only.

Summary of the clades:

- ⇨ **BTA clade** was supported only with *Acaronia* inside and only by the Bayesian method. In MP method, the clade got the bootstrap support lower than 50 %.
- ⇨ **CA clade** was supported in both MP and Bayesian analyses with the branch support of 59 % and 0.96 of posterior probability, respectively.
- ⇨ **NC clade** had not been formed, there is different topology
- ⇨ **KA clade** was unresolved in both MP and Bayesian analyses.

The monophylum of the genus *Krobia* was not resolved and also there was not the sister position of *Nannacara* and *Cleithracara,* which resulted in the nuclear gene analysis, and both concatenated datasets analyses (see more below). The **BTA clade** with the inner position of *Acaronia*, resulted as a sister taxon to *Laeatacara* genus. The **CA clade** formed the monophylum with the members of **KA clade** and the genus *Nannacara* with the questionable support of Bayesian posterior probability of 0.56 (and is unresolved in MP tree), see Figs. 3-4.

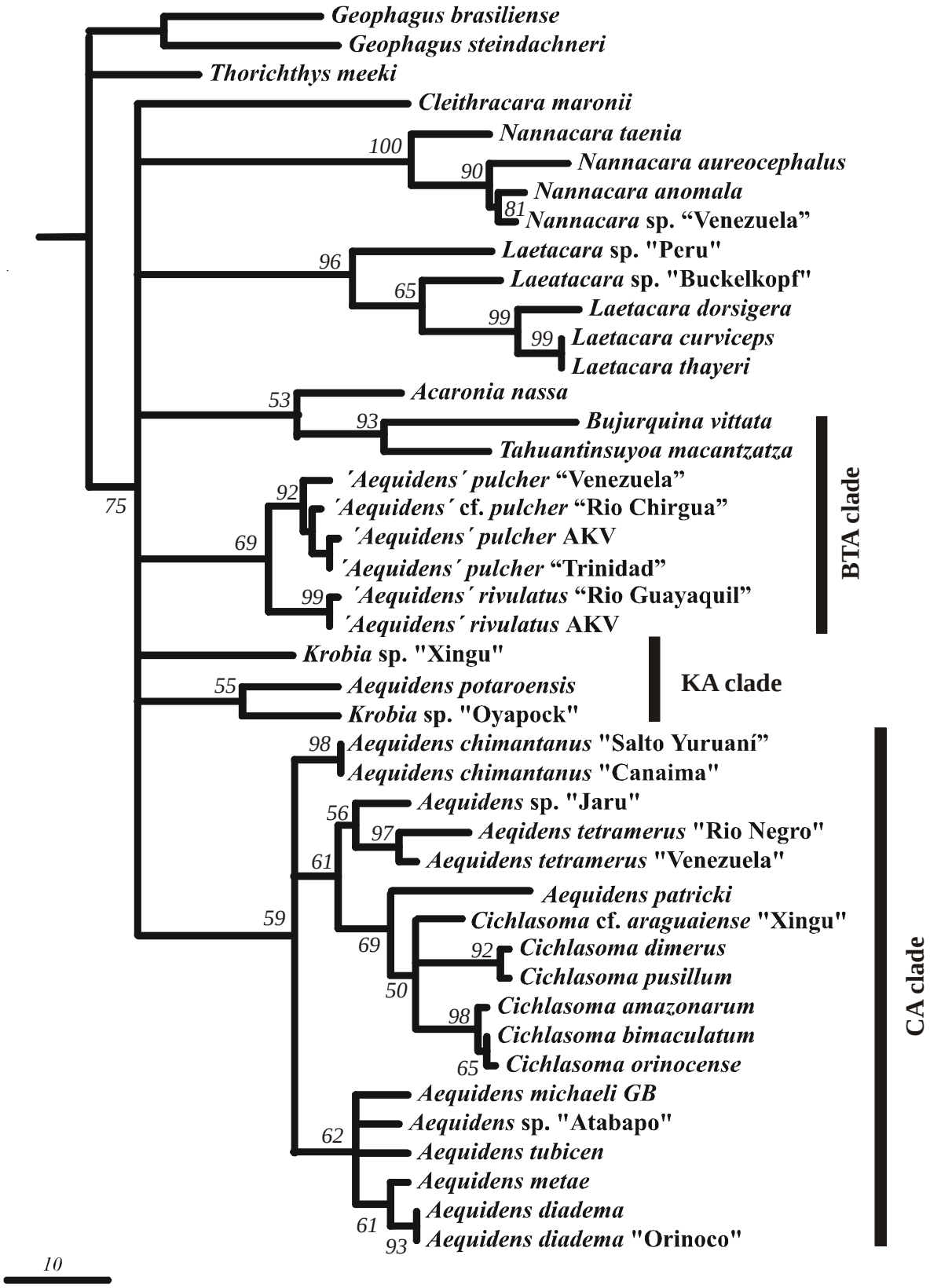


Fig. 3: The strict consensus of 61 best Maximum Parsimony trees based on a 612 bp long fragment of 16S rRNA gene. The tree length is 497 steps. The numbers in the nodes indicate the bootstrap supports counted for 1000 replicates. CI= 0.4849. RI=0.6859. The bar indicates the changes in sequences.

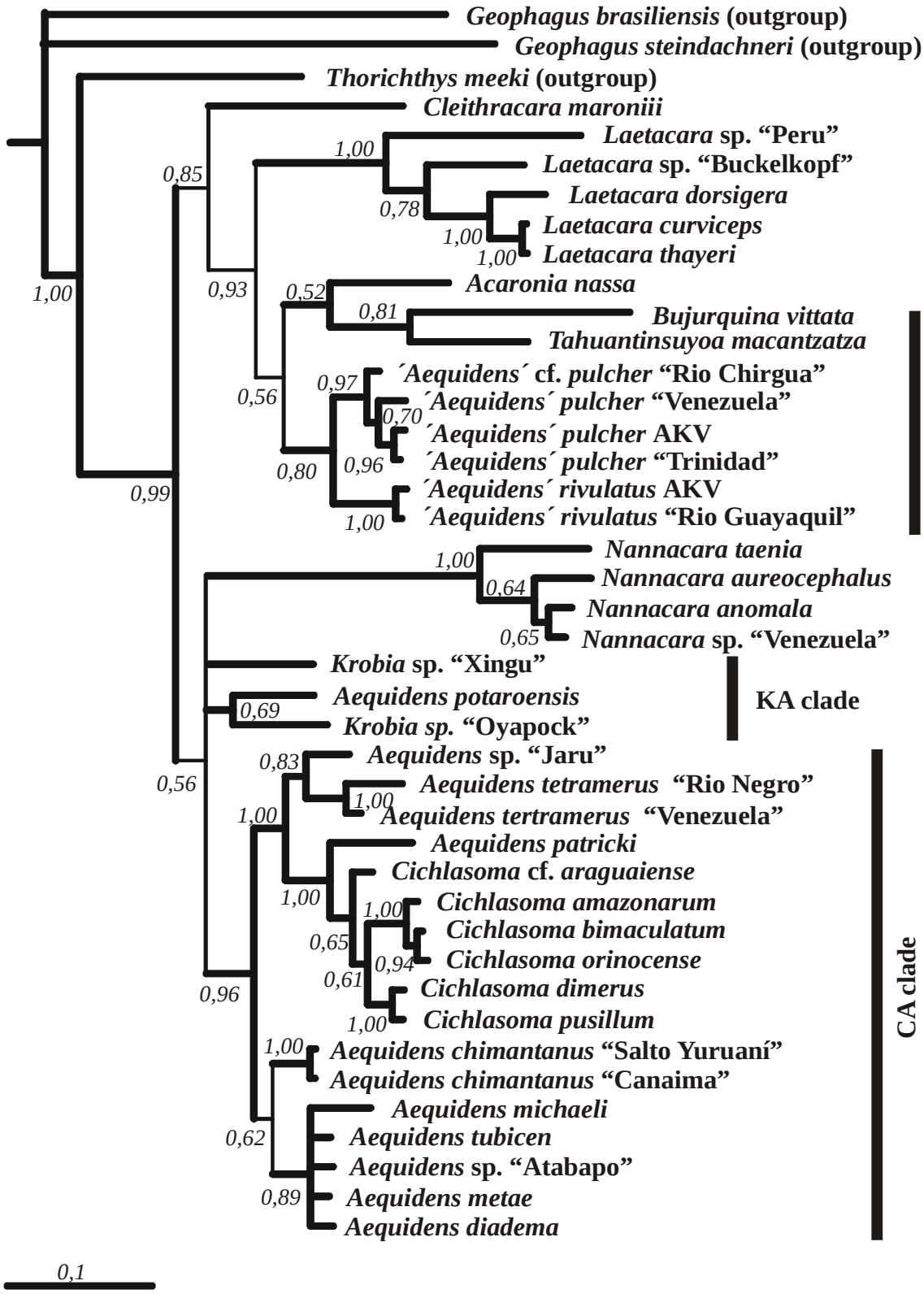


Fig. 4: The Bayesian tree based on a 612 bp long fragment of the 16S rRNA gene. The tree was performed for 1,000,000 generations, the last 7,500 trees used. The numbers in the nodes indicate the value of the Bayesian posterior probabilities. The thin lines indicate the branches not supported in the MP tree (Fig. 3).

4.2.3 The analysis of the mitochondrial cytochrome b gene

The fragment of 422 pb was used for the MP analysis and the fragment of 420 bp was used for Bayesian analyses for construction of the phylogenetic trees (Figs. 5-6). The reduced alignment of 40 sequences was used, three of them were downloaded from the Genebank, (see the list of samples in Appendix 2). The strict consensus of 26 best MP trees had very few well supported clades (see Fig. 5). There was a lot of unresolved positions, including also one outgroup species, and this tree supported practically only the generic monophylies, the deeper topology remained unresolved. The Bayesian tree was constructed using the algorithm „codon“ in the MrBayes software, which counts with the whole triplet as one character. The Bayesian tree (Fig. 6) differed from the MP tree (Fig. 5).

summary of the clades:

- ⇨ **BTA clade** was supported by the Bayesian analysis with the 0.97 support (see the Fig. 6), but the inner topology differed (*Tahuantinsuyoa* sister to the rest of species in the clade); MP analysis had the unresolved topology
- ⇨ **CA clade** was supported in both MP and Bayesian analyses (questionable supports of bootstrap 50 % and the maximum possible support of the Bayesian posterior probability of 1.00, respectively)
- ⇨ **NC clade** was unresolved in MP analysis and different topology resulted in Bayesian analysis.
- ⇨ **KA clade** was well supported in both MP and Bayesian analyses with the support of 67 % of bootstrap and 1.00 of posterior probabilitiy, respectively.

In the Bayesian analysis, there was a sister position of *Laetacara* and *Nannacara*. There was also the **CA clade** with the sister clade of *Cleithracara* but with the questionable support. The position of the outgroup species, *Thorichthys meeki,* was not outside the tribe Cichlasomatini but it resulted on the base of the tree with the sister **KA clade** (see Fig. 6). *Acaronia* standed as a basal taxon for all the rest of Cichlasomatini except for the **KA clade**.

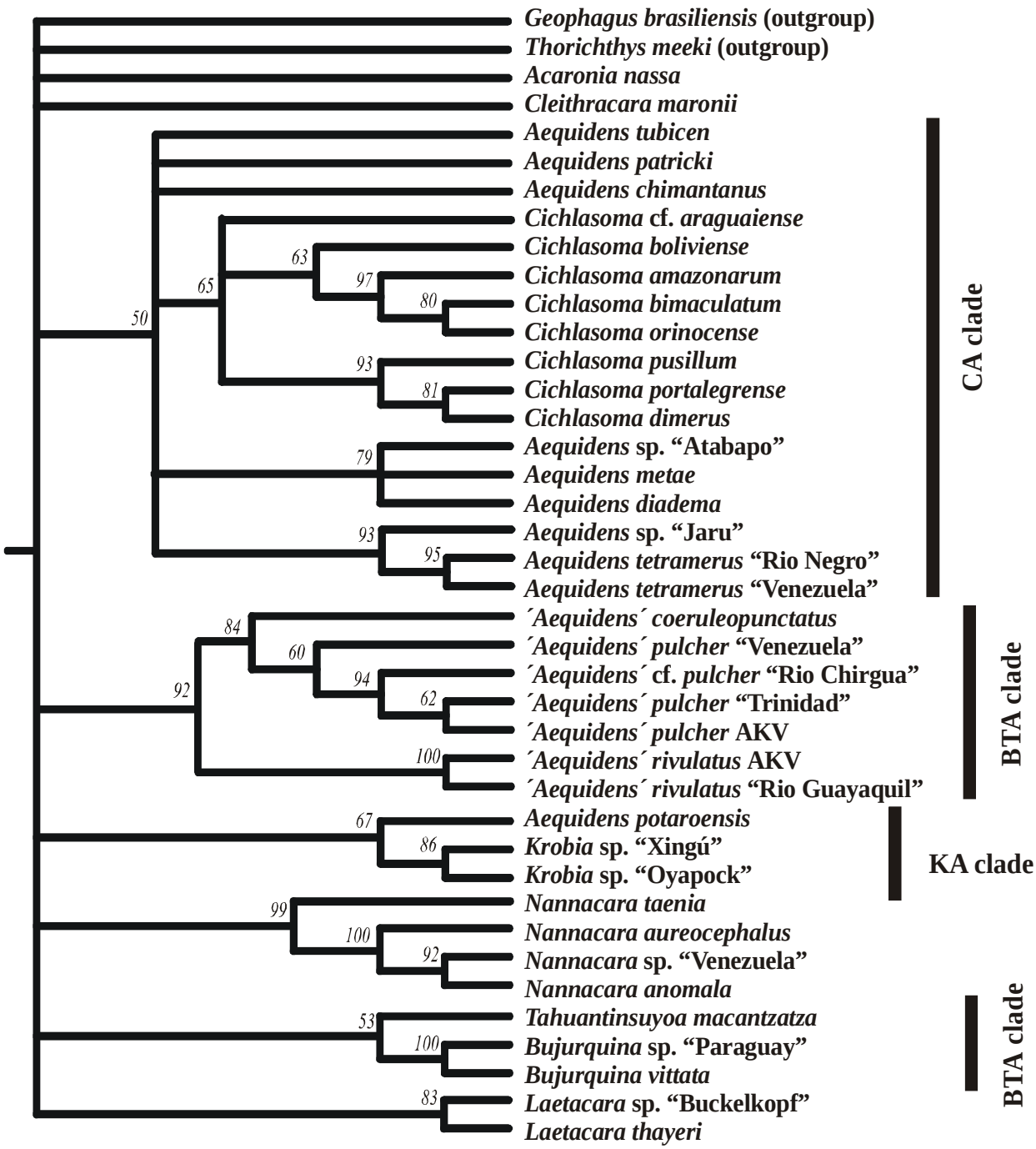


Fig. 5: The strict consensus of 26 Maximum Parsimony trees based on a 422 bp long fragment of the cytochrome b gene. The tree length is 859 steps. The numbers in the nodes indicate the bootstrap supports counted for 1000 replicates. CI= 0.3097. RI= 0.6191. The abbreviations of clades are given in text.

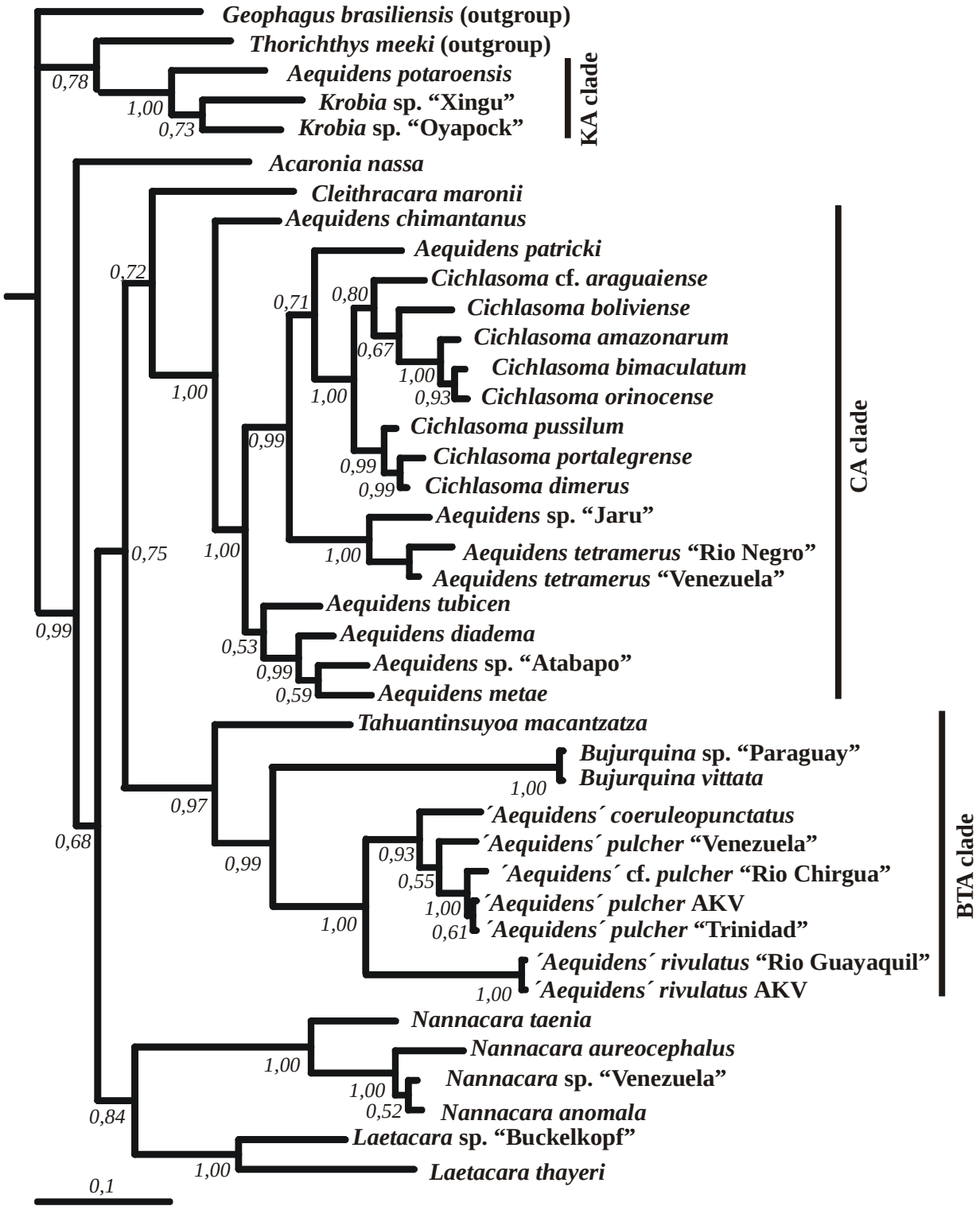


Fig. 6: The Bayesian tree based on a 422 bp long fragment of the cytochrome b gene. The model of „codon" was used for analysis. The tree was performed for 2,500,000 generations, the last 7,000 trees used. The numbers in the nodes indicate the Bayesian posterior probabilities. The abbreviations of clades given in text.

4.2.4 The analysis of the nuclear intron in ribosomal S7 gene

The dataset of sequences of the total length of 508 bp was used as an input for both MP and Bayesian analyses. In the alignment, ten deletions were observed (see the Table 4). The trees were constructed (See the Figs. 7-8) using the reduced alignment of 38 sequences. Between the MP tree and Bayesian tree there is no conflict, the Bayesian tree is just less unresolved than the MP tree (Figs. 7-8).

Summary of the clades:

- **BTA clade** was totally supported in both MP and Bayesian analyses (bootstrap 100 % and posterior probability of 1.00, respectively)
- **CA clade** was well supported in both analyses, with bootstrap of 88 % and the posterior porbability of 1.00.
- **NC clade** was well supported in both analyses, with bootstrap of 93 % and the posterior probability of 1.00
- **KA clade** was very well supported in both analyses, bootstrap support is 98 % and posterior probability value is 1.00.

In both MP and Bayesian analyses, the **CA clade** and **KA clade** were sister clades with the support of 86 % of bootstrap and 1.00 of posterior probability, respectively. *Acaronia* and *Laetacara* were sister genera here and they were sister taxa to the **CA clade** (with the questionable bootstrap support of 57 % and 0.66 of posterior probability). This sister position was in contrast to the mitochondrial genes (see also the Figs. 15-16) because in the mitochondrial genes the genera *Acaronia* and *Laetacara* were more relative to the **BTA clade**. The MP analysis solved also the sister position of **BTA clade** and **NC clades** with the bootstrap support of 57 %.

indels	taxons	length	position
deletion 1a	*Nannacara all*	14	176
deletion 1b	*Laetacara thayeri, L. dorsigera, L. sp.* "Buckelkopf", *Cleithracara*	7	176
deletion 2	*Acaronia*	4	218
deletion 3	*´Aequidens´ rivulatus, all*	4	314
deletion 4a	*Nannacara all*	5	316
deletion 4b	*Cleithracara*	9	316
deletion 5	*Acaronia*	10	322
deletion 6	*Cichlasoma all, Aequidens tetramerus, A. metae, A. patricki, A. sp. "Jaru", A. tubicen*	13	380
deletion 7	*Laetacara all*	8	466
deletion 8	*Aequidens tetramerus less A. tetramerus* "Rio Aguarico"	8	465

Table 4: The deletions observed in the nuclear S7 intron mapped on the tree (see Fig. 8 with mapped deletions). Deletions initiating in the same alignment position but having the different length are indicated as a, and b.

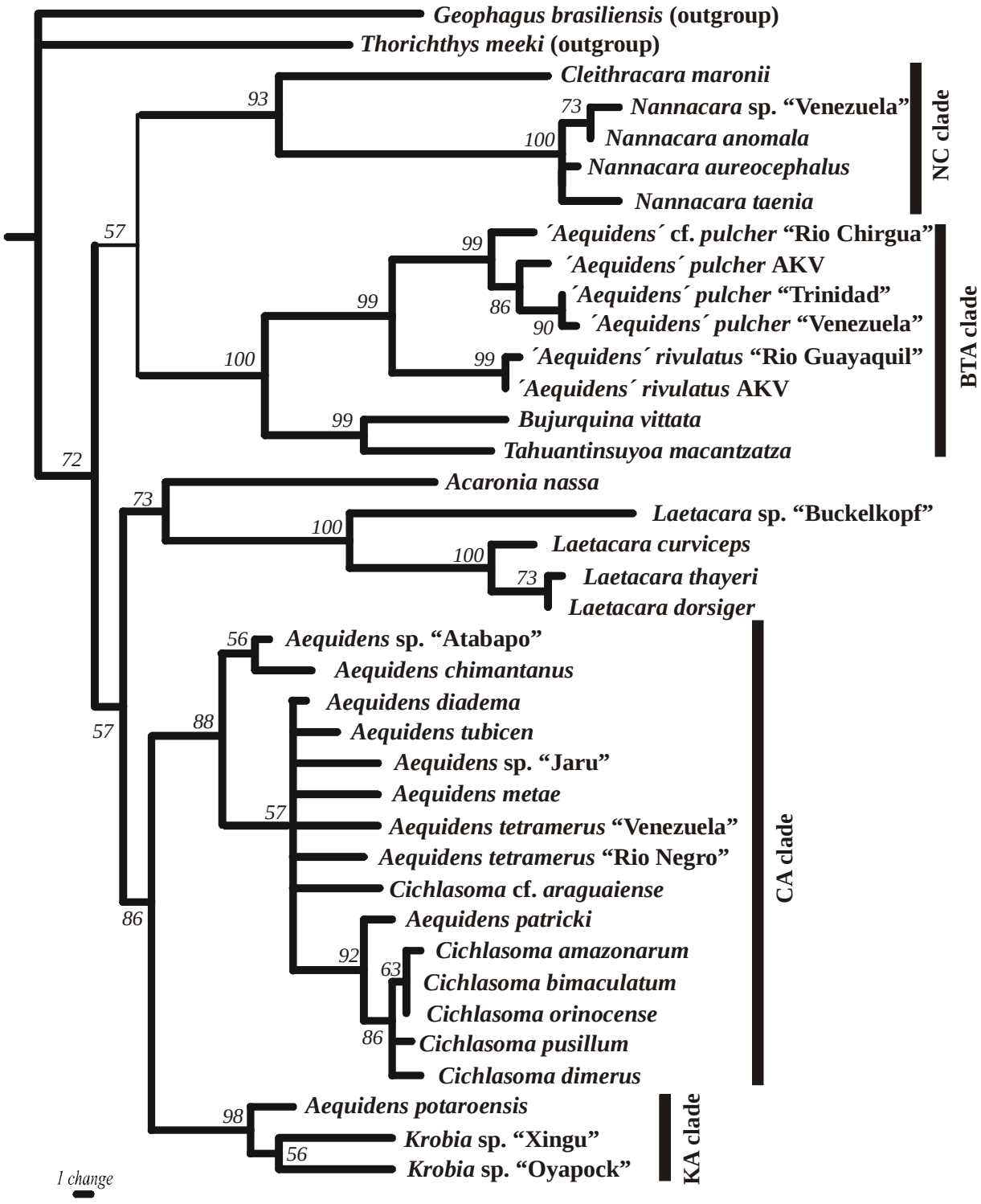


Fig. 7: The strict consensus of 144 best Maximum Parsimony trees based on a 508 bp long fragment of the nuclear marker, the first intron in ribosomal S7 gene. The tree length is 341 steps. The numbers in the nodes indicate the bootstrap supports counted for 1000 replicates. CI= 0.8211. RI= 0.8920. The bar below the tree marks the changes in the DNA sequence. The thin lines indicate the branches that did not resulted in the Bayesian analysis.

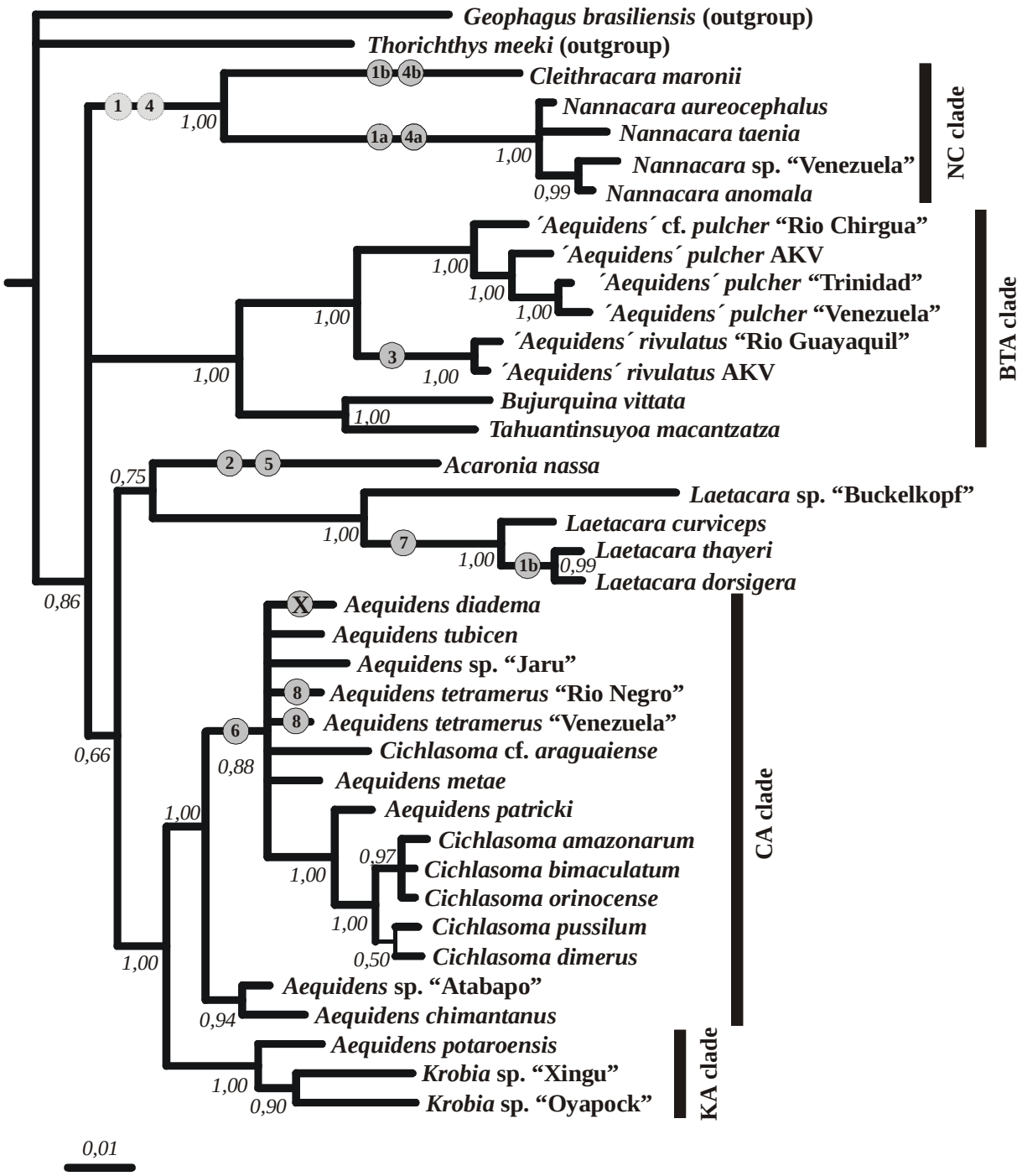


Fig. 8: The Bayesian tree based on the 508 bp long fragment of the nuclear marker, the intron in ribosomal S7 gene. The tree was performed for 2,000,000 generations, the last 15,000 trees used. The numbers in the nodes indicate the Bayesian posterior probabilities. The gray circles mark the position of deletion as mentioned in Table 4. The a- and b-marked circle of the same number indicate the deletions that initiate in the same position of alignment but differ in the length. See the Table 4. The light-gray circles with numbers 1 and 4 mark hypothetical position of deletion if the deletions 1a and 1b and 4a and 4b would originate by the same evolution event.

4.2.5 The results of the concatenation of genes

The Approximately Unbiased test (SHIMODAIRA, 2002) and Incongruence Length Difference Partition Homogeneity test (FARRIS ET AL, 1995) significantly supported the concatenation of mitochondrial genes only. The conflicting clades were explored and removed from the test.

Using the ILD PH test, the concatenation of all three genes was allowed only without the genera *Acaronia* and *Laetacara* and without five *Aequidens* species (*A. metae, A. chimantanus, A. diadema, A. patricki, A. sp. „Atabapo“*) (ILD=0,104; the null hypothesis about the homogeneity in the dataset was not rejected).

Using the AU test, the concatenation was allowed after the exclusion of *Acaronia* and ignoring of the inner topology in *Aequidens* genus (results of AU test, see Table 5).

data\topo	16s	cyt b	S7
16s	xxx	0.268	0.01
cyt b	0.136	xxx	0.0004
S7	4×10^{-10}	1×10^{-57}	xxx
S7 (-Aeq)	0.357	0.105	xxx

Table 5: The results of p-value of the AU test observed during the concatenation of the genes. In the lines, there are the tested datasets, in columns there are topologies of every gene used as the constraint. The gray patches indicate where the null hypothesis of homogeneity of the phylogenetic signal was rejected in the significance level of $p<0.05$. The concatenation of two mitochondrial genes (16S rRNA and cytochrome b) resulted as possible. The concatenation of nuclear S7 intron was possible only after the removal of *Acaronia* and the inner topology of *Aequidens* species.(i.e. ignoring their topology in 16S and cyt b topological constraints)

4.2.6 The analysis of concatenated mitochondrial genes, 16S rRNA and cytochrome b

The dataset of sequences of the total length of 1034 bp was used as an input for both MP and Bayesian analyses. The trees were constructed (see the Figs. 9-10) using the reduced alignment of 38 sequences.

The concatenation was allowed by the Approximetly Unbiased test (Shimodaira, 2002), see the p-values in the Table 5. In the Bayesian method, the model was counted separately for stem and loop positions in 16S rRNA and also for the first, second and third positions in the coding sequence of cytochrome b. There was no conflict in the MP tree and in the Bayesian tree, the MP tree was more unresolved, only.

Summary of clades:

- ⇨ **BTA clade** was supported by Bayesian analysis (0.73 of posterior probability), in MP has support lower than 50 %.
- ⇨ **CA clade** was very well supported in both MP and Bayesian analyses with support 88 % of bootstrap and 1.00 of posterior probability, respectively.
- ⇨ **NC clade** was supported by Bayesian analysis (posterior probability of 0.75), in MP analysis it got the support lower than 50 %.
- ⇨ **KA clade** was supported in both MP and Bayesian analyses (support of 82 % of bootstrap and 0.99 of posterior probability).

The **BTA clade** formed the monophylum with *Laetacara* and *Acaronia* genera. This monophylum was sister group to the **CA clade**. There was also the monophylum of the **NC clade** and **KA clade**, which resulted as a basal clade. These clade grouped together all Guyanan taxa (that live in Guyana rivers). It is *Cleithracara*, *Nannacara*, *Krobia* and *Aequidens potaroensis* (see Fig. 10).

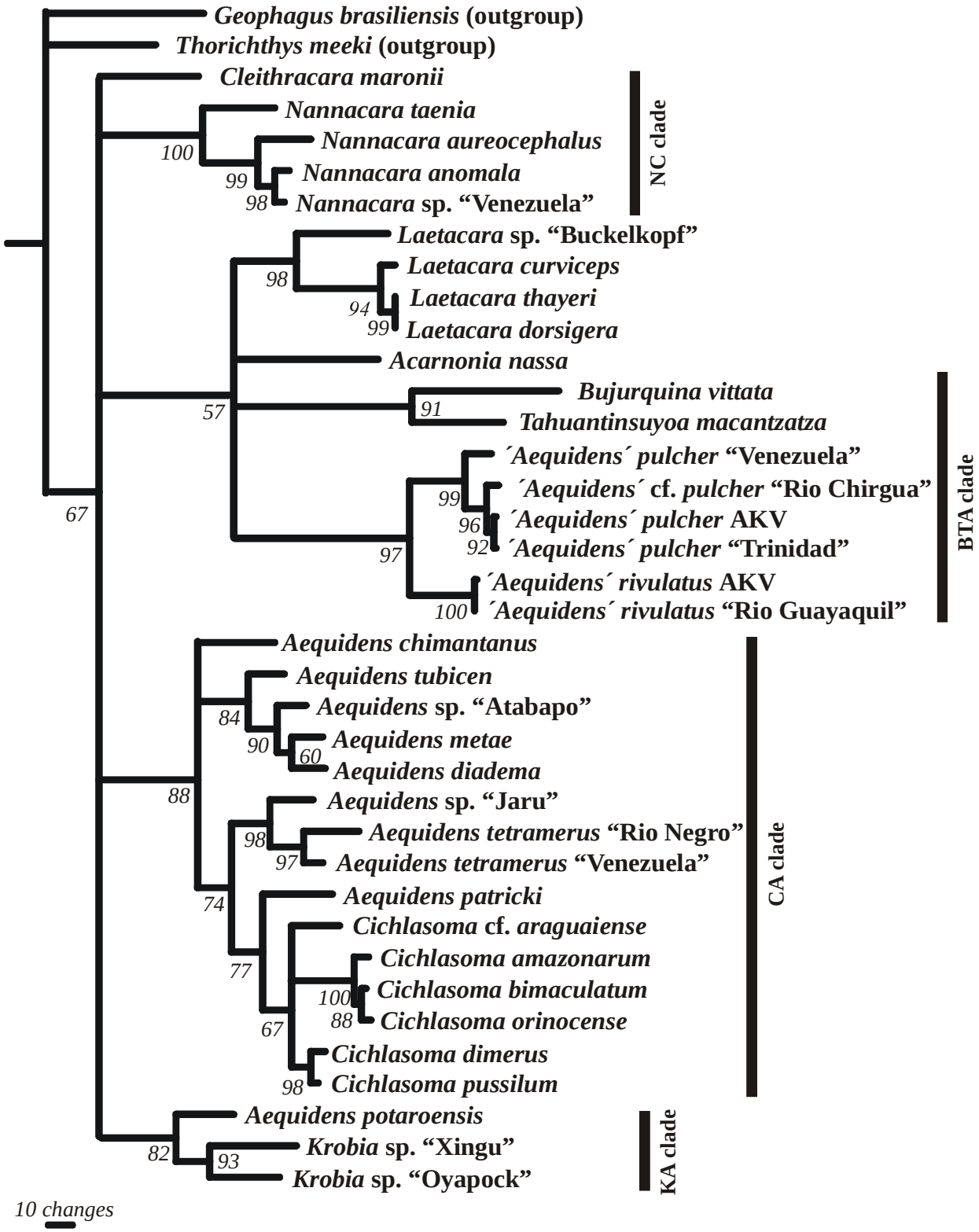


Fig. 9: The strict consensus of 6 best Maximum Parsimony trees based on a 1034 bp long combined "reduced alignment" dataset of both mitochondrial genes, 16S rRNA and cytochrome b. The tree length is 1285 steps. The numbers in the nodes indicate the bootstrap supports counted for 1000 replicates. CI=0.3821. RI= 0.6170. The bar below the tree marks the amount of changes in DNA sequence.

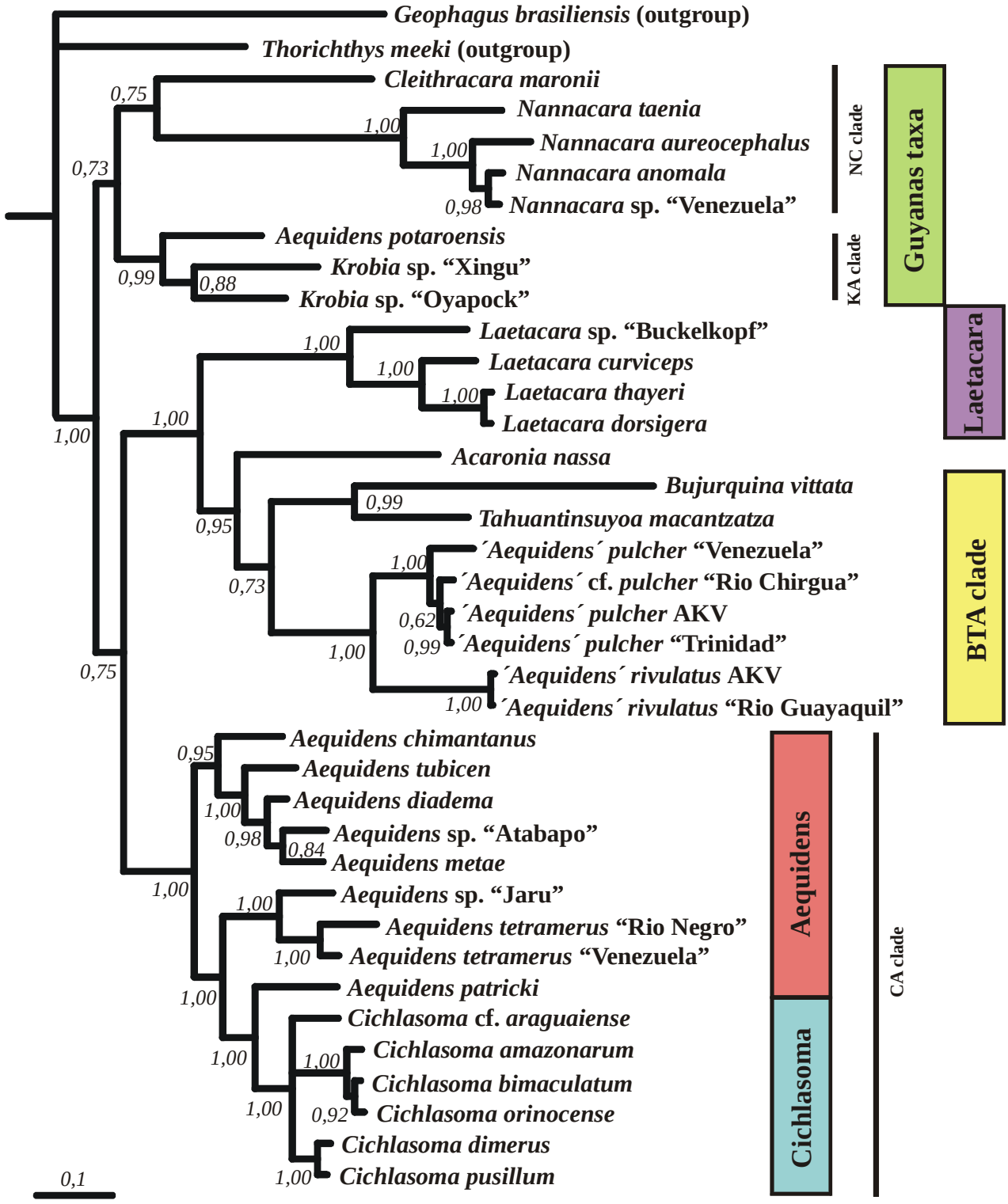


Fig. 10: The Bayesian tree based on a 1034 bp long fragment of concatenated both mitochondrial genes, 16S rRNA and cytochrome b. Constructed from the "reduced alignment" of 38 sequences. The model of GTR+I+G was used independently for each gene, each triplet position in the cytochrome b coding gene, for the stem and loop parts of 16S r RNA. The analysis was performed for 1,000,000 generations, the last 7,500 trees used. The numbers in the nodes indicate the Bayesian posterior probabilities.

4.2.7 The analysis of all three concatenated genes

The dataset of sequences of the total length of 1542 pb was used as an input for MP, ML and Bayesian analyses. The trees were constructed (see the Figs. 11-14) using the large alignment of 63 sequences (the Bayesian tree only) and the reduced alignment of 38 sequences. The concatenation of the whole alignment was allowed neither by ILD test implemented in PAUP (SWOFFORD, 2002) nor by the Approximetly Unbiased test (SHIMODAIRA, 2002), see the p-values in Table 5. With the limited conditions (see above), the ILD test allowed to concatenate all three genes if the genera *Acaronia, Laetacara* and five *Aequidens*-species were excluded, and the AU test allowed to concatenate all three genes if *Acaronia* were excluded and the inner topology of the genus *Aequidens* was ingnored.

Nevertheless, the concatenation was done without the problematic taxa as well as with the whole dataset. The trees were compared and the topology of rest of „unconflict" species remained unchanged, hence, only the full tree is published here, with the notice of conflict points.

In the Bayesian method, the model was counted separately for 1) stem and loop positions in 16S rRNA, 2) for the first, second and third positions in the coding sequence of cytochrome b, and also 3) for the intron in S7 gene. This means six independently tested partitions.

The MP, ML and the Bayesian trees there are only softly incongruent in the position of two *´Aequidens´ pulcher* species and in the position of several *Aequidens*-species. The MP tree is just less resolved than the ML and Bayesian tree (see Figs.12 - 14).

Summary of clades:

- **BTA clade** was very well supported in all MP, ML and Bayesian analyses with the bootstrap support of 98 % (MP), 100 % (ML) and the posterior probability value of 1.00 in both large alignment (LA) and reduced alignment (RA), respectively.
- **CA clade** was also very well supported in all analyses, the bootstrap value was 99 % (MP), 99 % (ML) and the Bayesian posterior probability value for both LA and RA observed 1.00.
- **NC clade** was well supported in all analyses, the bootstrap value was 85 % (MP), 93 % (ML) and the Bayesian posterior probability value for both LA and RA was 1.00.

⇨ **KA clade** was very well suppoerted in all analyses, the bootstrap value was 99 % (both MP and ML) and the Bayesian posterior probability values was 1.00 for both RA and LA.

In all MP, ML and Bayesian trees, the **BTA clade** was associated with the *Acaronia* and *Laetacara* species, the incongruence was found in the relationship to the **BTA clade**, in the MP and ML trees, *Acaronia* was the sister taxa to the BTA clade (bootstrap support 64 % in MP and 55 % in ML, respectively). In the Bayesian analysis, there resulted *Laetacara* as a sister clade to the **BTA clade** (0.55 of posterior probability). In the ML and Bayesian analysis, there was the sister position of **CA clade** and **KA clade** with the total value of posterior probability of 1.00 and 66 % of bootstrap, respectively, but in the MP tree this sister position got less than 50 % of bootstrap support and was considered to be unresolved. Within the **CA clade**, the monophyly of the genus *Cichlasoma* was unresolved in MP tree and was not supported in ML and Bayesian tree (the species *Aequidens patricki* resulted inside the *Cichlasoma* clade). The species *Aequidens chimantanus* clustered as a basal taxon of **CA clade** in MP and ML analysis. In Bayesian analysis of both LA and RA, the species *Aequidens chimantanus* clustered as a basal taxon to the clade of several *Aequidens*-species.

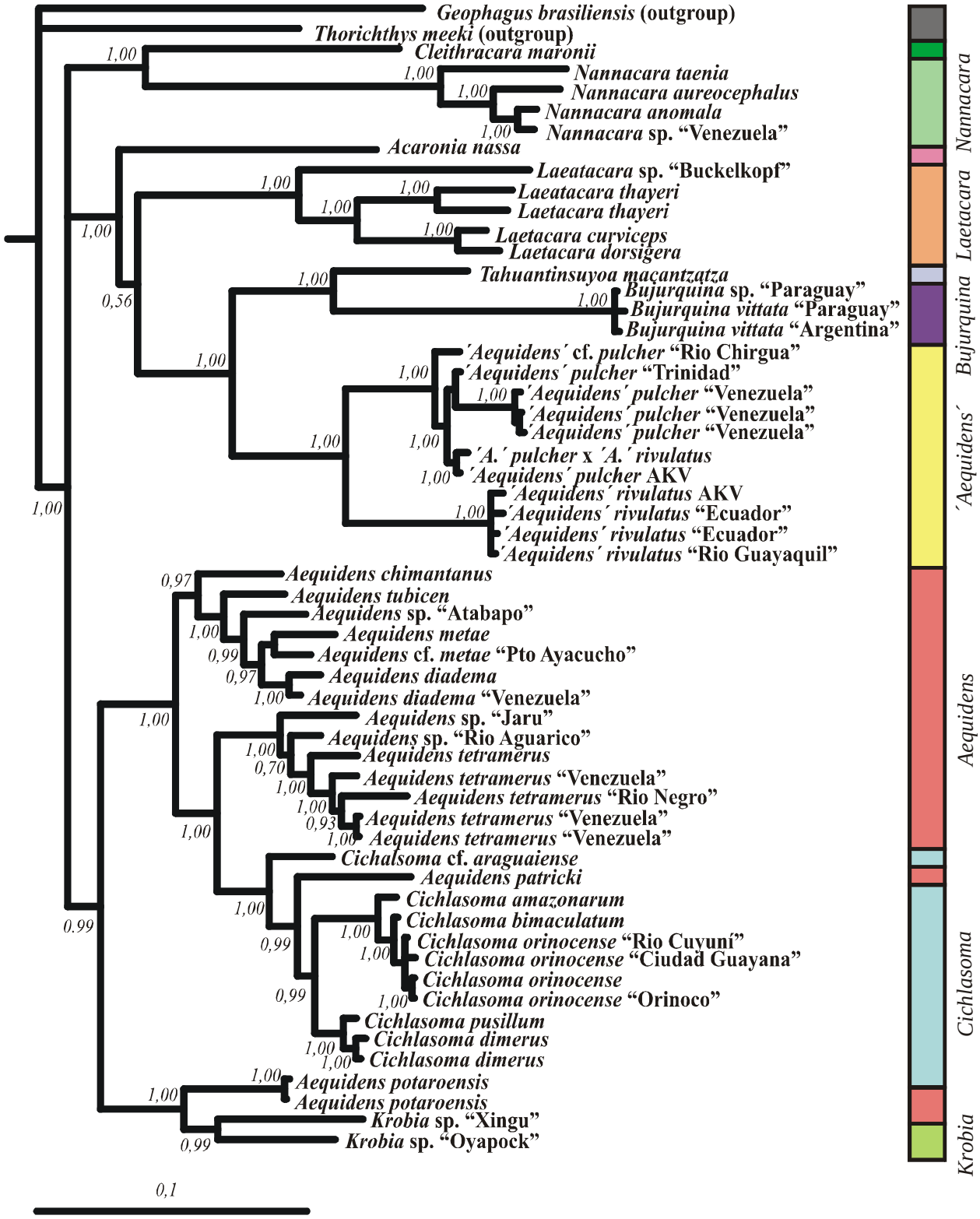


Fig. 11: The Bayesian tree based on the concatenated "large alignment" dataset of 63 sequences combined from all three genes. The model of GTR+I+G was used and the tree was performed for 2,000,000 generations, the last 15,000 trees used. The numbers in the nodes indicate the Bayesian posterior probabilities. The concatenation was allowed only with exclusion of *Acaronia*, *Laetacara* and five *Aequidens*-species (ILD test) or excluding *Acaronia* and the inner topology of *Aequidens* (AU test), respectively.

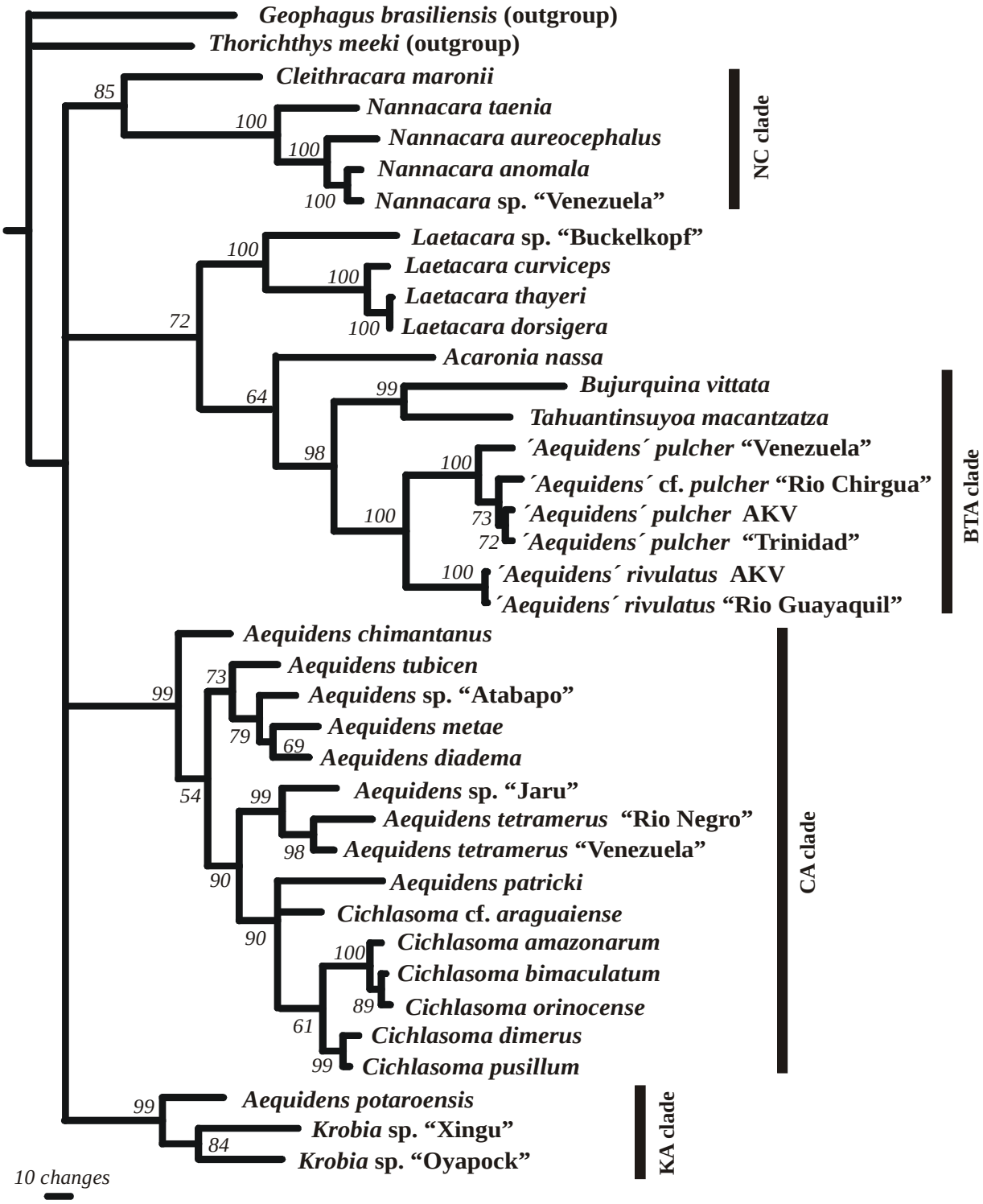


Fig. 12: The best Maximum Parsimony tree based on a 1542 bp long combined "reduced alignment" dataset of 38 sequences. Analysis based on both nuclear and mitochondrial markers (3 genes: 16S rRNA, cytochrome b, intron in S7 gene). The tree length is 1702 steps. The numbers in the nodes indicate the bootstrap supports counted for 1000 replicates. CI=0.4542. RI= 0.6577. The bar below the tree marks total amount of the changes in sequence of DNA. The concatenation was allowed only with exclusion of *Acaronia*, *Laetacara* and five *Aequidens*-species (ILD test) or excluding *Acaronia* and the inner topology of *Aequidens* (AU test), respectively.

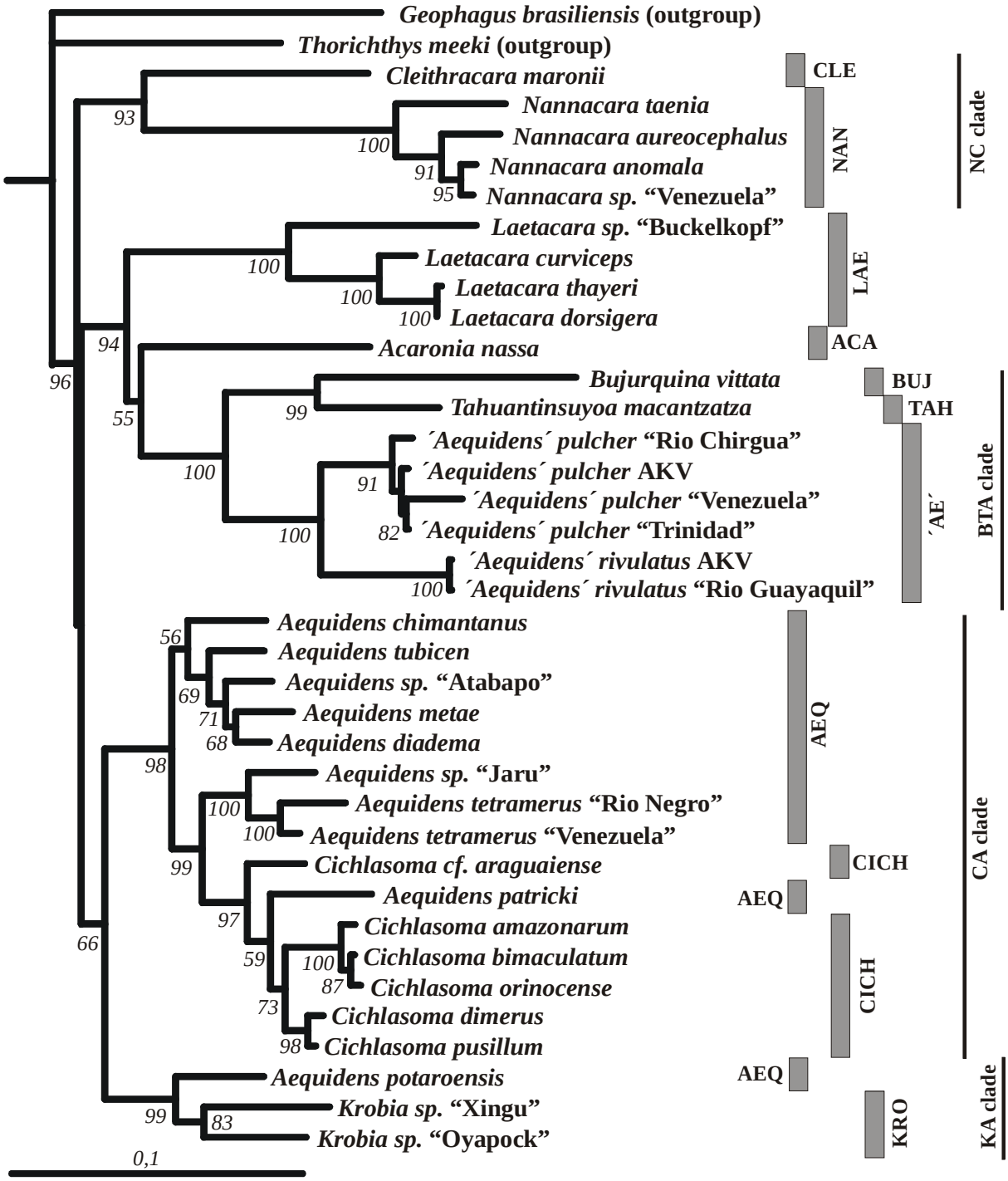


Fig. 13: The best Maximum Likelihood tree based on a 1542 bp long concatenated "reduced alignment" dataset of 38 sequences. Analysis based on the nuclear and mitochondrial markers (3 genes: 16S rRNA, cytochrome b, intron in S7 gene). The numbers in the nodes indicate the bootstrap supports counted for 339 replicates. The concatenation was allowed only with exclusion of *Acaronia*, *Laetacara* and five *Aequidens*-species (ILD test) or excluding *Acaronia* and the inner topology of *Aequidens* (AU test), respectively.

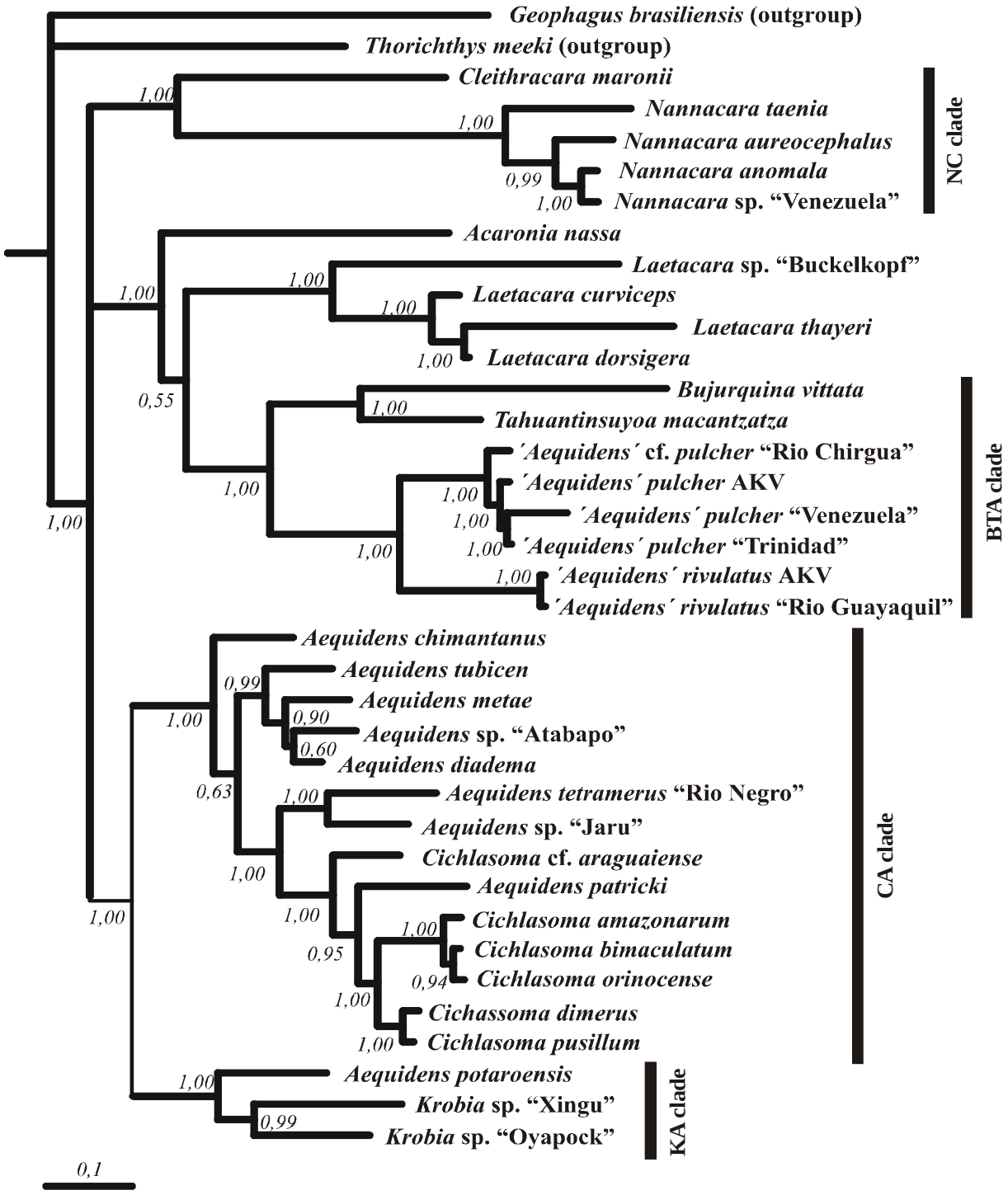


Fig. 14: The Bayesian tree based on a 1542 bp long concatenated "reduced alignment" dataset of 38 sequences. Analysis based on both nuclear and mitochondrial markers (3 genes: 16S rRNA, cytochrome b, intron in S7 gene). The model of GTR+I+G was used independently for each gene, each triplet position in cytochrome b coding gene, for the stem and loop parts of 16S r RNA. The analysis was run for 1,000,000 generations, the last 7,500 trees used. The numbers in the nodes indicate the Bayesian posterior probabilities. The thin line indicates the branches that resulted unresolved in the MP tree (Fig. 12). The concatenation was allowed only with exclusion of *Acaronia*, *Laetacara* and five *Aequidens*-species (ILD test) or excluding *Acaronia* and the inner topology of *Aequidens* (AU test), respectively.

4.2.8 Summary of phylogenetic relations

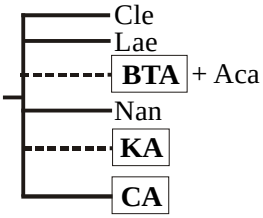
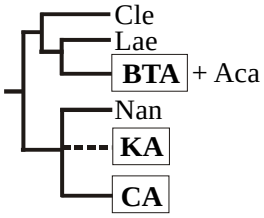
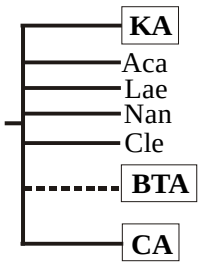
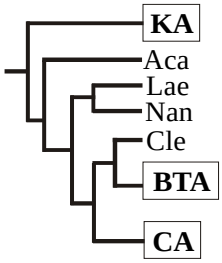
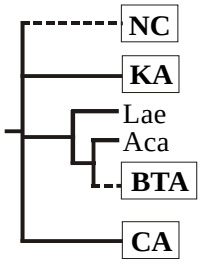
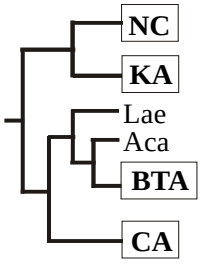
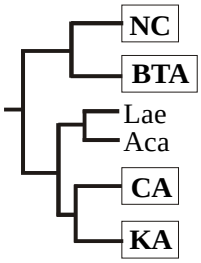
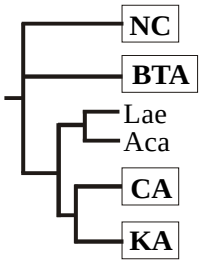
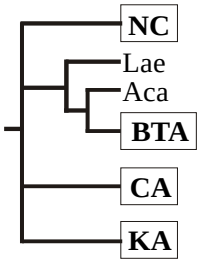
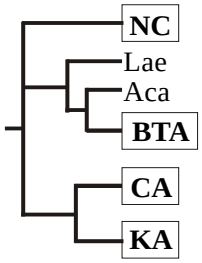
Gene / Analysis	Maximum Parsimony	Bayesian analysis
Mitochondrial 16S rRNA		
Mitochondrial Cytochrome b		
Mitochondrial concatenated 16S rRNA + cytochrome b		
Nuclear intron in S7 ribosomal protein		
Mitochondrial +nuclear concatenated 16S rRNA cytochrome b S7 intron		

Fig. 15 – The topologies of the major clades. The dashed line means the low support for the given clade. The names of clades are referred to in the text. Aca = *Acaronia*, Lae= *Laetacara*, Nan = *Nannacara*, Cle = *Cleithracara*.

topology\tree	LA	Reduced Alignment					Method
	KOMBI	16S	cytb	S7	Mitoch	KOMBI	
tribe Cichlasomatini	x	yes	unres.	yes	yes	yes	MP
	x	x	x	x	x	x	ML
	yes	yes	no	yes	yes	yes	Bayes
Nannacara	x	yes	yes	yes	yes	yes	MP
	x	x	x	x	x	yes	ML
	yes	yes	yes	yes	yes	yes	Bayes
Laetacara	x	yes	yes	yes	yes	yes	MP
	x	x	x	x	x	yes	ML
	yes	yes	yes	yes	yes	yes	Bayes
Aequidens	x	no	no	no	no	no	MP
	x	x	x	x	x	no	ML
	no	no	no	no	no	no	Bayes
Cichlasoma	x	unres.	yes	no	yes	yes	MP
	x	x	x	x	x	no	ML
	no	yes	yes	no	yes	no	Bayes
´Aequidens´	x	yes	yes	yes	yes	yes	MP
	x	x	x	x	x	yes	ML
	yes	yes	yes	yes	yes	yes	Bayes
Krobia	x	no	yes	yes	yes	yes	MP
	x	x	x	x	x	yes	ML
	yes	no	yes	yes	yes	yes	Bayes
Tahuantinsuyoa + Bujurquina	x	yes	yes	yes	yes	yes	MP
	x	x	x	x	x	yes	ML
	yes	yes	no	yes	yes	yes	Bayes
CN clade	x	no	unres.	yes	unres.	yes	MP
	x	x	x	x	x	yes	ML
	yes	no	no	yes	yes	yes	Bayes
BTA clade	x	no	unres.	yes	unres.	yes	MP
	x	x	x	x	x	yes	ML
	yes	no	yes	yes	yes	yes	Bayes
CA clade	x	yes	yes	yes	yes	yes	MP
	x	x	x	x	x	yes	ML
	yes	yes	yes	yes	yes	yes	Bayes
CA clade + KA clade	x	no	unres.	yes	no	unres.	MP
	x	x	x	x	x	yes	ML
	yes	no	no	yes	no	yes	Bayes

Table 6: The summary of observed topologies, in all three genes, all three methods and both alignments. ML = Maximum Likelihood method, MP = Maximum Parsimony method, Bayes = Bayesian methods, LA = large alignment of 63 sequences, x = the tree not constructed under the method, unres. = topology unresolved in this node. Mitoch = concatenated tree of two mitochodrial gene, KOMBI = concatenated tree of all three genes. The names of clades are referred to in the text.

	bootstrap > 90	Bayesian p.p. >95
	bootstrap 80-90	Bayesian p.p. 90-95
	bootstrap 70-80	Bayesian p.p. 80-90
	bootstrap 50-70	Bayesian p.p. 50-80

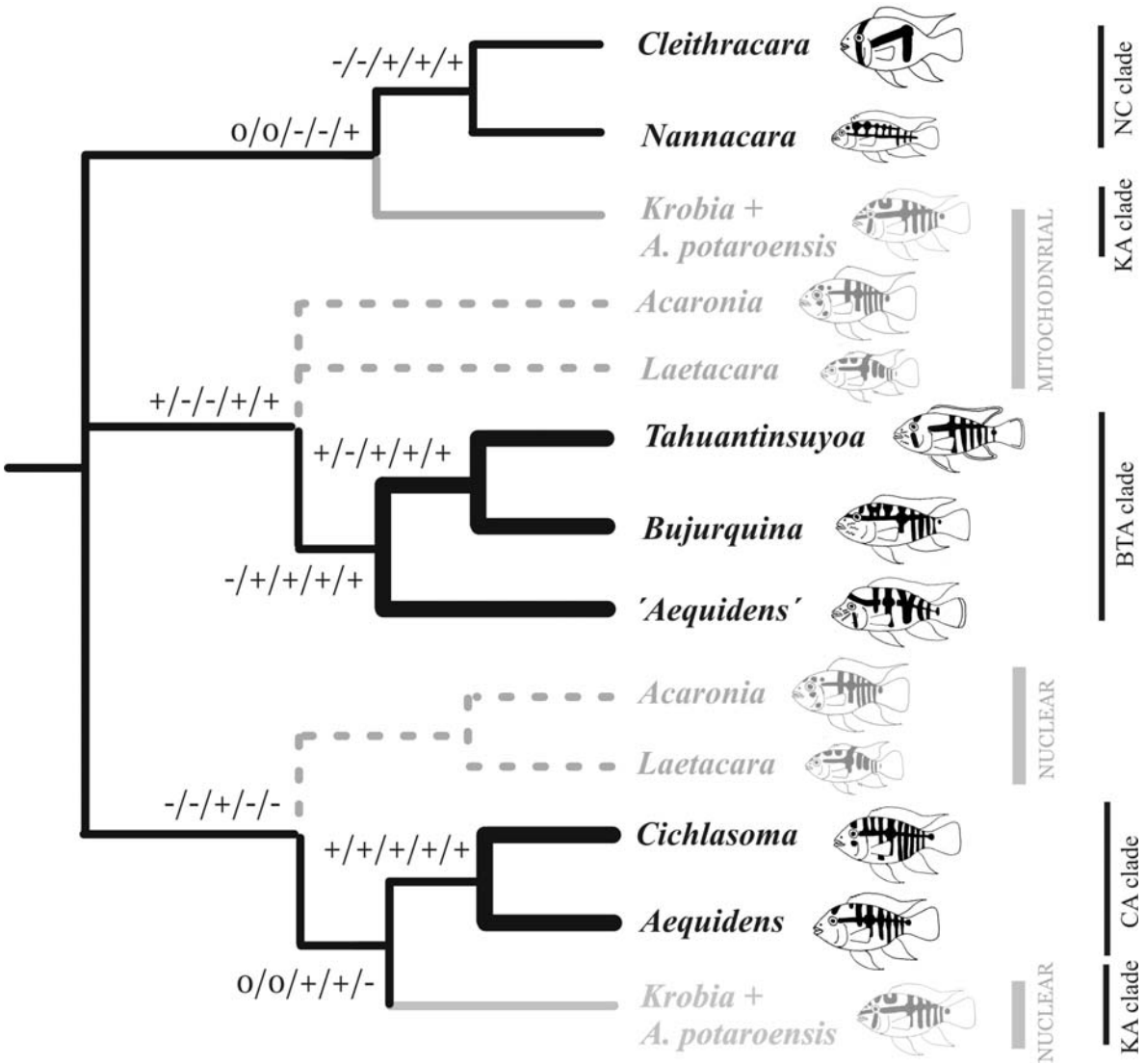


Fig. 16: The conclusion of the phylogenetic relations within the tribe Cichlasomatini shown at generic-level tree. There are included all tested genera and one species group. Two alternative positions of the *Acaronia* and *Laetacara* genera are marked by gray dashed line. The alternative hypothesis of *Krobia* and *A. potaroensis* position marked by gray full line. The black thick line indicates the clades very well supported. The signs in the nodes indicate the support from every gene and their combination in the following order: 16S rRNA (mt), cytochrome b (mt), S7 intron (nuc), concatenated tree from all three genes, concatenated tree from the mitochondrial genes. ´+´ indicates the support, ´-´ indicates not support, ´0´ indicates the unresolved situation in particular genes. Topology was considered as supported for genes with the same topology at least in one tree from the used methods (MP, Bayes). For more results see the trees from analyses (Figs. 3 – 14).

4.3 Conflicting points found out by Partitioned Bremer Support test

The Patritioned Bremer Support values were counted on the tree from concatenated all three genes. Six conflicting points were found on the concatenated cladogram of all three genes. (See Fig. 17). There is also one clade of the **KA clade** and **CA clade**, supported very well by the Bayesian posterior probability value (1.00) but less than 50% of bootstrap in parsimonious tree and zero-value of the Bremer Branch Support and Partitioned Bremer Support (see Fig. 17).

Found conflicts points:

1) *Acaronia*
    - 16S - sister to **BTA clade**
    - cyt b - unresolved on the base of cladogram
    - S7 - sister to *Laetacara* and together sister to **CA clade** + **KA clade**
    - concatenated - sister to (*Laetacara* + **BTA clade**)

2) *Laetacara*
    - 16S + cyt b – sister to (*Acaronia* + **BTA clade**)
    - S7 – sister to **CA clade** + **KA clade**
    - concatenated – sister to *Acaronia* + **BTA clade**

3) *Cleithracara*
    - 16S - sister to clade *Laetacara* + **BTA clade**
    - cyt b + S7 – forming **NC clade** with *Nannacara* genus
    - concatenated – sister to *Nannacara*, forming **NC clade**

4) The inner topology of *Aequidens tubicen, A. diadema, A. metae* and *A. sp. „*Atabapo“
    - 16S – unresolved
    - cyt b – monophylum of *A. diadema, A. metae, A. sp. „*Atabapo“
    - S7 - *A. sp. „*Atabapo“ basal to the others
    - concatenated - *A. tubicen* as a basal taxon to the three rest species. Conflict also in MP and Bayesian analyses in the inner topology of the rest three species.
5) *Cichlasoma* monophyly (two conflict points in the tree)

- 16S – *Cichlasoma* genus monophyletic, *Aequidens patricki* sister branch to *Cichlasoma*
- cyt b – *Cichlasoma* genus monophyletic
- S7 – *Cichlasoma* genus paraphyletic, *Cichlasoma araguaiense* is the basal branch, then *Aequidens patricki* and then the rest of *Cichlasoma* species

- concatenated - *Cichlasoma* paraphyletic like in S7 gene

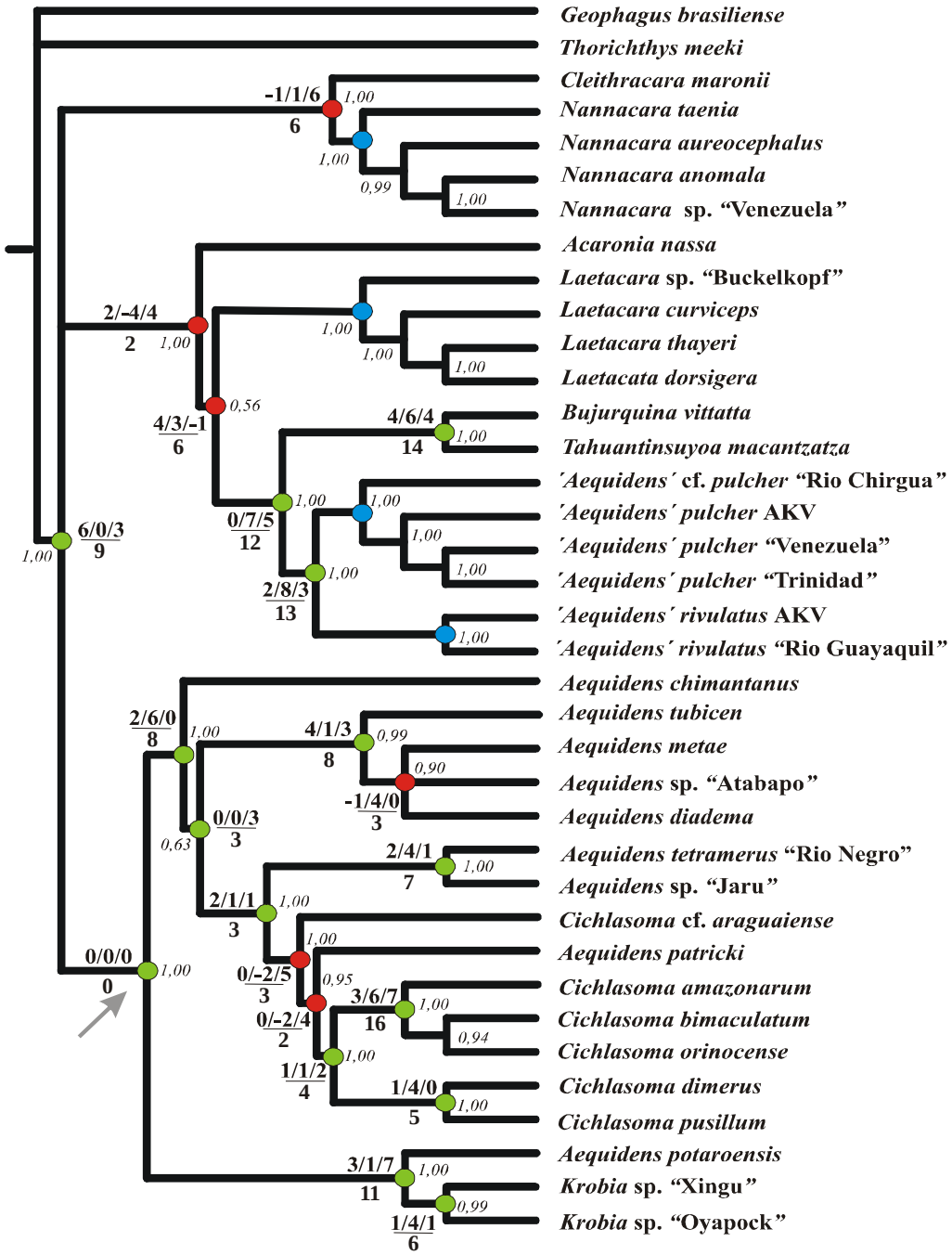


Fig. 17: The *Partitioned Bremer Support* (PBS) values tested on the tree topology from concatenated dataset of all three genes. The smaller numbers show the Bayesian posterior probabilities for each node.The larger numbers above lines show the values of PBS for selected nodes. Three values represent three genes in the following order: 16S rRNA, cytochrome b, S7 intron. The number under line indicates the total Bremer Support value for the node. The higher value the higher support for the node from the gene; the negative value means the conflict (the gene supports different more parsimonious topology). The red circles indicate the conflict nodes, the green circles indicate the nodes without conflict, the blue circle indicate the intrageneric or intraspecific nodes, which were not tested by PBS, because its topology was always constant in all trees. The gray indicator marks the node, where the observed Bayesian posterior probability support had value of 1.00 but both the parsimony bootstraping and Bremer Support values considered this node to be unresolved.

4.4 The test of alternative hypotheses

Thirty-eight hypotheses were tested: two concerned the tribe status, seven concerned the generic monophylies, ten regarding to the intergeneric relations and four to the relations of higher clades, 12 referring about the positions of unresolved taxa and three concerned about the geographic pattern in phylogenetic relations (see the Table 7). There are 12 topologies, resulting already in the concatenated tree, hence, the testing of the these topologies was unnecessary. However, they are mentioned here to demonstrate all possibilities in one table (e.g. all generic monophylies or all possible positions of unresolved taxa).

Following page:
Table 7: The results of the alternative hypotheses tested by AU-test on the concatenated dataset of all three genes. The gray patches indicate the topologies congruent with the concatenated tree, these topologies are listed here only to complete the results, their testing by AU-test was unnecessary. The column of 5% and 1% respectively, indicates the statistic significance level. The bold font indicates the accepted topologies on both significance levels. The last column with the „yes“ indicate the topologies, that have special comment mentioned in Chapter Discussion 5.5 and 5.6.

n	Hypothesis - monophylum:	AU	5%	1%	res	com
	Tribe monophyly:					
1)	**Cichlasomatini**	0,905	accept	accept	A	
2)	Cichlasomatini without *Acaronia*	0,012	**reject**	accept	5%	yes
	Generic status:					
3)	*Aequidens* genus	0,000005	**reject**	**reject**	1%	yes
4)	*Aequidens* genus (without *A. potaroense*)	0,005	**reject**	**reject**	1%	yes
5)	***Cichlasoma* genus**	0,454	accept	accept	A	yes
6)	***Nannacara* genus**	0,905	accept	accept	A	
7)	***Laetacara* genus**	0,905	accept	accept	A	
8)	***Krobia* genus**	0,905	accept	accept	A	
9)	***´Aequidens´* species group**	0,905	accept	accept	A	
	Intergeneric relations:					
10)	***Bujurquina* + *Tahuantinsuyoa***	0,905	accept	accept	A	
11)	**CN clade**	0,905	accept	accept	A	
12)	*Aequidens* + *´Aequidens´*	6×10^{-40}	**reject**	**reject**	1%	yes
13)	**BTA clade**	0,905	accept	accept	A	
14)	*Tahuantinsuyoa* + *´Aequidens´*	0,0003	**reject**	**reject**	1%	yes
15)	*Aequidens* + *Cichlasoma*	0,005	**reject**	**reject**	1%	yes
16)	**CA clade**	0,905	accept	accept	A	
17)	**CA clade + KA clade**	0,905	accept	accept	A	
18)	*Bujurquina* + *Krobia*	2×10^{-8}	**reject**	**reject**	1%	yes
19)	*(Bujurquina + Tahuantinsuyoa) + (KA clade)*	0,00003	**reject**	**reject**	1%	yes
	Position of Laetacara:					
20)	*Laetacara* + *Nannacara*	0,015	**reject**	accept	5%	
21)	***Laetacara* + NC clade**	0,103	accept	accept	A	
22)	***Laetacara* + BTA clade**	0,496	accept	accept	A	
23)	*Laetacara* + Krobia	0,0002	**reject**	**reject**	1%	
24)	*Laetacara* + KA clade	0,001	**reject**	**reject**	1%	
25)	***Laetacara* + CA clade**	0,086	accept	accept	A	
26)	***Laetacara* + CA clade + KA clade**	0,074	accept	accept	A	
	Position of Acaronia:					
27)	***Acaronia* + BTA clade**	0,905	accept	accept	A	
28)	***Acaronia* + Laeatacara**	0,453	accept	accept	A	
29)	***Acaronia* + Laetacara + BTA clade**	0,905	accept	accept	A	
30)	*Acaronia* + CA clade + KA clade	0,028	**reject**	accept	5%	
31)	***Acaronia* + NC clade**	0,065	accept	accept	A	
	NC clade position:					
32)	**NC clade + BTA clade**	0,087	accept	accept	A	
33)	**NC clade + CA clade + KA clade**	0,58	accept	accept	A	
34)	NC clade + *Laeatacara* + *Acaronia*	0,03	**reject**	accept	5%	
	Big Clades position:					
35)	(CA clade + KA clade) + (BTA clade)	0,029	**reject**	accept	5%	
	Geographic hypotheses of Guayana taxa:					
36)	*Aequidens potaroense* + *Aequidens chimantanus*	0,0001	**reject**	**reject**	1%	
37)	*Aequidens potaroense* + *A. chimantanus* + *Krobia*	0,012	**reject**	accept	1%	yes
38)	**CN clade + KA clade (Guayana taxa)**	0,376	accept	accept	A	yes

The description of this table given on the previous page.

4.5 The scenarios of the biogeographic history

The most parsimony explanation of my dataset resulted in the following scenario (see Fig. 19). The commentary of three wide-distributed species (*Acaronia nassa, Aequidens tetramerus* and *Cichlasoma bimaculatum*) was excluded.

The hypothetic common ancestor of the tribe Cichlasomatini originated from Amazon or Guyana province. Then two clades separated – one Guyanan and one Amazonian (mitochondrial scenario) or Guyanan taxa separated in two lineages (concatenated scenario; see Fig. 19). Within the Guyanan clade, two back-colonizations to the Amazon River basin occurred (Rio Xingu, *Krobia* sp. "Xingu", Rio Tocantins, *Nannacara taenia*). Other colonization to the Orinoco River basin happened (*Nannacara* sp. "Venezuela").

The Amazon clade diverged and the colonizations of other provinces occurred. The Orinoco River basin was colonized at least three times from the Amazon River basin *(´Aequidens´pulcher, Aequidens*-species and *Cichlasoma orinocense*), or probably the colonization by *Aequidens*-species occurred in two lineages. See the Fig. 19.

The Paraná River basin was colonized at least three times from the Amazon River basin (*Laetacara thayeri, Bujurquina vittata* and *Cichlasoma*-species).

The colonization of the Pacific slope of South America by *´Aequidens´* group probably occurred from the Orinoco River basin via the Maracaibo and Magdalena River basins, but the problem with the forming of Andes as a barrier was not yet clearly resolved, see below the results of the Molecular Clock estimation of the separation times.

The Cladogram Area tree (see Fig. 18) marked the one-clade position of the Pacific slope of South America, the Magdalena River and the Maracaibo provinces. Other clade was formed by the provinces of the Orinoco, Amazon and Guyana River basins with the sister branch of the Eastern Brazil Rivers. The rest of the provinces are remaining unresolved on the base of tree.

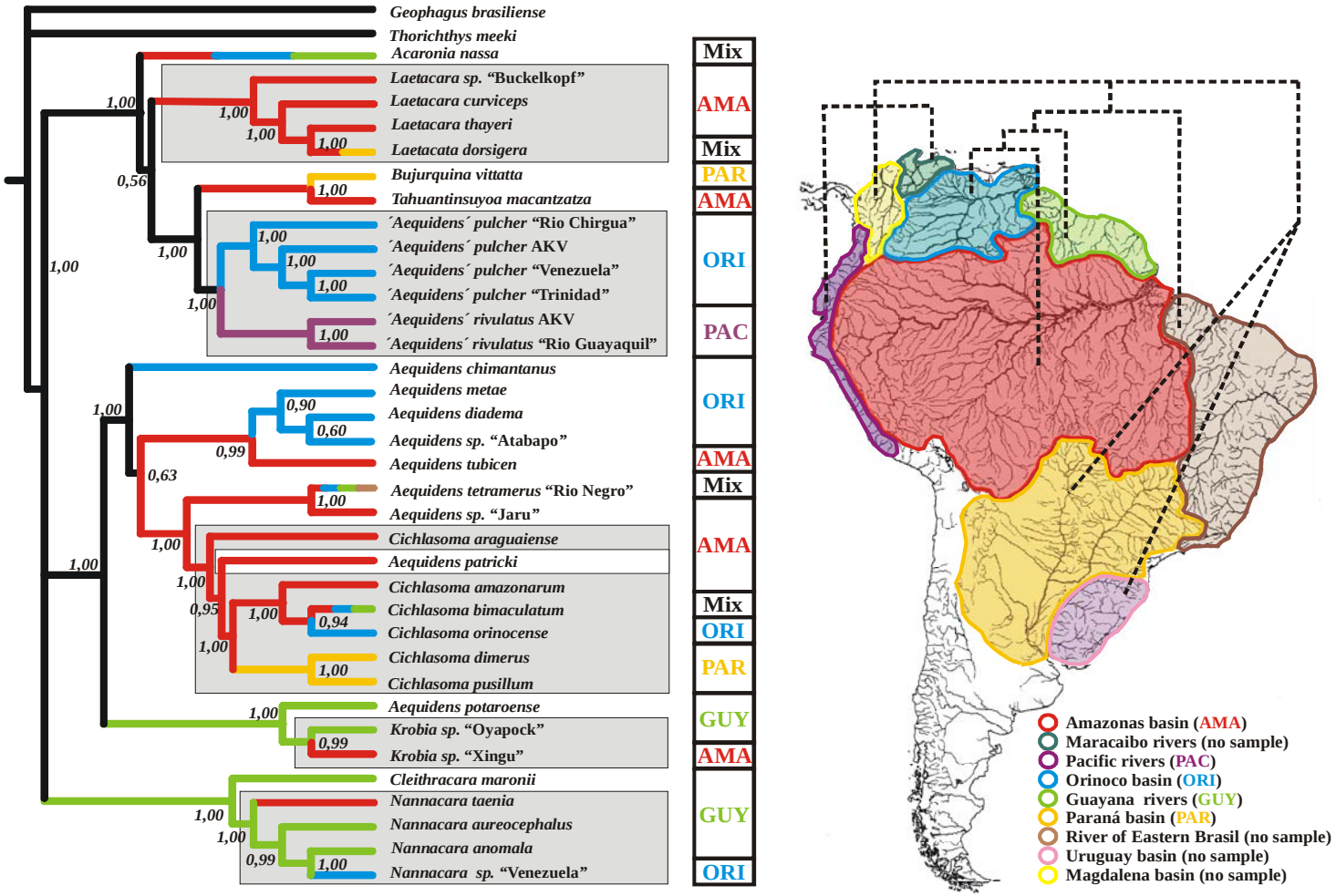


Fig. 18: The Bayesian tree based on the concatenated dataset of all three genes with the geographic distribution of species (left). The gray frames mark the generic clades.The map of the geographical fish faunal provinces (right). The black tree on the map shows the Cladogram Area based on presence/absence of species, genera and higher clades in the geographic province. The abbreviations in the column are explained below the map; Mix = the species with the more-than-one-province distribution.

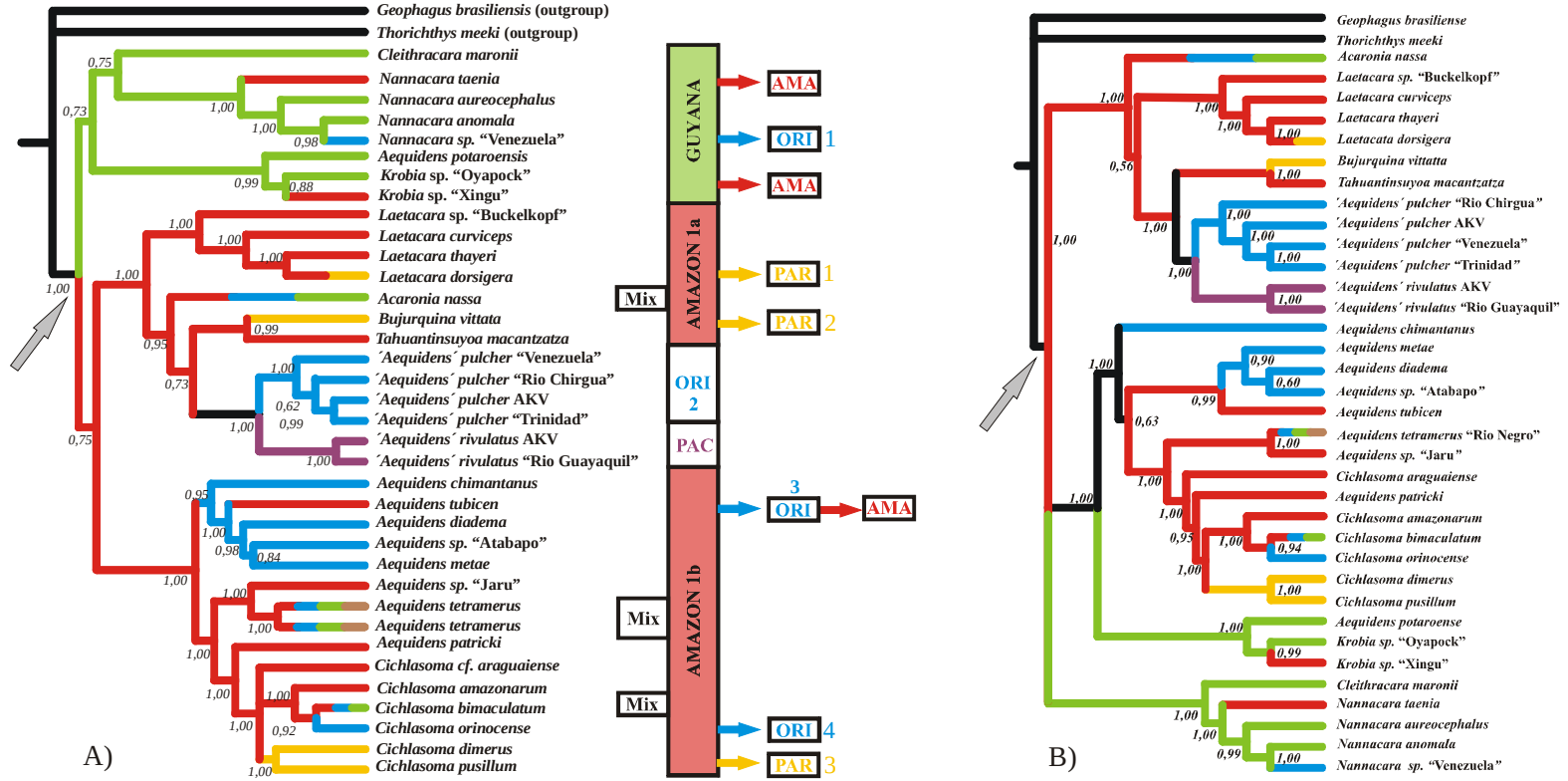


Fig. 19: The geographic distribution data mapped on the cladogram based on the A) concatenated mitochondrial genes B) concatenated dataset from all three genes. Shown the most parsimonious reconstruction, checked in the software WinClada, version 1.00.08 (Nixon, 2002).The gray indicator marks the position of a hypothetic common ancestor of the recent species belonging to the tribe Cichlasomatini. In both scenarios, the ancestor was Guyanan or Amazonian. In the mitochondrial scenario all Guyanan taxa clustered into one clade, in the all-three-genes scenario, two clades of Guyanan taxa were observed. The coloured indicators mark the colonization event. Three species with the large geographic distribution (marked as Mix) were ignored (*Acaronia nassa*, *Cichlasoma bimaculatum*, *Aequidens tetramerus*). AMA = Amazon river basin province, ORI = Orinoco river basin province, PAR = Paraná river basin province, PAC = the Pacific slope of South America. The coloures of the branches referred to the colours of the abbreviations of the provinces. The brown colour in *Aequidens tetramerus* indicates the province of the Eastern Brazil rivers. The black branches in the inner topology of the tree are unresolved (there was other identically parsimonious explanation).

4.6 The Molecular Clock results

The ML trees with the Molecular Clock algorithm enforced were constructed from every gene separately. The datasets of reduced alignments of 38 – 43 sequences were tested by the Likelihood Ratio Test (LRT) on the possibility of using clock analysis. Only the dataset of cytochrome b was significantly supported to behave clocklike. The datasets of 16S rRNA and S7 genes were rejected and the whole alignments could not be used for the clock analysis. See Table 8.

	-lnL	-lnL clock	Δ -lnL	**LRT: 2 x Δ -lnL**	samples	Degrees of freedom	**chi square on p=0.05**	A/R
16S rRNA	-3358.15	-3390.7	32.55	**65.1**	43	41	**56.94**	Reject
16S rRNA reduced	-2476.3	-2488.7	12.4	**24.8**	32	30	**43.77**	accept
cytochrome b	-3967.22	-3970.1	2.88	**5.56**	40	38	**53.38**	accept
S7 intron	-2700.6	-2727.1	26.5	**53**	38	36	**51**	Reject
S7 intron reduced	-1962.5	-1978.2	15.7	**31.4**	25	23	**35.17**	accept

Table 8: Results of the Likelihood Ratio Test (LRT), which compares the –lnL values of resulted trees. Only the value of the LRT observed for the cytochrome b gene was under the critical value for Chi-square on p=0.05 and the clock tree could be used for whole dataset. The alignment of other two genes, 16S rRNA and S7 intron had to be reduced to the dataset excluding the most distant taxa.

Therefore, one complete tree with 40 taxa (cytochrome b) and two reduced trees with 32 (16S) and 25 taxa (S7), respectively, were constructed under the assumption of Molecular Clock. Two points of interest were marked and time of separation of the clades was estimated. Subsequently, the context of separation of clades was tested with respect to the geological events. See the Figs. 20-21.

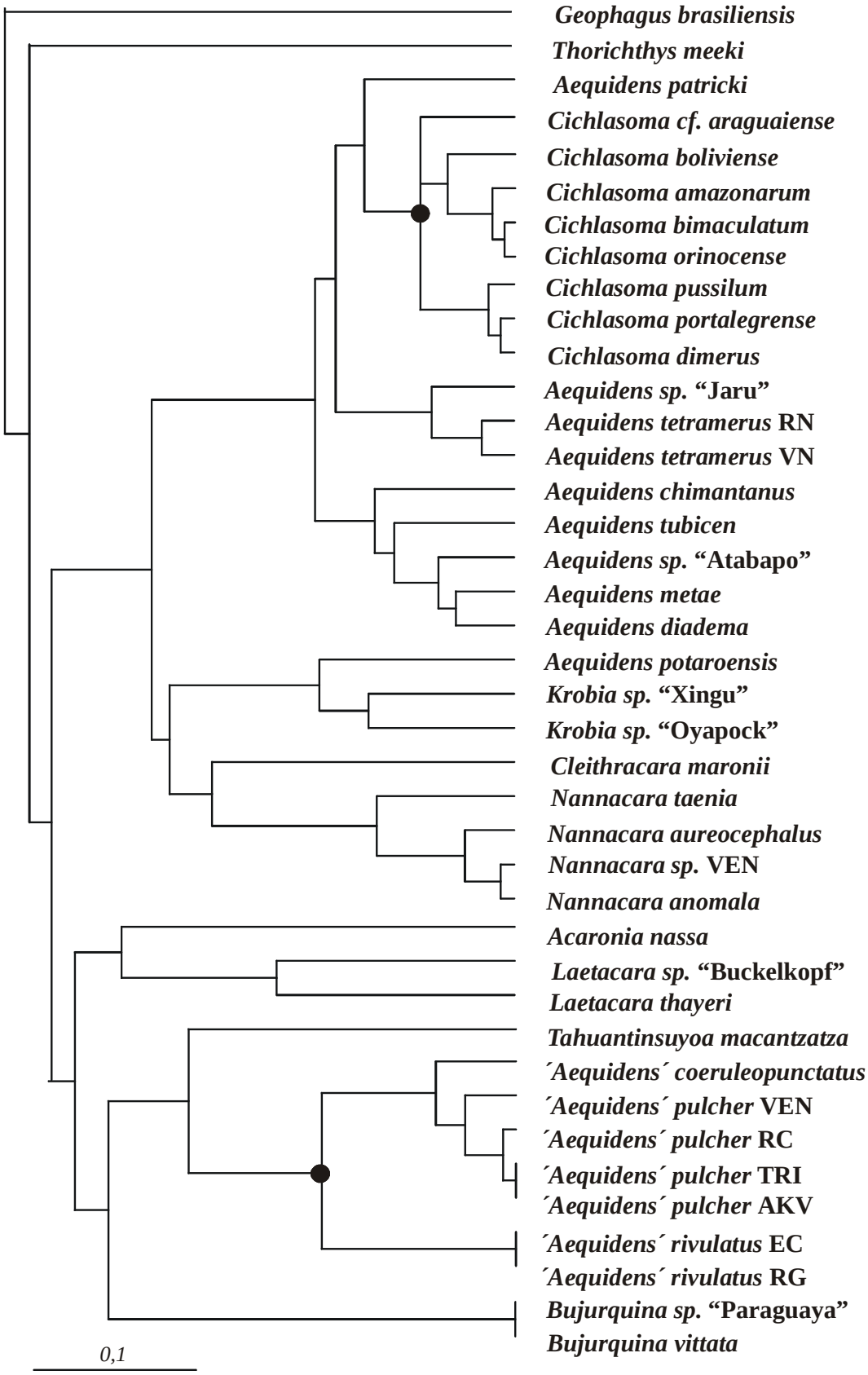


Fig. 20: The Molecular Clock tree based on the cytochrome b gene dataset. The circles indicate the tested nodes. The bar indicates the observed distances.

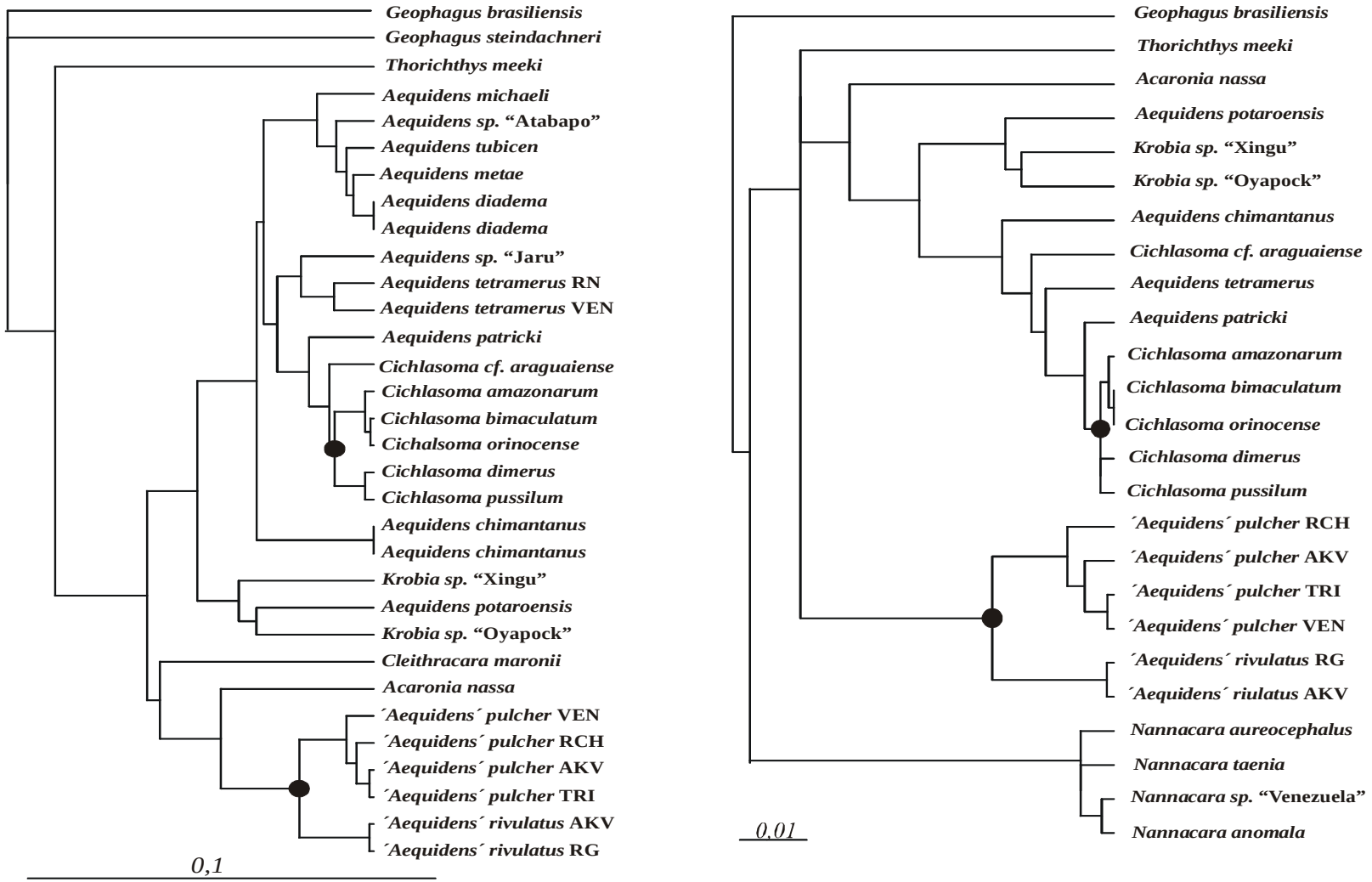


Fig. 21: The Molecular Clock trees based on the 16S rRNA dataset (left), and S7 intron dataset (right). The circles indicate the separation of two clades, which were tested by estimation of time in context to the geological event. The bar under the tree indicates the observed distances.

4.7 The mutation rate and the time of separation

There were strong incongruences in the amount of observed diversity among the gene markers, suggesting different rates of substitution. Two mitochondrial genes differed remarkably, the comparison of 16S rRNA and cytochrome b gene in *´Aequidens´ rivulatus* and *´Aequidens´ pulcher* indicated 6 – 12 times faster evolution in cytochrome b than in 16S rRNA.

The study on the as relative as possible group to Neotropical Cichlidae was accepted as the source of mutation rate. For the 16S rRNA gene in the study on Labridae (MABUCHI, 2004) was suggested mutation rate of 0,5% substitutions per My (Million years). The cytochrome b model used the mutation rate of 1,5% substitution per My suggested for African cichlids (STURMBAUER AND MEYER, 1993, KOBLMULLER ET AL., 2004). For the S7 intron, I used the only found mutation rate of 0,2 - 0,23% substitution per My suggested for Haemulidae (Perciformes; BERNARDI & LAPE, 2005).

The ratio of suggested mutation rates **1:3:0,4** (0,5% / 1,5% / 0,2-0,23%) from literature highly differed from the ratio of observed distances from my dataset. See the Table 9.

	16S	**cyt b JC**	**cyt b GTR**	**S7**
suggested	0,5	1,5		0,21
std.	**1**	**3**		**0,4**
´Aequidens´	2,15	12,5	25,35	4,2
std.	**1**	**5,8**	**11,8**	**2**
Cichlasoma	1,05	7,9	10,25	0,51
std.	**1**	**7,5**	**9,8**	**0,5**

Table 9: The ratio of observed distances in two tested clades based on the 16S rRNA gene, cytochrome b gene counted under model of Jukes-Cantor, cytochrome b counted under the GTR + I + G model, S7 intron; used mean values of intervals. The incongruence among the suggested and observed values is evident. Std. = standardized by 16S value.

4.8 The time estimations in context to the geological events

Two separation of species were tested in the context with the geological events:

1) In *´Aequidens´ pulcher* x *´Aequidens´ rivulatus* group was tested the eventual influence of Andes formation in North-western part of the continent to the species separation.
2) In the Amazon/Orinoco *Cichlasoma* species x the Paraná *Cichlasoma* species was tested the eventual influence of Paraná and Amazon basin separation to the lineage separation *Cichlasoma*-species.

The estimated time of the ´*Aequidens*´ lineage separation covered the interval of **1.2 – 11.5** Mya (Million years ago), which was not overlapping with the interval of the geological event – forming of Andes in the Northewestern part of the continent (30 – 11.8 Mya). The time of the *Cichlasoma* lineage separation was estimated in the interval of **0.4 – 4.1** Mya, which was also not overlapping with the time of Paraná and Amazon River separation (10 Mya). Therefore, all three genes suggested more recent separation of the species in question than predicted by tested geological events. Hence, these events probably were not responsible for the separation of the clades. (See the Table 10).

	16S rRNA	**cytochrome b**	**S7 intron**
´*Aequidens*´ *rivulatus* x ´*Aequidens*´ *pulcher*	1.2 - 3.1%	9.9 – 15.2% (27.7%)	3.8 – 4.6%
Cichlasoma from Amazon/Orinoco x *Cichlasoma* from Paraná River	0.4 - 1.7%	5.3 - 10.5% (12.3%)	0.4 - 0.62%
mutation rate	0.50%	1.50%	0.2 - 0.23%
divergence rate	1%	3.00%	0.4 - 0.46%
estimated divergence time of ´*Aequidens*´ separation	**1.2 - 3.1 Mya**	**3.3 – 5.07 (9.23) Mya**	**8.26 - 11.5 Mya**
estimated divergence time of *Cichlasoma* separation	**0.4 - 1.7 Mya**	**1.8 – 3.5 (4.1) Mya**	**0.86 – 1.55 Mya**
timing of forming of Andes as a barrier in North-western South America	**30 – 11.8 Mya** (Lundberg et al., 1998)		
timing of separation of Paraná and Amazonian drainages	**10 Mya** (Montoya-Burgos, 2003)		

Table 10: The observed intervals in datation of separation of clades in two tested events. The separation of ´*Aequidens* ´ *rivulatus* and ´*Aequidens*´ *pulcher* in context with the separation of Pacific and Atlantic basin of Northern South America; the estimated separation time in two lineages of *Cichlasoma*. The distances in 16S rRNA and S7 intron counted under the best fit model suggested by Modeltest (TrN+I+gamma for 16S rRNA and HKY+gamma for S7 intron), the cytochrome b distances counted by Jukes-Cantor model because of possibility of comparison; in the brackets, underlined, there is the maximum value for cytochrome b distance obtained by counting under the GTR + I + G model, as suggested by Modeltest.

4.9 The additional estimation of separation events

In the additional estimation there was tested the minimum and maximum suggested rate of 16S rRNA (RITCHIE ET AL., 1996, HUYSE ET AL., 2004), and the minimum and general teleost fish mutation rate of cytochrome b (CHANG ET AL., 2006, CARDENAS ET AL., 2005) .

Even using the extreme minimum and maximum values of the 16S rRNA gene, the results were in the interval **0.11 – 11.1** Mya for the *´Aequidens´* separation and **0.28 – 6.07** Mya for the *Cichlasoma* lineage separation, respectively. This timing was not overlapping with the interval of evolutionary events, such as in the first estimation.

However, using the extreme minimum rate of the cytochrome b gene and the general teleost fish mutation rate, the *´Aequidens´* lineage separation was estimated as **9.9 – 36.4** Mya and the *Cichlasoma* separation suggested the interval of **5.3 – 16.18** Mya. Both separation times overlapped with the interval of geological event and the possible influence of geological events to the species separation could not be rejected. See the Table 11.

	16S rRNA	**cytochrome b**
´Aequidens´ rivulatus x *´Aequidens´ pulcher*	1.2 - 3.1%	9.9 – 15.2% (27.7%)
Cichlasoma from Amazon/Orinoco x *Cichlasoma* from Paraná River	0.4 - 1.7%	5.3 - 10.5% (12.3%)
MIN estimated mutation rate	**0.14%** (Ritchie et al., 1996)	**0.38%** (Chang et al., 2006)
MIN divergence rate	0,28%	0,76%
MAX (16S) / GENERAL (cyt b) estimated mutation rate	**0.7%** (Huyse et al., 2004)	**0.5%** (Cardenas et al., 2005)
MAX / GENERAL divergence rate	1.4%	1.0%
divergence time of *´Aequidens´* separation	**MIN: 4.25 - 11.1 Mya**	**MIN: 13.02 – 20.0 (36.4) Mya**
	MAX: 0.28 - 1.21 Mya	**GENERAL: 9.9 – 15.2 (27.7) Mya**
divergence time of *Cichlasoma* separation	**MIN: 1.43 - 6.07 Mya**	**MIN: 6.97- 13.82 (16.18) Mya**
	MAX: 0.28 - 1.21 Mya	**GENERAL: 5.3 – 10.5 (12.3) Mya**
timing of creation of Andes as a barrier in Northern South America	**30 – 11.8 Mya** (Lundberg et al., 1998)	
timing of separation of Paraná and Amazonian drainages	**10 Mya** (Montoya-Burgos, 2003)	

Table 11: The table of the additionally estimated times using the extreme minimum and maximum values of mutation rates found in literature for 16S rRNA, and the minimum and the general teleost fish mutation rate for cytochrome b, respectively. The coloured values indicate the intervals that are overlapping with the interval of geological event. Only the mutation rates of cytochrome b gave the result of the possibly overlapping intervals.

5 Discussion

5.1 The dataset and analyses

Two of the selected molecular markers, mitochondrial cytochrome b and 16S rRNA genes, were both frequently used in previous cichlid phylogenetic studies (FARIAS ET AL., 1999, 2000, 2001, MARTIN & BERMINGHAM, 1998, SPARKS & SMITH, 2004). By contrast, the phylogenetic relations based on the nuclear intron in S7 gene have been recently studied in African cichlids (SCHELLY, 2006) and have not yet been published in Neotropical cichlids, up to date only ŘÍČAN (2005) used it in his PhD. thesis.

All three genes resolved well the phylogenetic relations up to intergeneric level. The deeper topology of major clades is then varying among genes. The parsimonious analyses of mitochondrial genes let the deeper topology unresolved (see Figs. 3, 5) whereas the nuclear S7 intron solved it with acceptable supports (Fig. 7). The incapability of solving the higher topology could be caused by the low number of characters due to short fragments of genes (ČERVENKA, 2005, STRAKA, 2005). The concatenation of mitochondrial genes solved more the deeper topology, although with several polytomies remaining. Generally, the Bayesian trees were more resolved in deeper topologies (see Figs. 3-4 and 12-14) and also supported the branches by the total value of 1,00 of posterior probability, but these branches were not supported in the parsimonious analysis. Possibly, as referred by PICKETT & RANDLE (2005), the posterior probability values as the branch support method are not necessarily correct.

In spite of the shared locus (mitochondria), phylogenetic signals of the mitochondrial genes have some conflicting points (see Figs. 3-6). But the incongruence between two mitochondrial genes is not so high to significantly reject the possibility of their concatenation by the Approximately Unbiased Test (SHIMODAIRA, 2002).

The incongruence in the phylogenetic signal between the nuclear S7 intron and both mitochondrial genes are more extensive and there are more points of conflict, hence, the concatenation was allowed neither by the Incongruence Length Difference Test (FARRIS ET AL., 1995) nor by the AU test (SHIMODAIRA, 2002). The concatenation could be applied only with excluding of *Acaronia* and *Laetacara* genera and with excluding of several *Aequidens* species for ILD test (then the hypotheses of homogeneity was not rejected), or excluding *Acaronia* genus and the ignoring the inner topology of whole *Aequidens* genus (except the *A. potaroensis*) for AU test, respectively.

There are several papers that impeach the reliability of the ILD test as the unbiased method of identification of the incongruence in the dataset (DOWTON & AUSTIN, 2002, BARKER & LUTZONI, 2002 and YODER ET AL., 2001).

5.2 Phylogeny of the tribe Cichlasomatini

The monophyly of the tribe sensu KULLANDER (1998) was not supported. The *Acaronia* genus considered by KULLANDER (1998) to be an outstanding tribe Acaroniini falls inside the tribe Cichlasomatini. This finding is also supported by the previous molecular studies on the Neotropical cichlids (FARIAS ET AL., 1999, 2000, 2001, SPARKS & SMITH, 2004), which did not focus on this tribe, however. The morphology-based uncertainty and an outstanding position of *Acaronia* could be caused by the large divergences in the jaw apparatus, due to *Acaronia´*s piscivory (KULLANDER, 1983b).

The monophyly of the Cichlasomatini + *Acaronia* was supported in all analysis with one exception of the Bayesian analysis of cytochrome b gene dataset (Fig. 6), where the outgroup species from the tribe Heroini, *Thorichthys meeki,* clustered with the **KA clade** as a basal taxon to the rest of the Cichlasomatini species. The sequence of *T. meeki* was later checked with the downloaded sequence from the GeneBank and these two sequences were not identical, but the inner position of outgroup still remained if the analysis was repeated using this GeneBank sequence. This abnormality could be caused by the insufficient length of used fragment of cytochrome b (420 bp only, which means 140 triplets for the Bayesian model "codon").

In the generic levels, the monophyly of each genus was tested (except of *Bujurquina*, *Tahuantinsuyoa* and *Acaronia*, where only one species was included in the analyses and except of *Cleithracara*, because it is the monotypic genus). The monophyly was strongly supported for the genera *Laetacara*, *Nannacara* and *Krobia,* and for the group of *´Aequidens´* species. The generic status of *Cichlasoma* is questionable because of the *Aequidens patricki* clustering inside the genus according to the nuclear gene. This is the conflict point between the signals from the nuclear and mitochondrial genes, but this could also be caused by the fact, that both *Aequidens patricki* and *Cichlasoma cf. araguaiense* (clustering as more basal than the *A. patricki*) could be considered as the long branch taxa, which may represent a problem for the phylogenetic algorithms (ZIMA ET AL., 2004).

By contrast, the monophyly of *Aequidens* has never been found and was rejected also by AU test (p = 0,000005). First, this is caused by the species *Aequidens potaroensis*,

which always clustered together with the *Krobia* species and outside the **CA clade** *(Cichlasoma + Aequidens). A. potaroensis* is the only species of the genus *Aequidens* from my dataset, which lives in the Guyana rivers, the same geographic province as the genus *Krobia*. The contamination or mismatch of samples is not considerable because the analysis was based on four specimens from different localities, which were analysed in different time. Moreover this pattern resulted in all three genes. However, even if *Aequidens potaroensis* was excluded, the monophyly of *Aequidens* genus was not supported (not resulted in any analysis and the AU test rejected it in the p=0,005). The genus *Aequidens* (without *A. potaroensis*) is the paraphylum with the inner group of the genus *Cichlasoma*. This resulted from both mitochondrial genes and also from the nuclear gene separately. In the concatenated tree of all three genes, the genus *Aequidens* also formed paraphylum (or polyphylum with *A. potaroensis* included). However, the concatenation was allowed only with the exclusion of several taxa. If the ILD test was applied, the paraphyly of the *Aequidens* could be postulated, because there were excluded only several species of *Aequidens* genus and the rest supported well the paraphyletic status. With respect to the AU test, however, there was ignored the whole *Aequidens* topology, hence, no conclusion about the status of the genus *Aequidens* cannot be appointed from the concatenated tree.

These results about the *Aequidens* polyphyletic (with *A. potaroensis*) or paraphyletic (*A. potaroensis* excluded) status have not yet been published in any paper.

5.3 The higher phylogenetic relations

There are four major clades that result in several genes and with various values of support. The **BTA clade** is the monophylum of the genera *Bujurquina, Tahuantinsuyoa* and *´Aequidens´*. It is very well supported in almost all analyses (see Figs. 3-14 in the Chapter Results).

The inner topology is the sister position of *Bujurquina* and *Tahuantinsuyoa* genera, and *´Aequidens´* as a basal taxon to them. The possible sister position of *Bujurquina* and *Tahuantinsuyoa* was already suggested by KULLANDER (1986) when the genera were established.

Later, KULLANDER (1998) published the study based on the morphology, where the genera *´Aequidens´* and *Tahuantinsuyoa* were sister clades, but the genus *Bujurquina* was sister to the genus *Krobia*. (See also Fig. 2 in Introduction Chapter). This sister position neither resulted in any of my analyses nor was it accepted by the AU test ($p=2x10^{-8}$) even

if the monophyly of today's generic clades ((*Bujurquina* + *Tahuantinsuyoa*) and *(Krobia* + *Aequidens potaroensis*)) was tested (p=0,00003, see also Table 7 in the Chapter Results). In FARIAS ET AL. (1999, 2000, 2001), the genus *Bujurquina* appears as sister species to the genera *Laetacara* and *Acaronia*, which is not in conflict with my data (see the Figs. 9 - 14), because in this study, they did not analysed any *Tahuantinsuyoa* species, and the only *´Aequidens´* sp. included to their analysis was not determined in the speceis level. I suppose that the *´Aequidens´* sp. used in these studies (FARIAS ET AL., 1999, 2000, 2001) belonged to the genus *Aequidens* more likely than to the *´Aequidens´* species group. Firstly, there was the relative position to the genus *Cichlasoma* (in FARIAS ET AL., 2001) and, furthermore, they suggested the sister position of *´Aequidens´* and *Aequidens* (FARIAS ET AL., 1999, 2000). However, the sister position of *Aequidens* and *´Aequidens´* was also tested in this thesis by the AU test with the highly significant result (rejected on $p=6x10^{-40}$).

Nevertheless, I have to point out here, that from the genus *Bujurquina*, only one species of *Bujurquina vittata* (and three samples) was analysed. Moreover, this is the only species of the genus *Bujurquina* with the Paraná drainage distribution, and it could cause the larger distance from remaining Amazonian *Bujurquinas*. For the further research, the other species of the genus *Bujurquina* should be added to the analyses before postulate any conclusion about their taxonomic and phylogenetic relations.

Therefore, for the facts mentioned above, I can reasonably consider the **BTA clade** to be a very well supported monophyletic group.

The **CA clade**, which is the monophylum of *Cichlasoma* and *Aequidens* (*A. potaroensis* excluded), is very well supported in all analyses. The possible sister position of *Cichlasoma* and *Aequidens* genera was already suggested in KULLANDER (1983a) and was vaguely supported also in the first molecular studies (FARIAS ET AL., 1999, 2000).

Because of the support in all analyses, the **CA clade** should be considered to be a very well supported monophyletic group.

However, the incongruence in mitochondrial and nuclear genes was found in the inner topology of the clade. First conflicting point is in the monophyly of *Cichlasoma* genus supported by both mitochondrial genes but rejected by the nuclear gene (see above). Other conflicts are in the inner topology of *Aequidens* genus, (see Figs 3 – 14).

The **KA clade** is the monophylum of *Krobia* genus and *Aequidens potaroensis*. Because of just two samples of *Krobia* species analysed, I cannot definitely set, if the *Aequidens potaroensis* is the inner group of *Krobia* genus or the sister group. In cytochrome b and S7 intron, the position was sister to the *Krobia* genus, in 16S rRNA, however, there was a sister position of *Aequidens potaroensis* and *Krobia* sp. "Oyapock". The idea of relationship of the genus *Krobia* and the species *Aequidens potaroensis* was already mentioned in KULLANDER (1983a), at that time like *´Aequidens´ guianensis* group. Later, when KULLANDER (1989) established the genus *Krobia*, this species was excluded from this group. No molecular study to date analysed any species of *Krobia*, hence, only the morphological studies are available.

The **NC clade** is the monophylum of *Nannacara* and *Cleithracara maronii*, which is the only species of the nominotypic genus *Cleithracara*. This clade was supported by both concatenated analyses; however, the incongruence in the grouping of *Cleithracara* and the *Nannacara* genus is evident (see. Fig. 3-15). Neither the cytochrome b gene nor the 16S rRNA gene supported this topology. By contrast, the nuclear S7 intron supported well this clade. The hypothesis of this sister position was already suggested by KULLANDER (1998) based on the morphological characters.

The phylogenetic relations among clades were not satisfactorily resolved (see Fig. 15) the insufficient length of fragments may be the possible reason.

5.4 The position of the conflicting genera

Two genera, *Acaronia* and *Laetacara,* were found out as the species with conflicting position during the concatenation by the ILD test. The AU test suggested only *Acaronia* as a conflicting taxon.

There is strong conflict between nuclear and mitochondrial genes in the position of the genera *Acaronia* and *Laetacara*. According to the nuclear marker, the genera *Acaronia* and *Laetacara* clustered as the sister groups and formed monophylum with the sister **CA clade** and **KA clade**. The mitochondrial genes supported the sister position of both genera to the **BTA clade**. The same topology as in mitochondrial genes resulted from the concatenated cladogram (see Fig. 15 – 16).

Concerning the genus *Acaronia*, I cannot postulate any conclusion, except of the fact that the mitochondrial gene had stronger signal than the nuclear. The sister position of *Acaronia* to the genus *Bujurquina* has already been published in FARIAS ET AL. (1999, 2000, 2001), but always based on the mitochondrial data only.

The genus *Laetacara* was not recognized as a conflicting taxon by the AU test. Therefore, the position of *Laetacara* resulted as relative to the **BTA clade** in both concatenated cladograms (nuclear + mitochondrial and two mitochondrial genes). The congruent topology was also observed in FARIAS ET AL. (1999, 2000).

5.5 The comparison of morphological concept (KULLANDER, 1998) with the results of the present study

The results of this molecular-based work have both congruent and conflicting points with the morphology-based taxonomic concept (KULLANDER, 1998).

Firstly, this study corroborates the suggested relative position of *Cichlasoma* and *Aequidens* (**CA clade** in this work) in the morphological concept after KULLANDER (1998). Also the sister position of *Cleithracara* and *Nannacara* (herein **NC clade**) was supported from nuclear and both concatenated cladograms.

By contrast, strong incongruence between the morphological concept (KULLANDER, 1998) and the molecular-based study was found in the sister position of *Krobia* and *Bujurquina* (as suggested in KULLANDER, 1998; see above). The other conflict point was already mentioned, it is the inner position of the genus *Acaronia*, which was considered by KULLANDER (1998) to belong into the different tribe. Both topologies never resulted during my analyses, nor were supported by alternative-hypotheses testing by AU test (see above).

Last but not least, there are two additional morphology-based topologies, suggested already by KULLANDER (1983a), which were, however, rejected later by the same author (KULLANDER, 1989, 1998). It is the sister position of the genera *Tahuantinsuyoa* and *Bujurquina* and the relative position of the genus *Krobia* and the species *Aequidens potaroensis*. Both these suggestions were newly corroborated in my molecular based study.

5.6 The additional comments to the alternative hypotheses

The hypotheses concerning the alterative phylogeneic partitioning of the dataset were tested by the AU-test and already mentioned above. It remains to test the geographic hypotheses. The monophyly of the Guyanan taxa was significantly supported by the AU-test, although it resulted only from the information contained in the mitochondrial genes. The fact, that this topology was not rejected even for the concatenated dataset, is very important, because we can postulate, that the Guyanan taxa probably originated from one common ancestor.

5.7 Hypothetic scenarios of the biogeographic history

The hypothetic scenario of the biogeographic history within the tribe Cichlasomatini was postulated (see Fig. 19 and the Chapter Results 4.5). Not all questions were clearly resolved. Firstly, the hypothetic common ancestor could be Guyanan or Amazonian, both hypotheses were found out as identically parsimonious. Further investigation has to be done including also the species from other tribes of the subfamily.

Three species with the wide geographic distribution (*Acaronia nassa, Aequidens tetramerus* and *Cichlasoma bimaculatum*) were excluded from the commentary of the hypothetic scenario, because their intraspecific phylogenetic relations are not yet known. Only further research focused on the population study of these species could answer this question.

The hypothetic direction of colonization was not clearly resolved in several branches (*´Aequidens´*, *Aequidens*), because more identically parsimonious hypotheses were found out. This was probably caused by the lack of several species of these genera in the sampling. The further investigation with more included species of the genus *Aequidens* and the group *´Aequidens´* could help to resolve these problems.

The Cladogram Area tree (see Fig. 18 in the Chapter Results) shew the one-clade position of the Pacific slope of South America , the Magdalena river and the Maracaibo provinces. This is probably due to the presence of the only group of *´Aequidens´* species in these provinces (see also the map in the Appendix 4.3). Other clade was formed by the provinces of the Orinoco, Amazon, Guyana and Eastern Brazil basins. The rest of provinces (Uruguay river and Eastern Brazil rivers) remained unresolved on the base of tree. This was probably caused by the low amount of the species living in these provinces in comparison to other provinces. See also the maps in the Appendix 4.1 – 4.10.

5.8 The Molecular Clock estimations

5.8.1 The substitution rates used in this study

The substitution rates for every used gene have already been estimated by other researchers, but their values differed considerably. I used the selected mutation rates found in literature for the most relative group to Neotropical cichlids. I also did other additional estimation using the extreme rates found in literature to demonstate the large interval in suggested rates and their influence on the estimated timing of studied lineage separation.

Generally, the **mutation rate** estimates for the cytochrome b vary, e.g. from 0,38% (CHANG ET AL., 2006 in Cyprinidae) to 1,4% substitution per My in Scorpaenidae

(KOCHZIUS ET AL., 2003). The general teleost fish cytochrome b is considered to have the **divergence rate** of 1% per My, which means the **mutation rate** of 0,5% of substitution per My per lineage (CARDENAS ET AL., 2005). However, to my knowledge, the study on the perciform fish (family Badidae) deals with the mutation rate of 0,8% per My (RUBER ET AL., 2004).

In African cichlids, there is a lot of studies concerning of datation of their evolutionairy events, but the calibration of molecular clock is difficult, due to their adaptive radiation and lack of the fossil material (SALZBURGER ET AL., 2002, BRANDSTATTER ET AL., 2005). The estimation of divergence rate established in STURMBAUER & MEYER (1993) was based on the Jukes-Cantor algorithm for distance counting. The divergence rate was estimated as 3% per My (= 1,5% substitution per My of mutation rate, respectively). The following studies more or less corroborate this estimation of divergence rate, all of them using the same algorithm, because of the comparison (KOBLMULLER ET AL., 2004).

Because to my knowledge, there was no estimation for divergence rates in Neotropical cichlids, the African cichlid estimation for the cytochrome b gene had to be used. Therefore, the distances were constructed under the GTR + I + G model (suggested by Modeltest) and also by the Jukes-Cantor model to comparison (used by authors of African cichlid studies). The Jukes-Cantor model, however, could highly underestimate the real divergence of sequences.

The substitution rate of 16S rRNA gene varies in literature in interval from 0,14% (RITCHIE ET AL., 1996 in Trematomidae) to 0,7% (HUYSE ET AL., 2004 in Gobiidae) of substitutions per My. In Labridae, which is the family mostly relative to Cichlidae, the divergence rate was suggested on 1% per My (= 0,5% of substitution per My per lineage; MABUCHI ET AL., 2004). Hence, I had to use the same rate of 0,5% per My, because there was no estimation for cichlids to date.

The only study using both the 16S rRNA and cytochrome b markers together for the Molecular Clock application concerned the Siluriformes (Sisoridae), where the mutation rate for cytochrome b was suggested on 0,91% per My and for 16S rRNA was suggested on 0,23% of substitution per My (GUO ET AL., 2005), suggesting, approximately, four times faster mutation rate in the cytochrome b relative to 16S rRNA. It highly differs from the ratio of the mutation rates estimated separately (found in literature), and also from the observed results. (See the Chapter Results 4.6 – 4.9).

To my knowledge, the only estimation of substitution rate of nuclear intron in S7 ribosomal gene was published in the study from BERNARDI & LAPE (2005) suggesting the rate of 0,2 - 0,23% substitutions per My for *Anisotremus* (Haemulidae, Perciformes).

With respect to the large intervals in the mutation rates, it was very difficult to select the best fitting rates to the Neotropical Cichlidae.

5.8.2 The estimated times of separation

With the respect to the results of the estimated times as shown in Tables 10 – 11 in the Chapter Results, and using the selected suggestions of the mutation rate, the conclusion was:

1) The colonization of the Pacific slope of South America by *´Aequidens´* genus had to occurr later than the formation of Andes as a barrier appeared. This result is based on all three genes using the selected mutation rates. However, when the minimum and the general teleost fish mutation rate for cytochrome b was used, the intervals are overlapping, suggesting that last period of Andes formation in the Northwestern part of the continent together with the higher sea level and the gulf occurring in this area, probably could promote the divergence of both species *´Aequidens´ pulcher* and *´Aequidens´ rivulatus*.
2) The colonization of the Paraná river basin by *Cichlasoma* ancestor also had to happen after the separation of this basin from the Amazonia. This result is based on all three genes using the selected mutation rates. However, when the minimum and the general teleost fish mutation rate for cytochrome b was used, the possibly influence of Paraná and Amazon separation to the *Cichlasoma* lineage separation was observed. The later colonization from the Amazon to the Paraná basin possibly could occur via the "change points" of fish fauna (see the Chapter Introduction 2.6.3).

Due to the large interval of suggested mutation rates in literature and large incongruences observed in my dataset during the time estimation, the results from the Molecular Clock analyses are not yet well prepared for eventual publication in the scientific press. The further investigation focused on the better Molecular Clock calibration has to be done.

6 Conclusions

Phylogenetic relations:

- ⇨ Four monophyletic clades were identififed, the **BTA clade** (*Bujurquina + Tahuantinsuyoa + ´Aequidens´*), the **CA clade** (*Cichlasoma + Aequidens* (without *A. potaroensis*)), both very well supported, then the **KA clade** (*Krobia + A. potaroensis*) and the **NC clade** (*Nannacara + Cleithracara*), which was not, however, observed in all trees. The topology among these clades is not well resolved.
- ⇨ The position of the rest of the genera, *Acaronia* and *Laetacara,* remains unresolved, due to the conflict of phylogenetic signal of three genes during their concatenation.
- ⇨ There are both congruent and conflicting points with the morphological taxonomic concept (KULLANDER, 1998) in the intergeneric phylogenetic relations.

Generic level:

- ⇨ The *Nannacara*, *Laetacara* are both monophyletic genera (resulted in nuclear and mitochondrial genes).
- ⇨ The *Cichlasoma* genus is probably monophyletic (supported by AU test from concatenated cladogram), however, one species from *Aequidens* genus (*Aequidens patricki*) clustered inside the *Cichlasoma* genus in the nuclear gene analysis.
- ⇨ *Aequidens* as a genus is not monophyletic, species of the genus *Cichlasoma* form its inner group
- ⇨ The species of *Aequidens potaroensis* does not belong to *Aequidens* genus, it rather appears to be the species of *Krobia* genus.

Biogeography:

- ⇨ The hypothetic common ancestor of the recent species of the tribe Cichlasomatini probably originated in the Amazon or Guyana river system.
- ⇨ The colonization of trans-Andean province by *´Aequidens´ rivulatus* ancestor occurred probably in **1.2 – 11.5** Mya and, therefore, the separation of the *´Aequidens´* lineages could not be caused by forming of Andes in the northwestern part of the continent, which occured in **30 – 11.8** Mya.
- ⇨ The separation of the Paraná clade and the Amazon/Orinoco clade of *Cichlasoma* species occured in **0.4 – 4.1** Mya and probably was not caused by the separation of Paraná river system in Late Miocene (**10** Mya).

7 Literature

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8 Appendix

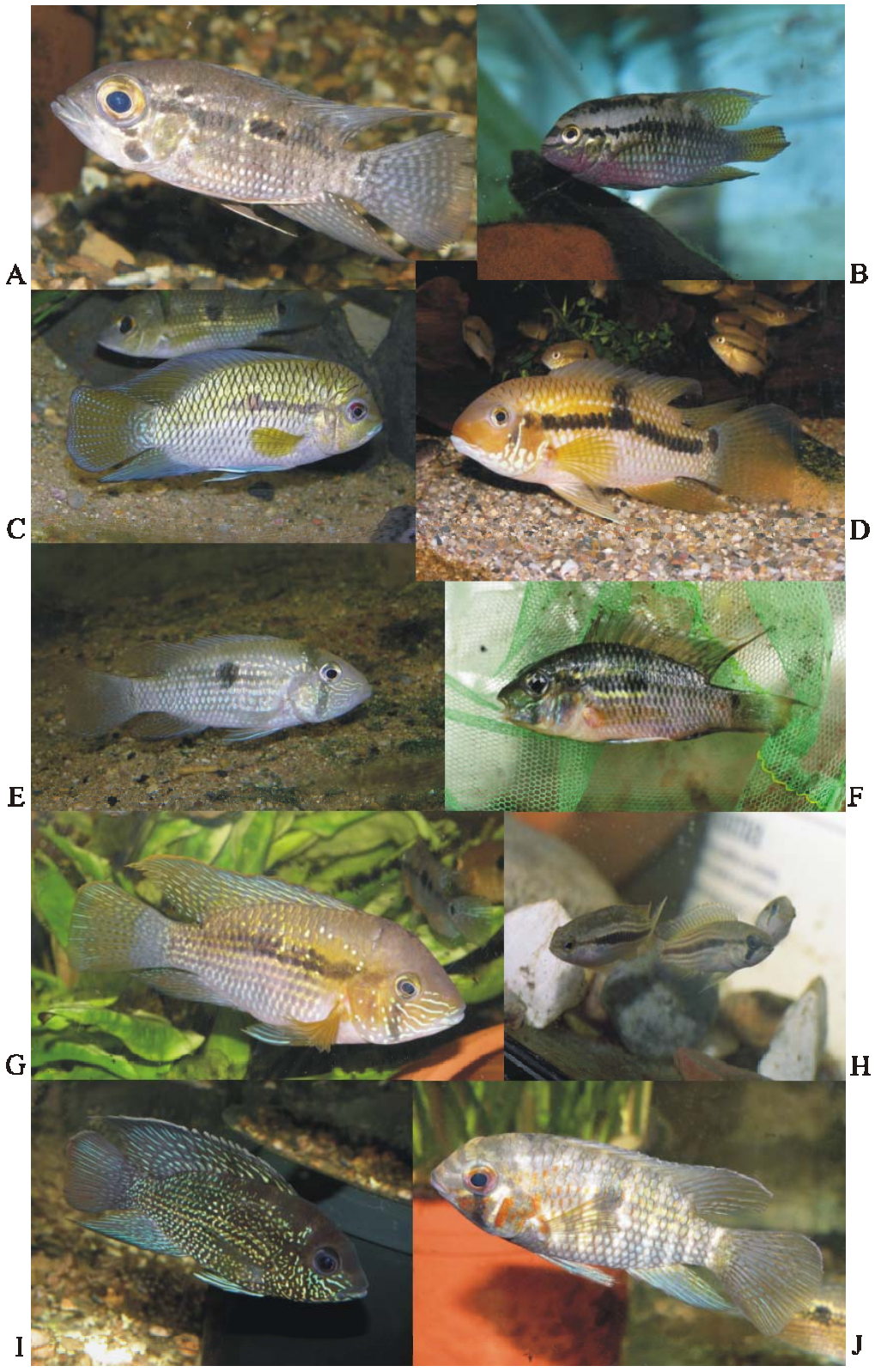
Appendix 1 - The representatives of the genera included in this study

Appendix 2 - The list of samples used in the present study

Appendix 3 - The list of valid described species belonging to the tribe Cichlasomatini

Appendix 4 - Maps of the geographic distribution of the tribe Cichlasomatini

Appendix 5 - CD-ROM with the input NEXUS file and PDF file of the thesis



Appendix 1: The representatives of the genera included in this study. A – *Acaronia nassa*, B – *Laetacara dorsigera*, C – *Cichlasoma pusillum*, D – *Aequidens patricki*, E – *Tahuantinsuyoa macantzatza*, F – *Bujurquina vittata*, G – the hybrid of ´*Aequidens*´ *rivulatus* and ´*A.*´ *pulcher*, H – *Nannacara taenia*, I - ´*Aequidens*´ cf. *pulcher* „Rio Chirgua", J – *Krobia* sp. „Oyapock". The foto of *Cleithracara* is missing. Foto by author.

Appendix 2: The table of samples used in the present study. AN° = access number to GeneBank, RA = samples that were used in the reduced alignment.

taxon name	source/locality	country/AN°	tribus	RA
Geophagus brasiliensis	aquarium stock	AKV	outgroup	yes
Geophagus steindachneri	aquarium stock	AKV	outgroup	no
Thorichthys meeki	aquarium stock	AKV	outgroup	yes
´A.´ pulcher x ´A.´ rivulatus	aquarium stock	AKV	Cichlasomatini	no
´Aequidens´ pulcher	aquarium stock	AKV	Cichlasomatini	yes
´Aequidens´ pulcher	Cunaviche, Los Llanos	Venezuela	Cichlasomatini	yes
´Aequidens´ pulcher	Los Llanos	Venezuela	Cichlasomatini	no
´Aequidens´ pulcher	San Vicente, Los Llanos	Venezuela	Cichlasomatini	no
´Aequidens´ cf. *pulcher*	Rio Chirgua, Carabobo	Venezuela	Cichlasomatini	yes
´Aequidens´ pulcher	Trinidad	Trinidad	Cichlasomatini	yes
´Aequidens´ rivulatus	Ecuador	Ecuador	Cichlasomatini	no
´Aequidens´ rivulatus	Ecuador	Ecuador	Cichlasomatini	no
´Aequidens´ rivulatus	Rio Guayaquil	Ecuador	Cichlasomatini	yes
´Aequidens´ rivulatus	aquarium stock	AKV	Cichlasomatini	yes
Acaronia nassa	aquarium stock	AKV	Cichlasomatini	yes
Aequidens diadema	Tobogán, Orinoco	Venezuela	Cichlasomatini	yes
Aequidens diadema	aquarium stock	AKV	Cichlasomatini	yes
Aequidens chimantanus	Canaima	Venezuela	Cichlasomatini	yes
Aequidens chimantanus	Mayupa, upper Canaima	Venezuela	Cichlasomatini	no
Aequidens chimantanus	Rio Yuruani, Gran Sabana	Venezuela	Cichlasomatini	no
Aequidens chimantanus	Salto Kamá	Venezuela	Cichlasomatini	no
Aequidens metae	aquarium stock	AKV	Cichlasomatini	yes
Aequidens patricki	aquarium stock	AKV	Cichlasomatini	yes
Aequidens potaroensis	Las Claritas	Venezuela	Cichlasomatini	yes
Aequidens potaroensis	Las Claritas	Venezuela	Cichlasomatini	no
Aequidens sp. "Atabapo"	Rio Atabapo	Venezuela	Cichlasomatini	yes
Aequidens sp. "Jaru"	Rio Jaru, Rondonia	Brazil	Cichlasomatini	yes
Aequidens tetramerus	Las Claritas	Venezuela	Cichlasomatini	yes
Aequidens tetramerus	aquarium stock	AKV	Cichlasomatini	no
Aequidens tetramerus	Cunaviche, Los Llanos	Venezuela	Cichlasomatini	no
Aequidens tetramerus	Rio Aguarico, Amazonas	Ecuador	Cichlasomatini	no
Aequidens tetramerus	Rio Negro	Brazil	Cichlasomatini	yes
Aequidens tetramerus	Cunaviche, Los Llanos	Venezuela	Cichlasomatini	no
Aequidens tetramerus	aquarium stock	AKV	Cichlasomatini	no
Aequidens tubicen	Cachoeira Porteira, Rio Trombetas	Brazil	Cichlasomatini	yes
Bujurquina sp.	Paraguay	Paraguay	Cichlasomatini	no
Bujurquina vittata	PN Chaco	Argentina	Cichlasomatini	yes
Bujurquina vittata	Paraguay	Paraguay	Cichlasomatini	no
Cichlasoma amazonarum	Iquitos	Peru	Cichlasomatini	yes
Cichlasoma bimaculatum	Rio Tacutu, Bonfim	Brazil	Cichlasomatini	yes
Cichlasoma cf. *araguaiense*	Rio Xingu, Altamira	Brazil	Cichlasomatini	yes
Cichlasoma cf. *metae*	Puerto Ayacucho	Venezuela	Cichlasomatini	no
Cichlasoma dimerus	Rio Paraguay	Paraguay	Cichlasomatini	yes
Cichlasoma dimerus	Paraguay	Paraguay	Cichlasomatini	no
Cichlasoma orinocense	delta Orinoka	Venezuela	Cichlasomatini	yes
Cichlasoma orinocense	Las Claritas	Venezuela	Cichlasomatini	no
Cichlasoma orinocense	Ciudad Guayana	Venezuela	Cichlasomatini	no
Cichlasoma orinocense	Venezuela	Venezuela	Cichlasomatini	no
Cichlasoma pusillum	aquarium stock	AKV	Cichlasomatini	yes

Cleithracara maronii	aquarium stock	AKV	Cichlasomatini	yes
Krobia sp. "Oyapock"	Oyapock River	French Guyana	Cichlasomatini	yes
Krobia sp. "Xingu"	Rio Xingu, Cachoeira Parati	Brazil	Cichlasomatini	yes
Laetacara curviceps	aquarium stock	AKV	Cichlasomatini	yes
Laetacara dorsigera	aquarium stock	AKV	Cichlasomatini	yes
Laetacara sp. "Buckelkopf"	Rio Tapajos, Jacaréancanga, Pará	Brazil	Cichlasomatini	yes
Laetacara sp. "Peru"	Peru	Peru	Cichlasomatini	no
Laetacara thayeri	aquarium stock	AKV	Cichlasomatini	no
Laetacara thayeri	aquarium stock	AKV	Cichlasomatini	yes
Nannacara anomala	aquarium stock	AKV	Cichlasomatini	yes
Nannacara aureocephalus	aquarium stock	AKV	Cichlasomatini	yes
Nannacara sp.	delta Orinoco	Venezuela	Cichlasomatini	yes
Nannacara taenia	aquarium stock	AKV	Cichlasomatini	yes
Tahuantinsuyoa macantzatza	aquarium stock	AKV	Cichlasomatini	yes
Aequidens michaeli	GeneBank	AF049001	Cichlasomatini	yes
´Aequidens´ coeruleopunctatus	GeneBank	AF236043	Cichlasomatini	yes
Cichlasoma boliviense	GeneBank	AF009952	Cichlasomatini	yes
Cichlasoma portalegrense	GeneBank	U88854	Cichlasomatini	yes

Genus *Acaronia* Myers, 1940
***Acaronia nassa* (Heckel, 1840)**
Acaronia vultuosa Kullander, 1989

Genus *Aequidens* Eigenmann and Bray, 1894
Aequidens chimantanus Inger, 1956
Aequidens diadema (Heckel, 1840)
Aequidens epae Kullander, 1995
Aequidens gerciliae Kullander, 1995
Aequidens mauesanus Kullander, 1997
Aequidens metae Eigenmann, 1922
Aequidens michaeli Kullander, 1995
Aequidens pallidus (Heckel, 1840)
Aequidens paloemeuensis Kullander&Nijssen, 1989
Aequidens patricki Kullander, 1984
Aequidens plagiozonatus Kullander, 1984
Aequidens potaroensis Eigenmann, 1912
Aequidens rondoni (Ribeiro, 1918)
***Aequidens tetramerus* Heckel, 1840**
Aequidens tubicen Kullander&Ferreira, 1991
Aequidens viridis (Heckel, 1840)

"*Aequidens*" *rivulatus* group:
"Aequidens" rivulatus (Guenther, 1859)
"Aequidens" sapayensis (Regan, 1903)
"Aequidens" biseriatus (Regan, 1913)

"*Aequidens*" *pulcher* group:
"Aequidens" coeruleopunctatus (Kner, 1863)
"Aequidens" latifrons (Steindachner, 1879)
"Aequidens" pulcher (Gill, 1858)

"*Aequidens*" *hoehnei* group:
"Aequidens" hoehnei (Ribeiro, 1918)

Genus *Bujurquina* (Kullander, 1986)
Bujurquina apoparuana Kullander, 1986
Bujurquina cordemadi Kullander, 1986
Bujurquina eurhinus Kullander, 1986
Bujurquina hophrys Kullander, 1986
Bujurquina huallagae Kullander, 1986
Bujurquina mariae (Eigenmann, 1922)
Bujurquina labiosa Kullander, 1986
Bujurquina megalospilus Kullander, 1986
Bujurquina moriorum, Kullander, 1986
Bujurquina ortegai Kullander, 1986
Bujurquina oenolaemus Kullander, 1987
Bujurquina peregrinabunda Kullander, 1986
Bujurquina robusta Kullander, 1986
Bujurquina syspilus (Cope, 1871)
Bujurquina tambopatae Kullander, 1986
Bujurquina vittata (Heckel, 1840)
Bujurquina zamorensis (Regan, 1905)

Genus *Cichlasoma* Swainson, 1839
Cichlasoma amazonarum Kullander, 1983
Cichlasoma araguaiense Kullander, 1983
***Cichlasoma bimaculatum* (Linnaeus, 1758)**
Cichlasoma boliviense Kullander, 1983
Cichlasoma dimerus (Heckel, 1840)
Cichlasoma orientale Kullander, 1983
Cichlasoma orinocense Kullander, 1983
Cichlasoma paranaense Kullander, 1983
Cichlasoma portalegrense (Hensel, 1870)
Cichlasoma pusillum Kullander, 1983
Cichlasoma sanctifranciscense Kullander, 1983
Cichlasoma taenia (Bennett, 1831)

Genus *Cleithracara*
Cleithracara maronii (Steindachner, 1882)

Genus *Krobia* Kullander and Nijssen, 1989
Krobia guianensis (Regan, 1905)
Krobia itanyi (Puyo, 1943)

Genus *Laetacara* (Kullander, 1986)
Laetacara dorsigera (Heckel, 1840)
Laetacara curviceps (Ahl, 1924)
***Laetacara thayeri* (Steindachner, 1875)**
*Laetacara flavilabris (*Cope, 1870)

Genus *Nannacara* Regan, 1905
Nannacara adoketa Kullander & Prada-Pedreros, 1993
***Nannacara anomala* Regan, 1905**
Nannacara aureocephalus, Allgayer, 1983
Nannacara bimaculata Eigenmann, 1912
Nannacara quadrispinnae Staeck & Schindler, 2004
Nannacara taenia Regan, 1912

Genus *Tahuantinsuyoa* (Kullander 1986)
Tahuantinsuoya chipi Kullander, 1991
Tahuantinsuoya macantzatza Kullander, 1986

Appendix 3: The list of valid described species belonging to the tribe Cichlasomatini, in the alphabetical order. The type species marked by the bold font. After FROESE & PAULY (2006) and STAWIKOWSKI & WERNER (1998).

Appendix 4: The geographic distribution of studied genera.

4.1 genus *Acaronia*

4.2 genus *Aequidens*

4.3 genus´*Aequidens*´

4.4 genus *Bujurquina*

4.5 genus *Cichlasoma*

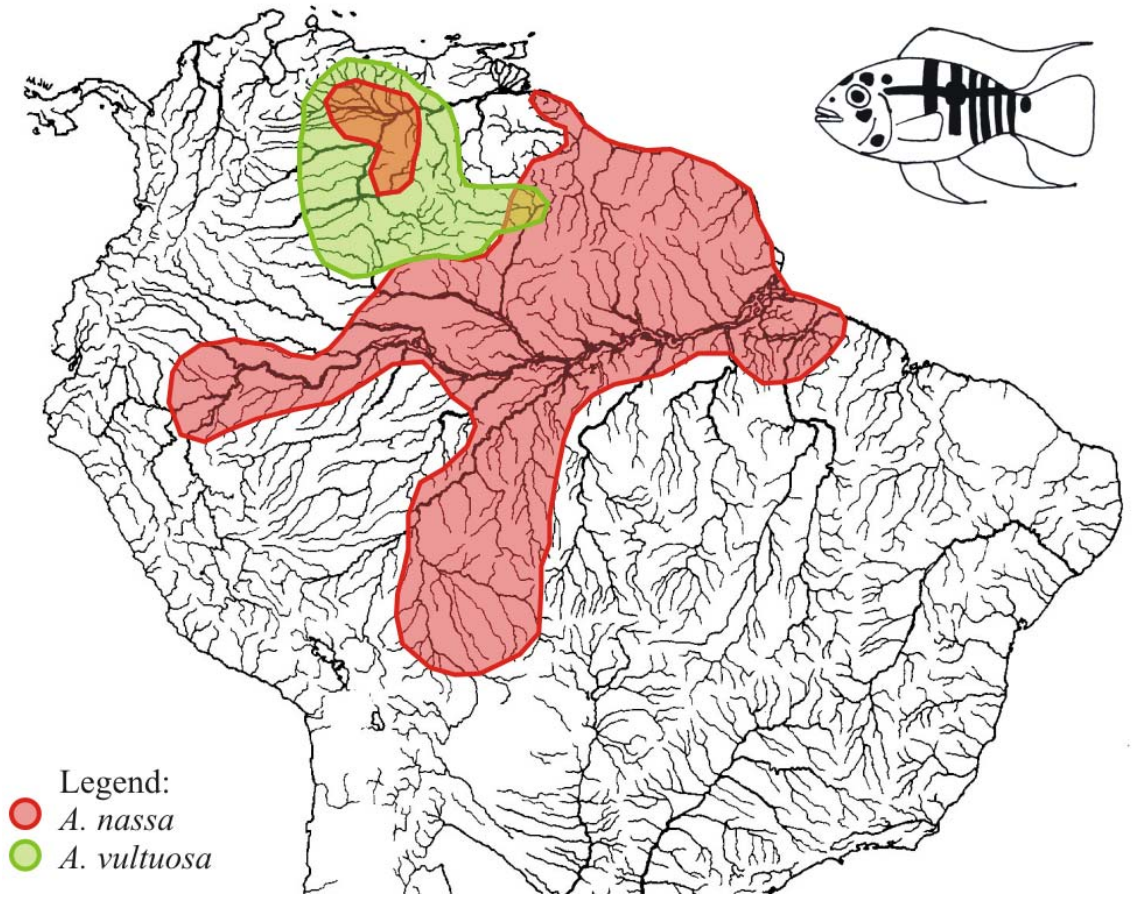
4.6 genus *Cleithracara*

4.7 genus *Krobia*

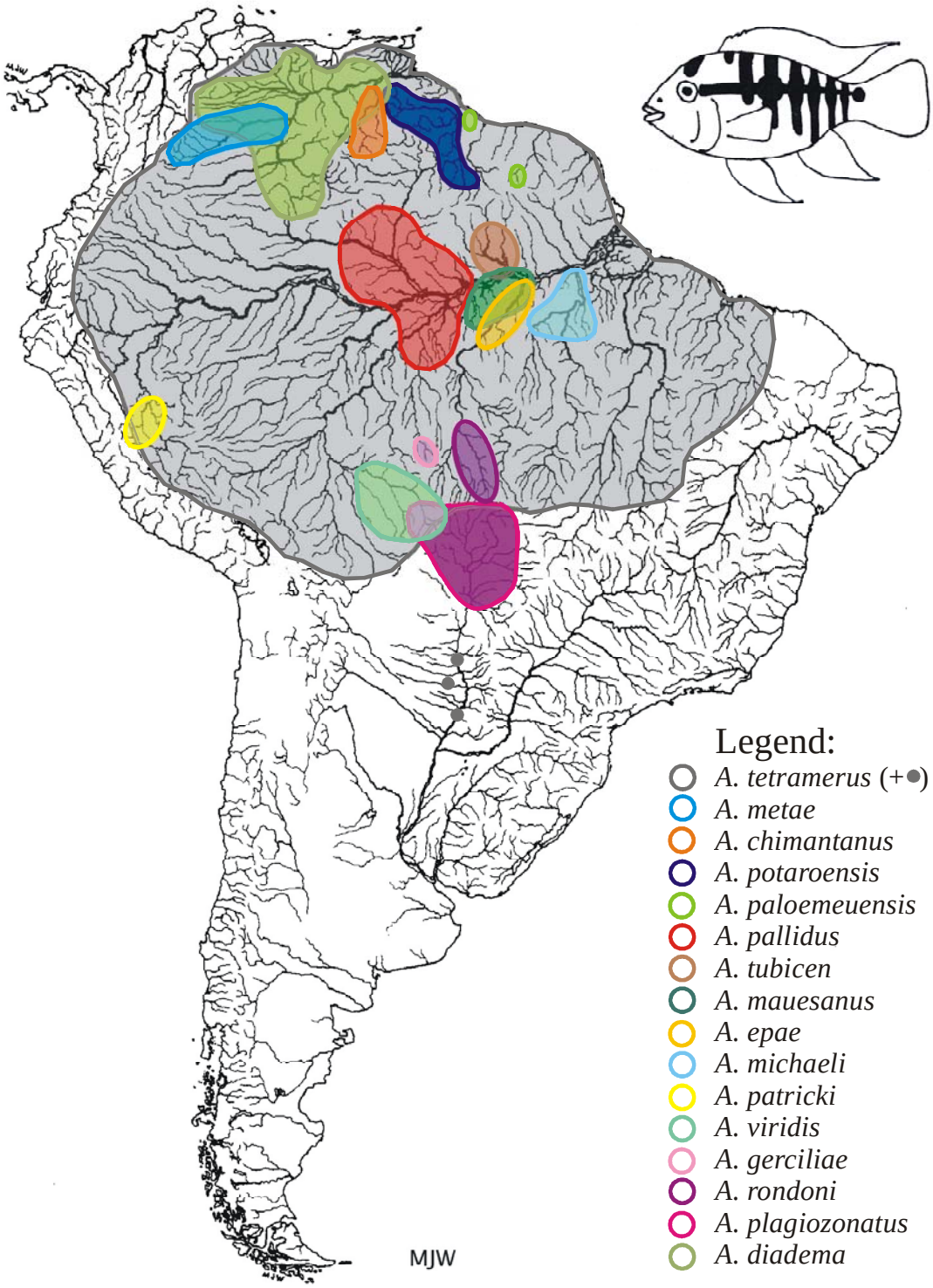
4.8 genus *Laetacara*

4.9 genus *Nannacara*

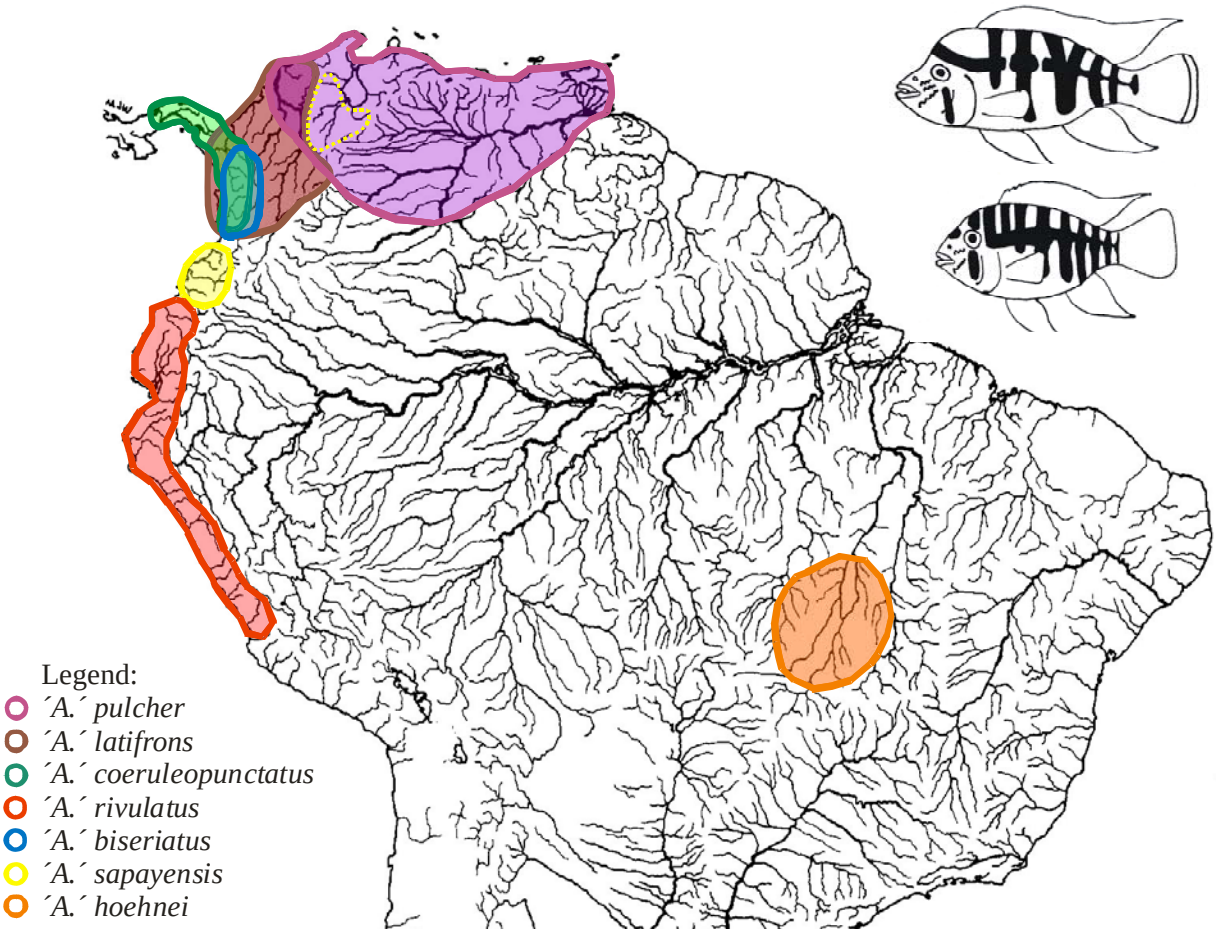
4.10 genus *Tahuantinsuyoa*



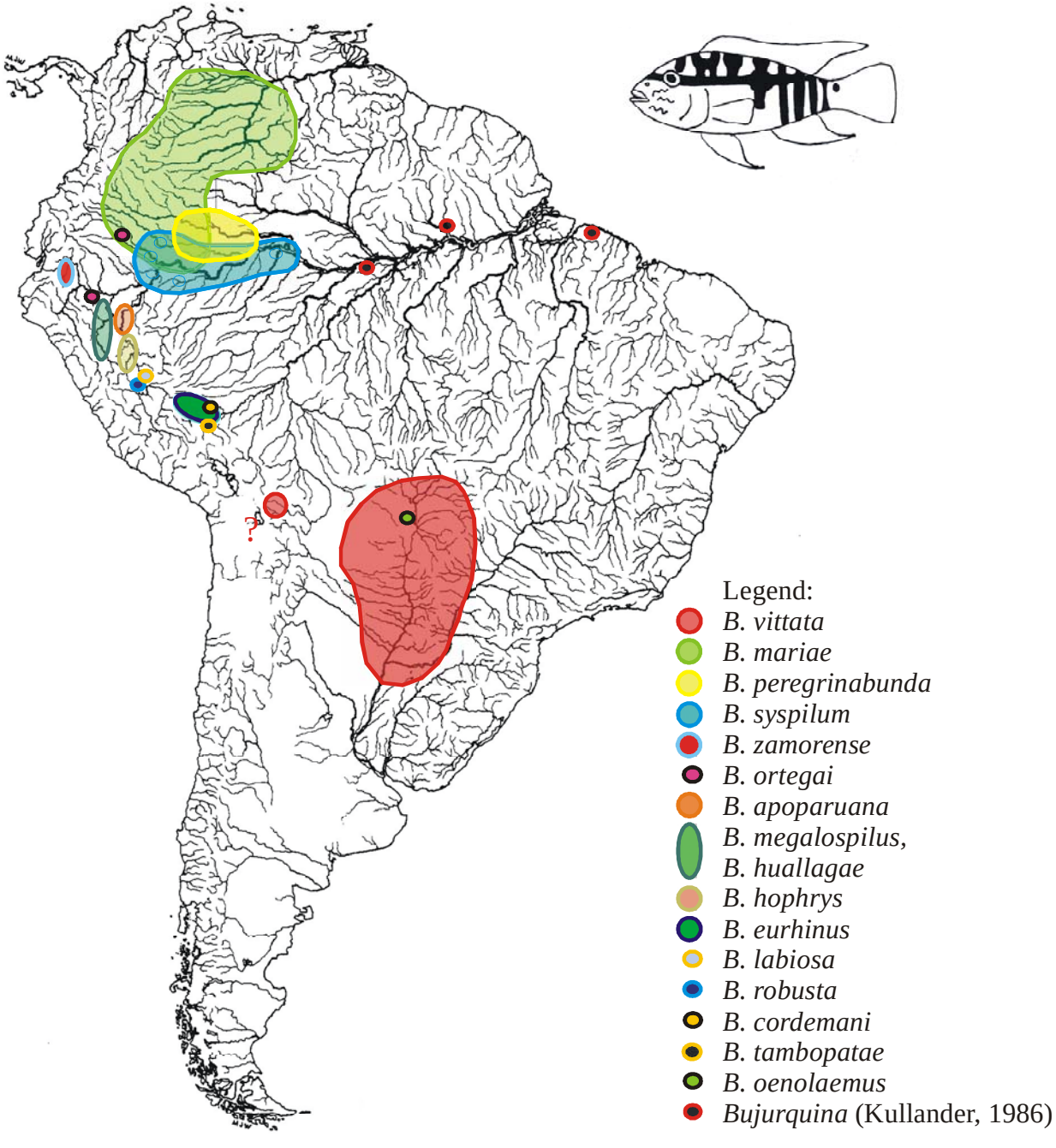
Appendix 4.1: Geographic distribution of the genus *Acaronia*. After the Neodat II database, FROESE & PAULY (2006), and STAWIKOWSKI & WERNER (1998). The black and white map background downloaded from the Neodat website (available on www.neodat.org). The drawing designed by author.



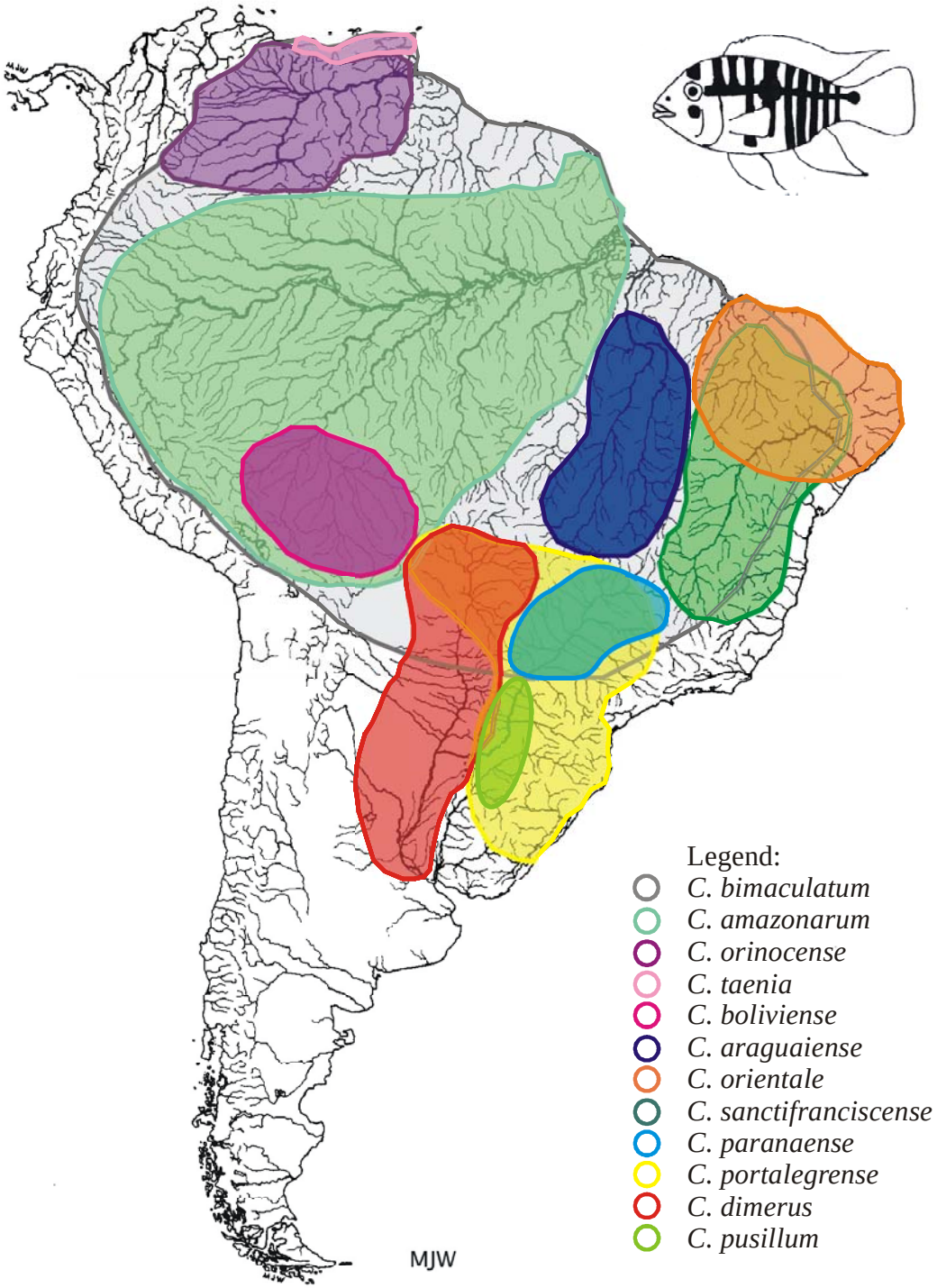
Appendix 4.2: Geographic distribution of the genus *Aequidens*. After the Neodat II database, FROESE & PAULY (2006), and STAWIKOWSKI & WERNER (1998). The black and white map background downloaded from the Neodat website (available on www.neodat.org). The drawing designed by author.



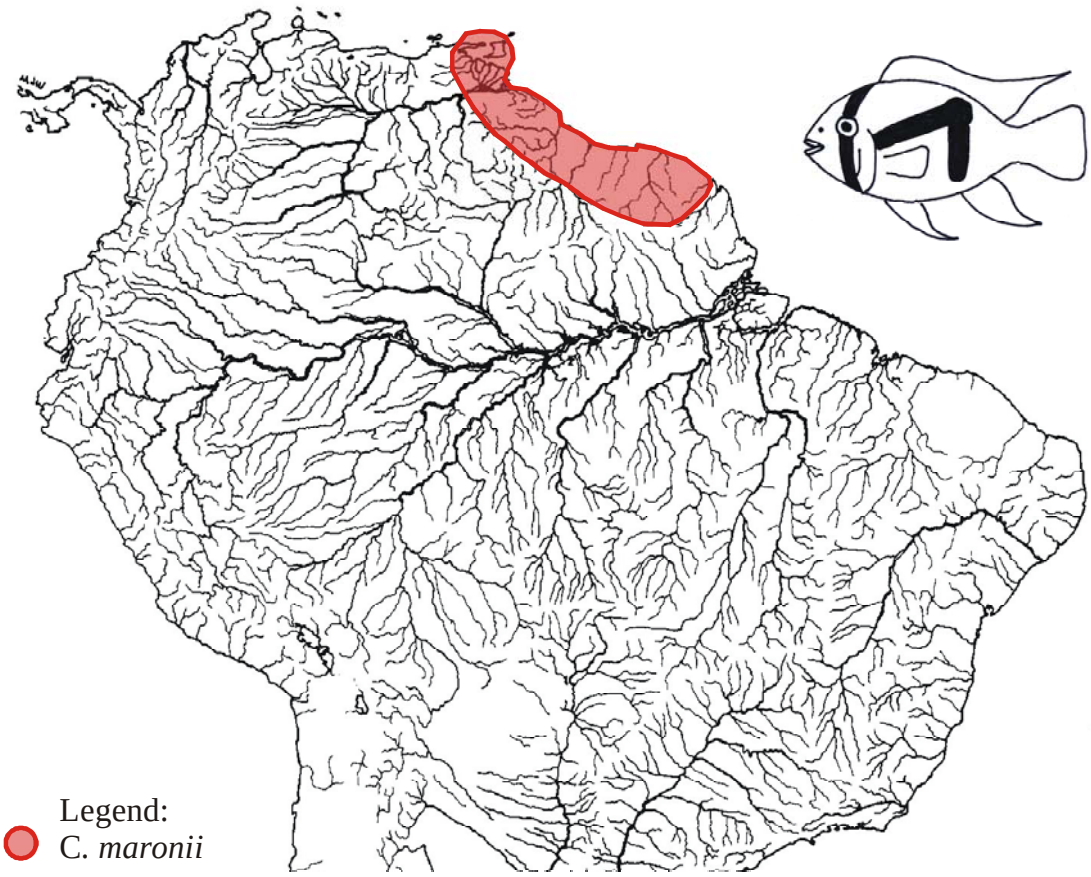
Appendix 4.3: Geographic distribution of the *´Aequidens´* species group. After the Neodat II database, FROESE & PAULY (2006), and STAWIKOWSKI & WERNER (1998). The black and white map background downloaded from the Neodat website (available on www.neodat.org). The yellow dashed line indicates the questinable data observed the from Neodat II database. The drawing designed by author.



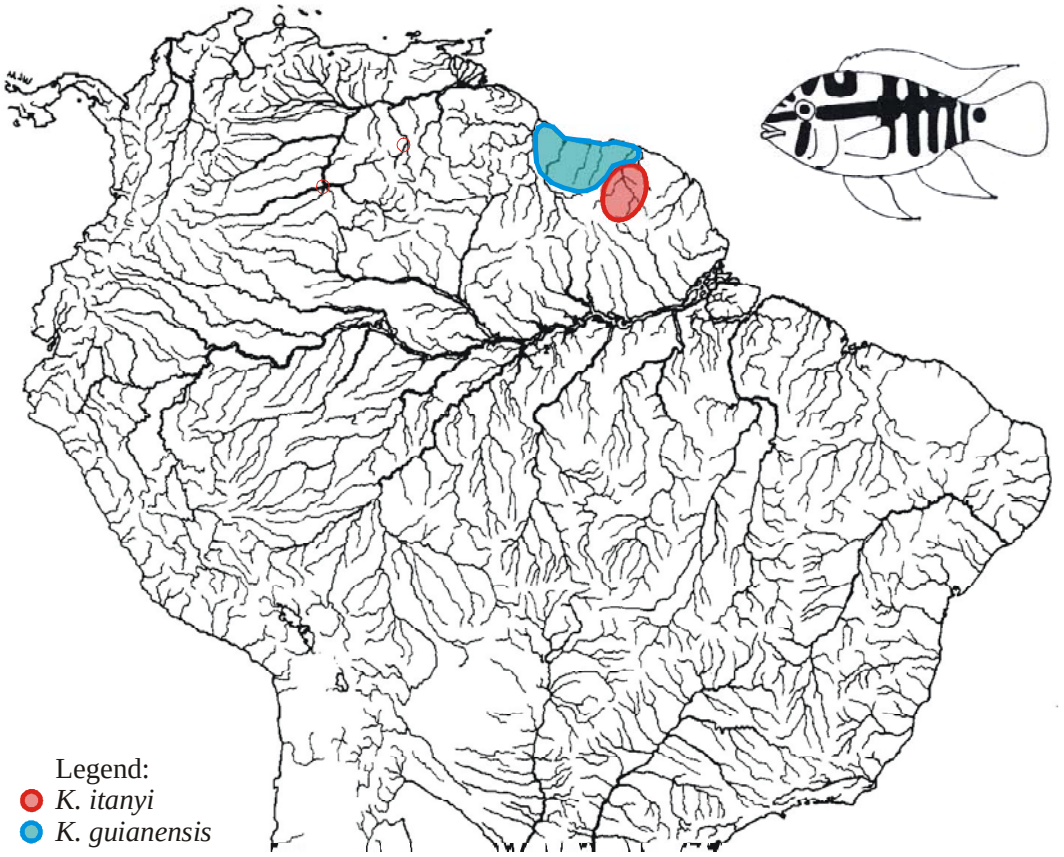
Appendix 4.4: Geographic distribution of the genus *Bujurquina*. After the Neodat II database, FROESE & PAULY (2006), KULLANDER (1986) and STAWIKOWSKI & WERNER (1998). The black and white map background downloaded from the Neodat website (available on www.neodat.org). The points marked as *Bujurquina* (KULLANDER, 1986) indicate the data from KULLANDER (1986) about presence of the genus *Bujurquina* without the species determination. The drawing designed by author.



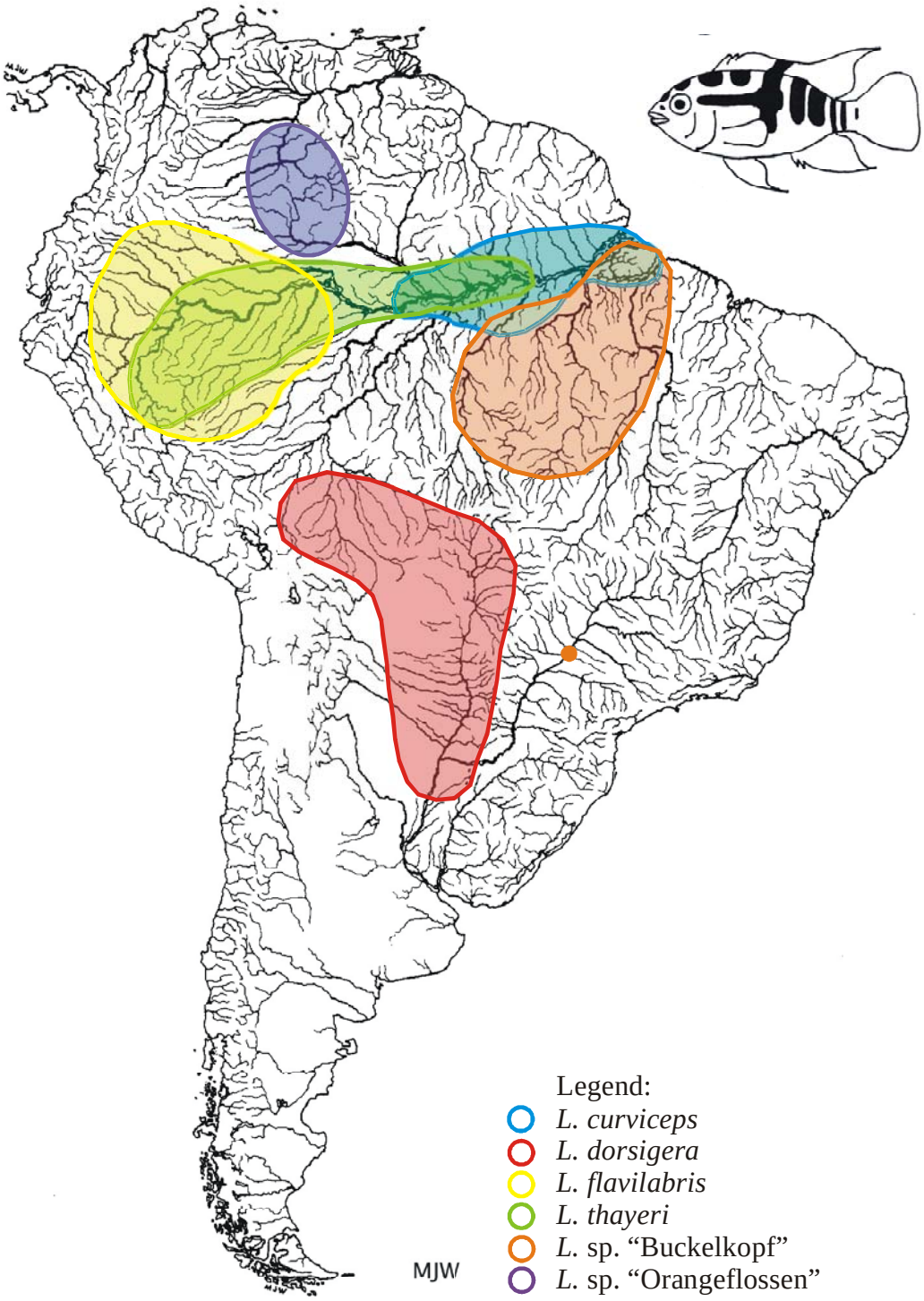
Appendix 4.5: Geographic distribution of the genus *Cichlasoma*. After the Neodat II database, FROESE & PAULY (2006), KULLANDER (1983a), and STAWIKOWSKI & WERNER (1998). The black and white map background downloaded from the Neodat website (available on www.neodat.org). The drawing designed by author.



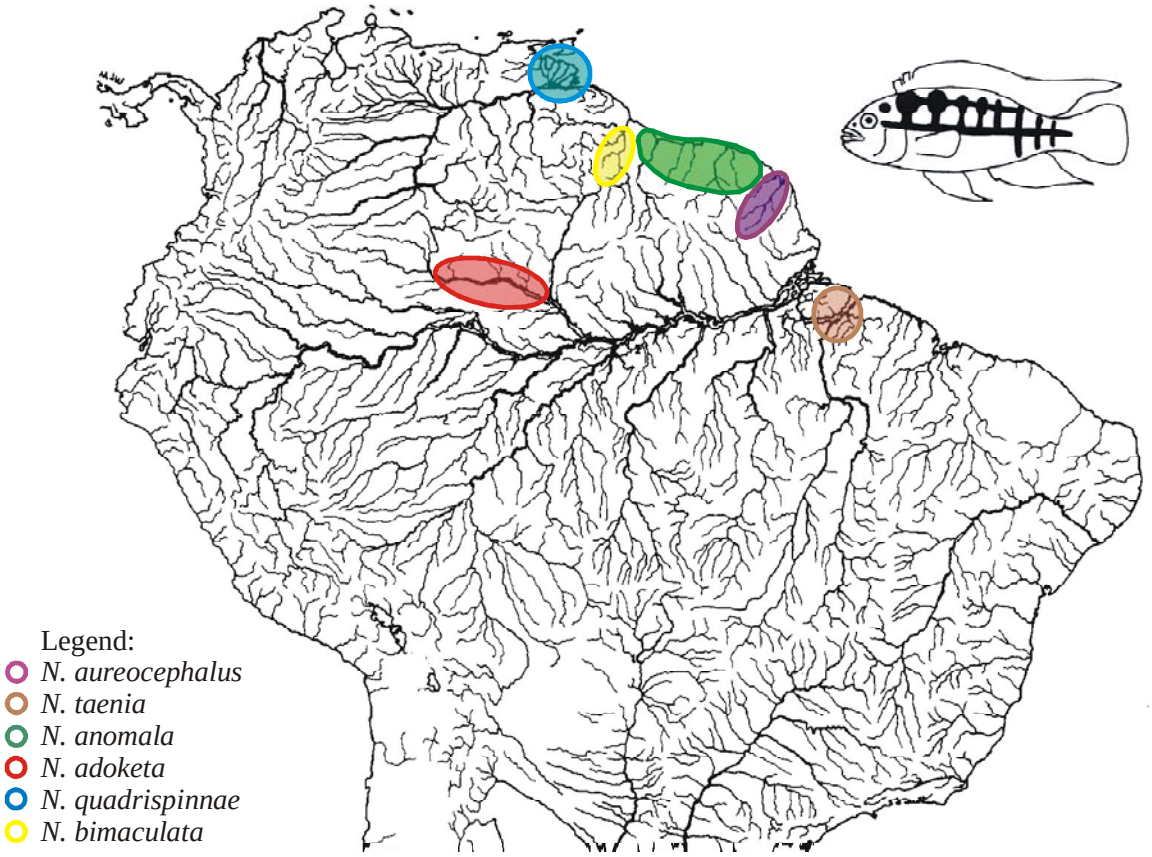
Appendix 4.6: Geographic distribution of the species *Cleithracara maronii*. After the Neodat II database, FROESE & PAULY (2006), and STAWIKOWSKI & WERNER (1998). The black and white map background downloaded from the Neodat website (available on www.neodat.org). The drawing designed by author.



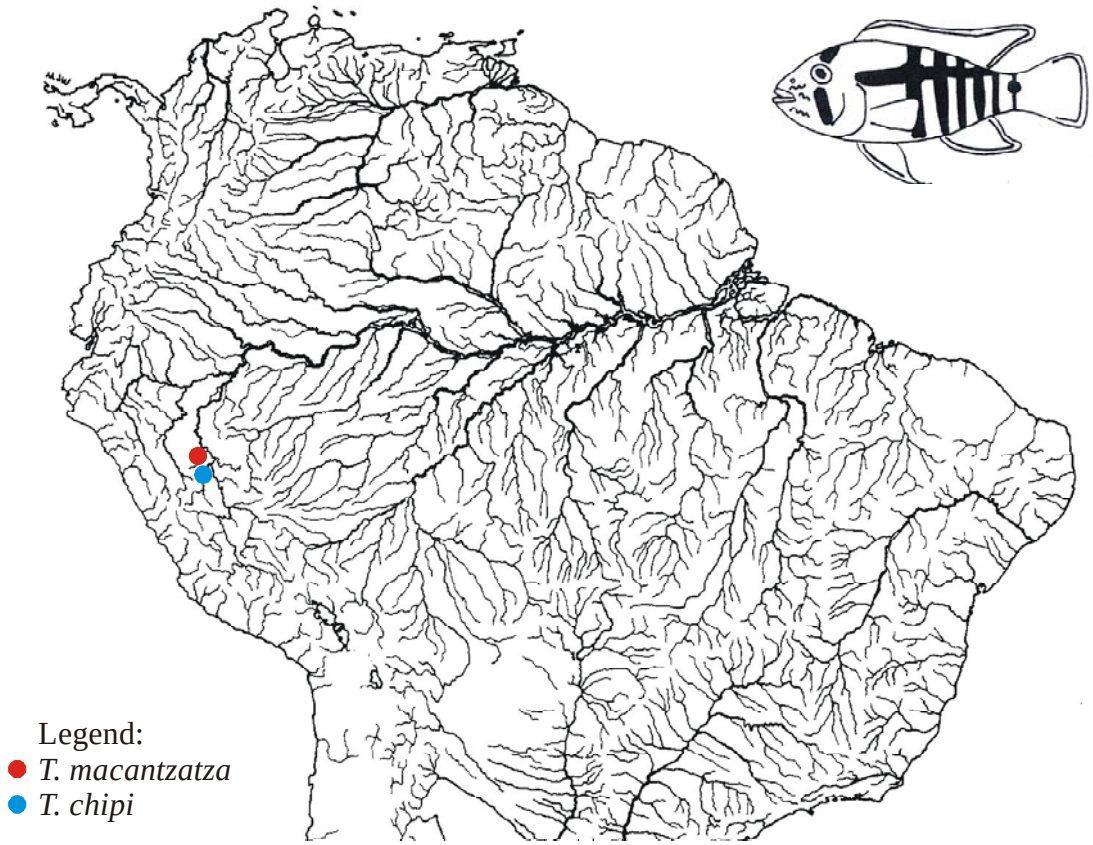
Appendix 4.7: Geographic distribution of the genus *Krobia*. After the Neodat II database, Froese & Pauly (2006), and STAWIKOWSKI & WERNER (1998). The small red circles indicate the questionable data from the Neodat II database. The black and white map background downloaded from the Neodat website (available on www.neodat.org). The drawing designed by author.



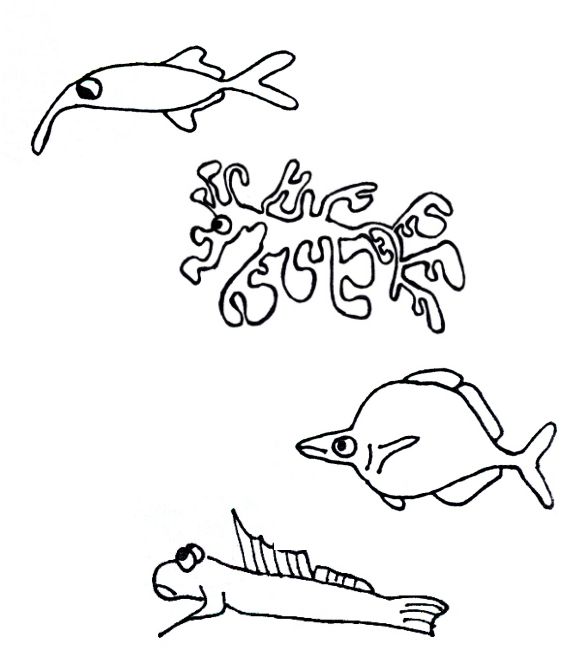
Appendix 4.8: Geographic distribution of the genus *Laetacara*. After the Neodat II database, FROESE & PAULY (2006), and STAWIKOWSKI & WERNER (1998). The black and white map background downloaded from the Neodat website (available on www.neodat.org). The drawing designed by author.



Appendix 4.9: Geographic distribution of the genus *Nannacara*. After the Neodat II database, FROESE & PAULY (2006), and STAWIKOWSKI & WERNER (1998). The black and white map background downloaded from the Neodat website (available on www.neodat.org). The drawing designed by author.



Appendix 4.10: Geographic distribution of the genus *Tahuantinsuyoa*. After the Neodat II database, FROESE & PAULY (2006), and STAWIKOWSKI & WERNER (1998). The black and white map background downloaded from the Neodat website (available on www.neodat.org). The drawing designed by author.



... ryby se dělí na cichlidy a necichlidy...