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Revision of the Genus *Symphysodon* Heckel, 1840 (Teleostei: Perciformes: Cichlidae) based on molecular and morphological characters

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Abstract

Systematics of the cichlid genus *Symphysodon* has been investigated and three species are recognised: *S. discus* Heckel, 1840 (synonym: *S. discus willischwartzi* Burgess, 1981); *S. aequifasciatus* Pellegrin, 1904 (synonyms: *S. discus* var. *aequifasciata* Pellegrin, 1904; *S. aequifasciata aequifasciata* sensu Schultz, 1960; S. Discus Tarzoo – sic – Lyons, 1960); and *S. haraldi*, Schultz, 1960 (synonyms: *S. aequifasciata haraldi* Schultz, 1960; *S. aequifasciata axelrodi* Schultz, 1960). The present revision is based on DNA sequences of partial mitochondrial control regions of 48 specimens of *Symphysodon* from 20 different locations in the central and lower Amazon basin, which revealed three genetically distinct clades of *Symphysodon*. One of these genetic clusters is composed of specimens that morphologically are *S. discus*, but also of *S. haraldi* and natural hybrids of *S. discus x S. haraldi*. This indicates that either the *"discus"* clade is composed, at least partially, of hybrids or, alternatively, that a *"haraldi"* phenotype evolved (or was retained) independently in this clade. The other two clades consist of *S. aequifasciatus* and *S. haraldi*.

The definition of the three species is supported by extensive field studies over the last 40 years, investigating distributional patterns and documenting adaptation of each species to a distinct type of water, characterised by unique chemical parameters. In addition, *S. discus* and *S. aequifasciatus* display distinct colorations and colour patterns, with nine vertical bars on each flank. In *S. discus* the first, and particularly the fifth and ninth bars are prominent and/or wider, while all bars are typically of equal width in *S. aequifasciatus*. The latter species is also recognised by its rust-brown or red dots on the body, ranging from a few spots to a dense cover all over, rarely forming red spotted lines or being present in the anal fin region only. *Symphysodon haraldi* displays a wide range of colours, colour patterns and a larger number of vertical bars (8 up to 16), which may differ substantially in shape. Except for hybrids of *S. discus x S. haraldi*, *S. haraldi* does not resemble the other two species. A study of geographic distribution patterns of the three species was carried out throughout the central and lower Amazon basin: in the western Amazon in almost every tributary of the Solimões and the Marañon Rivers to Iquitos, and in the eastern part in most tributaries of the Amazon River down to its mouth. The valid names of the three species are: *S. discus* – the Heckel discus; *S. aequifasciatus* – the green discus; and *S. haraldi* – the blue discus. The "brown" or "common" discus of the aquarium trade is the same as the "blue" discus.

In *S. aequifasciatus* a congruence of genetic and morphological (colour) characters has been found, whereas some specimens that would phenotypically be assigned to *S. haraldi*, genetically group also with the *S. discus* clade. Only future studies using nuclear DNA markers will allow untangling the evolutionary history of the phenotypcially heterogeneous *S. "discus"* clade.

Zusammenfassung

Eine Untersuchung der Discus-Gattung *Symphysodon* zeigte, dass drei Arten gültig sind: *S. discus* Heckel, 1840 (Synonym: *S. discus willischwartzi* Burgess, 1981); *S. aequifasciatus* Pellegrin, 1904 (Synonyme: *S. discus* var. *aequifasciata* Pellegrin, 1904; *S. aequifasciata aequifasciata* sensu Schultz, 1960, S. Discus Tarzoo – sic – Lyons, 1960); sowie *S. haraldi* Schultz, 1960 (Synonyme: *S. aequifasciata haraldi* Schultz, 1960; *S. aequifasciata axelrodi* Schultz, 1960). Die vorliegende Revision basiert auf DNA-Sequenzierungen partieller mitochondrialer Kontrollabschnitte bei 48 Exemplaren von *Symphosodon* von 20 verschiedenen Fundorten im mittleren und unteren Amazonasbecken, bei denen sich drei genetisch getrennte Kladen der Gattung *Symphysodon* zeigten. Eines dieser genetischen Cluster umfasst Exemplare, die morphologisch der zuerst beschriebenen Discus-Art entsprechen: *S. discus*, aber auch Exemplare, die *S. haraldi* und Naturhybriden aus *S. discus x S. haraldi* ähnlich sind. Dies deutet darauf hin, dass entweder die Klade „*discus*" zumindest teilweise aus Hybriden besteht oder dass sich in dieser Klade unabhängig ebenfalls ein „*haraldi*"-Phänotyp entwickelt hat (bzw. erhalten blieb). Die anderen beiden Kladen werden von den bekannten Formen von *S. aequifasciatus* bzw. *S. haraldi* gebildet.

Die Diagnosen der drei Arten wurde durch umfangreiche Freilandstudien während der letzten 40 Jahre abgesichert. Dazu gehörten Untersuchungen der Verteilungsmuster und die Dokumentation von Anpassungen der drei Arten an unterschiedliche Gewässertypen mit distinkten chemischen Parametern. Hinzu kommt, dass zwei der drei Arten *(S. discus* und *S. aequifasciatus)* gut unterscheidbare Grundfarben und Farbmuster aufweisen. Die Vertreter dieser beiden Arten besitzen jeweils neun senkrechte Streifen, aber bei *S. discus* sind der erste, besonders aber der fünfte und neunte Streifen besonders auffällig und/oder breit. Bei *S. aequifasciatus* hingegen sind die neun Streifen typischerweise gleich breit; außerdem lässt sich diese Art an ihren rostbraunen bis dunkelroten verstreuten Flecken erkennen, von denen nur wenige vorhanden sein können, die sich aber auch über den gesamten Körper erstrecken können oder in seltenen Fällen gar rote Linien aus Flecken bilden oder auf den Analflossenbereich beschränkt sind.

Die dritte Art *(S. haraldi)* kann die unterschiedlichsten Farben und Muster zeigen. Die Zahl der senkrechten Streifen kann stark variieren (8 bis zu 16), und die Streifenbreite kann ganz unterschiedlich sein, von breit bis sehr schmal. Diese Art ist von den anderen beiden gut zu unterscheiden (die Hybriden zwischen *S. haraldi x S. discus* unberücksichtigt).

Im zentralen und unteren Amazonasbecken wurden die geografische Verteilung der drei Arten eingehend untersucht: am westlichen Amazonas nahezu in allen Nebenarmen des Solimões und des Marañon bis Iquitos und im östlichen Teil des Amazonas in den meisten Nebenflüssen des Amazonas bis hin zur Mündung.

Die gültigen Namen der drei Arten sind: *S. discus* – Heckel-Discus; *S. aequifasciatus* – Grüner Discus; und *S. haraldi* – Blauer Discus. Bei dem im Aquarienhandel üblichen Namen „Brauner" oder „Gewöhnlicher Discus" handelt es sich um dieselbe Art wie beim „Blauen Discus".

Nur bei *S. aequifasciatus* zeigt sich eine Übereinstimmung zwischen genetischen und morphologischen (Farb-) Merkmalen; hingegen sind einige Exemplare, die man phänotypisch *S. haraldi* zuordnen würde, genetisch gesehen zur Klade *S. discus* zu zählen. Nur durch weitere Untersuchungen mit Hilfe von radioaktiven DNA-Markern wird man die Evolution der phänotypisch uneinheitlichen *S. „discus"*-Klade klären können.

Résumé

La systématique du genre *Symphysodon* a été étudiée et trois espèces sont été reconnues: *S. discus* Heckel, 1840 (synonyme: *S. discus willischwartzi* Burgess, 1981); *S. aequifasciata* Pellegrin, 1904 (synonymes: *S. discus* var. *aequifasciata* Pellegrin, 1904; *S. aequifasciata aequifasciata* sensu Schultz, 1960; S. Discus Tarzoo – sic – Lyons, 1960); et *S. haraldi* Schultz, 1960 (synonymes: *S. aequifasciata haraldi* Schultz, 1960; *S. aequifasciata axelrodi* Schultz, 1960). Cette révision se base sur des séquençages ADN mitochondriaux partiels de régions de contrôle de 48 spécimens de *Symphysodon* de 20 localités différentes situées dans le bassin central et inférieur de l'Amazone, ce qui a révélé trois clades de *Symphysodon* génétiquement distincts. Un de ces groupes génétiques se compose de spécimens qui sont morphologiquement des *S. discus*, mais aussi de *S. haraldi* et d'hybrides naturels de *S. discus x S. haraldi*. Ceci révèle soit que le clade *"discus"* se compose, du moins en partie, d'hybrides, soit, alternativement, qu'un phénotype *"haraldi"* a évolué (ou a été retenu) indépendamment dans ce clade. Les deux autres clades sont constitués de *S. aequifasciatus* et de *S. haraldi*.

La délimitation de ces trois espèces s'appuie sur des études de terrain extensives, au long de ces 40 dernières années, qui ont porté sur leur mode de distribution et sur l'adaptation de chaque espèce à un type d'eau particulier, caractérisé par des paramètres chimiques propres. En outre, *S. discus* et *S. aequifasciatus* ont des couleurs et des patrons de coloration différents, avec neuf barres verticales sur les flancs. Pour le *S. discus*, la première et surtout la cinquième et la neuvième barre sont accentuées et/ou plus larges, alors que toutes les barres sont typiquement de même largeur pour *S. aequifasciatus*. Cette dernière espèce se distingue aussi par ses taches brun rouille ou rouges sur le corps, pouvant varier de quelques-unes à une répartition dense sur tout le corps, pouvant former rarement des lignes pointillées rouges ou présentes seulement dans la région de l'anale. *Symphysodon haraldi* arbore une large gamme de couleurs, de patrons de coloration et un plus grand nombre de barres verticales (8 jusqu'à 16), dont la forme peut varier notablement. Hormis les hybrides de *S. discus x S. haraldi*, *S. haraldi* ne ressemble pas aux deux autres espèces. Une étude des modes de distribution géographique des trois espèces a été menée tout au long du bassin central et inférieur de l'Amazone: dans l'Amazone occidental, dans presque chaque tributaire du Solimões et du Marañon jusqu'à Iquitos, et dans sa partie orientale, dans la plupart de affluents de l'Amazone jusqu'à son embouchure. Les noms scientifiques des trois espèces sont: *S. discus* – le Discus Heckel; *S. aequifasciatus* – le Discus vert; *S. haraldi* – le Discus bleu. Le "Brun" ou Discus "commun" du commerce aquariophile est de la même espèce que le "Bleu".

Pour *S. aequifasciatus*, une concordance des caractères génétiques et morphologiques (couleur), à été découverte, alors que certains spécimens, qui seraient assimilés au groupe *S. haraldi* du point de vue phénotypique, se regroupent aussi dans le clade *S. discus* du point de vue génétique. Seules de futures études au moyen de marqueurs nucléaires de l'ADN pourront dévoiler la vraie histoire évolutive du clade *S. "discus"*, hétérogène sur le plan phénotypique.

Sommario

È stata studiata la sistematica del genere discus *Symphysodon*. I risultati portano a riconoscere tre specie: *S. discus* Heckel, 1840 (sinonimo: *S. discus willischwartzi* Burgess, 1981), *S. aequifasciatus* Pellegrin, 1904 (sinonimi: *S. discus* var. *aequifasciata* Pellegrin, 1904, *S. aequifasciata aequifasciata* sensu Schultz, 1960, S. Discus Tarzoo – sic – Lyons, 1960) e *S. haraldi* Schultz, 1960 (sinonimi: *S. aequifasciata haraldi* Schultz, 1960; *S. aequifasciata axelrodi* Schultz, 1960). Questa revisione, che ha rivelato tre linee geneticamente distinte di *Symphysodon*, è basata su analisi di sequenza di una parte delle regioni di controllo del DNA mitocondriale, effettuata su 48 esemplari provenienti da 20 differenti località del bacino centrale e meridionale delle Amazzoni. Uno di questi gruppi genetici è composto di esemplari che morfologicamente appartengono a *S. discus*, ma anche a *S. haraldi* e ad ibridi naturali *S. discus x S. haraldi*. Ciò indica che il gruppo filetico *"discus"* è composto, almeno parzialmente, di ibridi o, in alternativa, che il fenotipo *"haraldi"* si è evoluto (o è stato conservato) indipendentemente in questo gruppo. Le altre due linee corrispondono, rispettivamente, a *S. aequifasciatus* e *S. haraldi*.

La separazione delle tre specie è confortata da oltre 40 anni di studi sul campo, che hanno documentato la loro distribuzione e la loro capacità adattativa ai differenti tipi di acque, caratterizzate da parametri chimici unici. Inoltre *S. discus* e *S. aequifasciatus* hanno una colorazione ben riconoscibile, ognuna caratterizzata da nove bande verticali. In *S. discus* la prima e specialmente la quinta e la nona sono ben marcate e/o più estese rispetto a *S. aequifasciatus*, nel quale le barre sono tipicamente uguali in larghezza. Quest'ultima specie può essere anche riconosciuta per una serie di macchie puntiformi dal color ruggine al rosso scuro, che possono variare da poche file, talora presenti solo nella regione della pinna anale, ad un numero tale da estendersi su tutto il corpo, formando in rari casi quasi delle linee rossastre. *Symphysodon haraldi* ha una colorazione molto variabile e il disegno e il numero delle bande verticali (8 fino a 16) possono differire in modo sostanziale. Se si escludono gli ibridi *S. haraldi x S. discus*, *S. haraldi* non è simile alle altre due specie. La distribuzione geografica e le barriere idrogeologiche delle tre specie sono state studiate lungo tutto il bacino centrale e meridionale del Rio delle Amazzoni: nella parte occidentale del bacino è stata considerata la maggior parte degli affluenti dei fiumi Solimões e

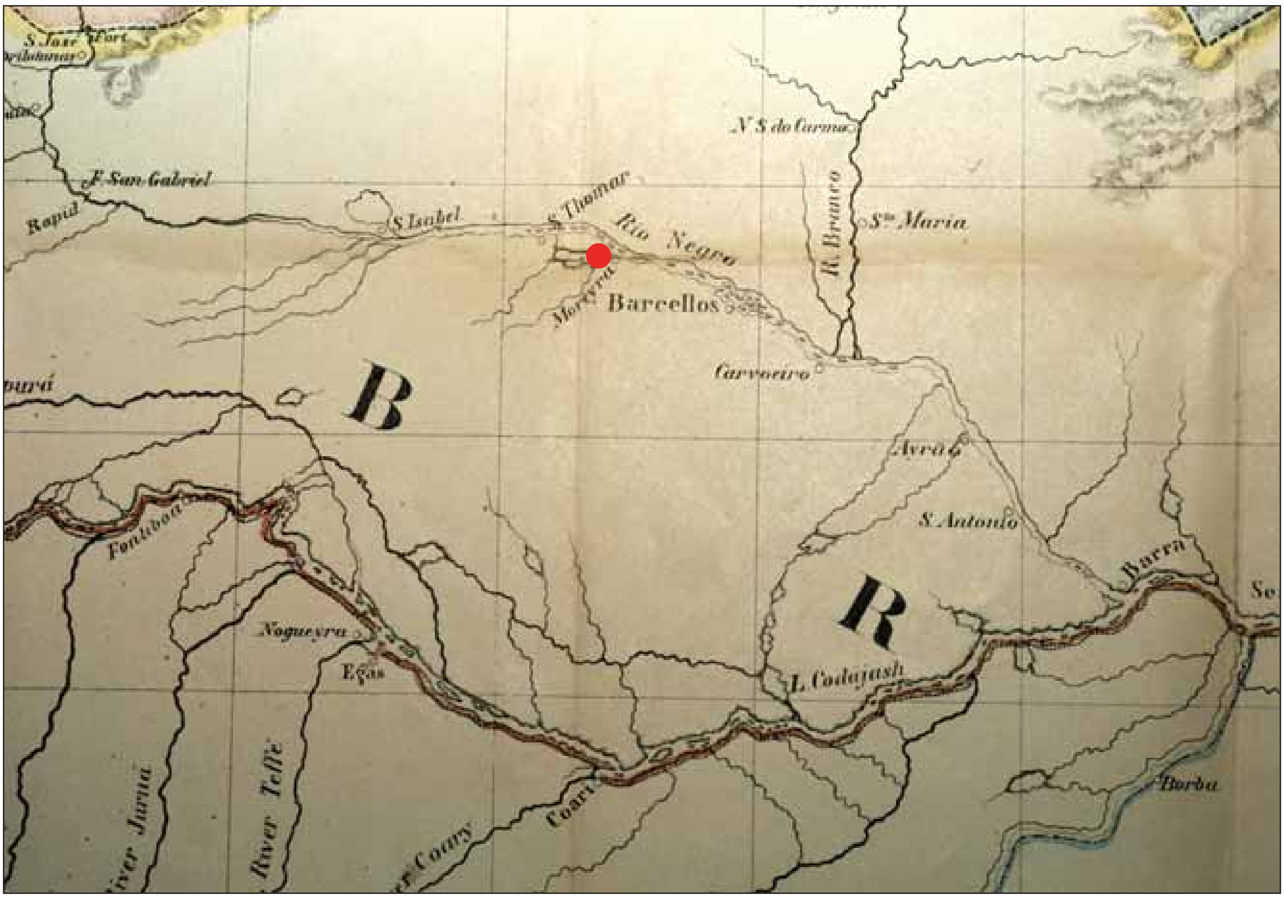


Fig. 1. Map of 1854 with the type locality of *Symphysodon discus* Heckel, 1840 (red spot) collected by Natterer at Moreya (= Moreré) northwest of Barcellos (= Barcelos). Photo by N. Khardina from *Exploration from the Valley of the Amazon* by W. L. Herndon & L. Gibbon, 1854.

Marañon fino all'Iquitos e nella parte orientale la maggior parte degli affluenti del Rio delle Amazzoni fino alla foce. I nomi validi delle tre specie sono: *S. discus* – il discus di Heckel; *S. aequifasciatus* – il discus verde; *S. haraldi* – il discus blu. Il cosiddetto "discus marrone" o "discus comune" diffuso nel mercato dei pesci d'acquario rappresenta la stessa specie nota come "discus blu".

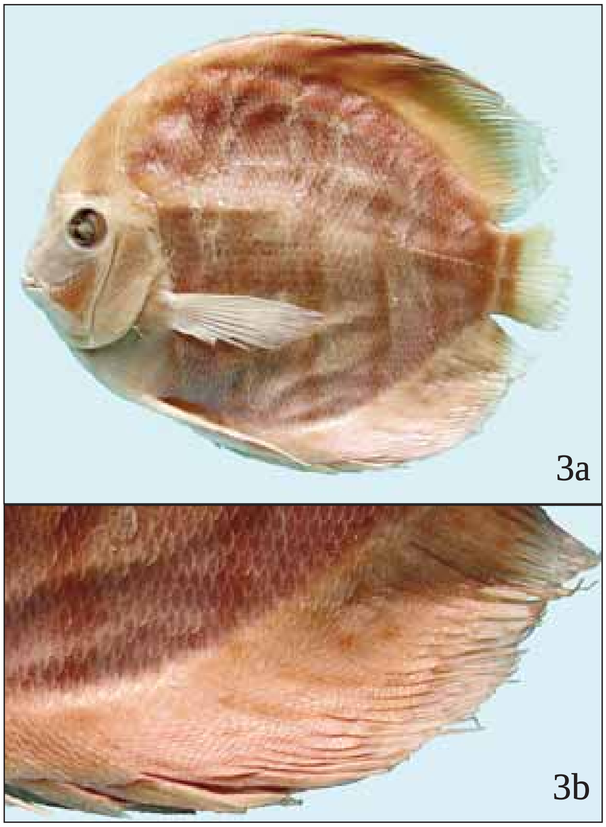
I dati mostrano che solo *S. aequifasciatus* mostra congruenza dei tratti genetici e morfologici (colorazione), mentre alcuni esemplari che potrebbero essere assegnati fenotipicamente a *S. haraldi* sembrano appartenere al gruppo genetico di *S. discus*. Solo ulteriori studi con marcatori di DNA nucleare potranno districare la vera storia evolutiva dell'eterogenea linea filetica di *S. "discus"*.

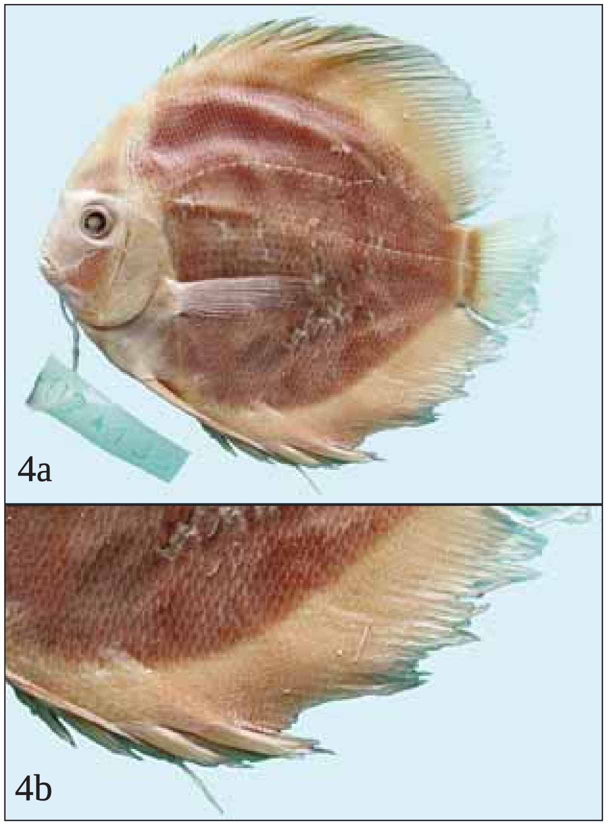
HISTORY

The first discus was collected by the Austrian naturalist Johann Baptist Natterer (1787-1843) between 1832 and 1833 (different dates are given, such as 2 November 1833) during the ninth of his 10 journeys. Natterer collected a single specimen near the mission station of Moreré, on the Rio Moreré (which on maps of 1854 is called Rio Moreyra, Fig. 1). On some recent maps it may still be found as "Moreira", but the mission station has long disappeared, as has the river name Moreyra. In 1840 this specimen was described as *S. discus* (Fig. 2) by the German naturalist Johann Jakob Heckel, working at the Natural History Museum in Vienna. Heckel erected a new genus for this unusually shaped fish: *Symphysodon*, and gave the type locality as "Moreré am Rio-negro" (= Moreyra, today Moreira), which is northwest of Barcelos at 63°30'W 0°35'S (not Barra, or Barra do Rio Negro, nor Manaus, as stated in recent publications. The correct type locality for *S. discus* Heckel, 1840 was re-instated by Géry & Bleher 2004).

The second discus was described by Pellegrin in 1903 (published 1904) as *Symphysodon discus* var. *aequifasciata* var. nov. Pellegrin had three (or four) specimens at hand, collected by the Frenchman Dr Clement Jobert around 1878. Two of the specimens were from Teffé (= Tefé) (MNHN 1902/134-135, Figs 3 a, b; 4 a, b) and one from Santarem (= Santarém) (MNHN 1902/130, Fig. 5 a, b). It seems that there was a fourth specimen, which Pellegrin included in his original description, from Calderón (Pellegrin inserted "Calderón" in black ink into his already printed description), but the latter specimen was never found in the Muséum National d'Histoire Naturelle (MNHN). Pellegrin noted that there seem to be two "types" in Jobert's collection: "La coloration de ces spécimens

Fig. 2. *Symphysodon discus* Heckel, 1840, Holotype, NMW 35612, 98.6 mm SL. The specimen was collected by J. Natterer at Moreré (= Moreira), northwest of Barcelos, Amazon, Brazil. Photo by N. Khardina, courtesy of NHM.





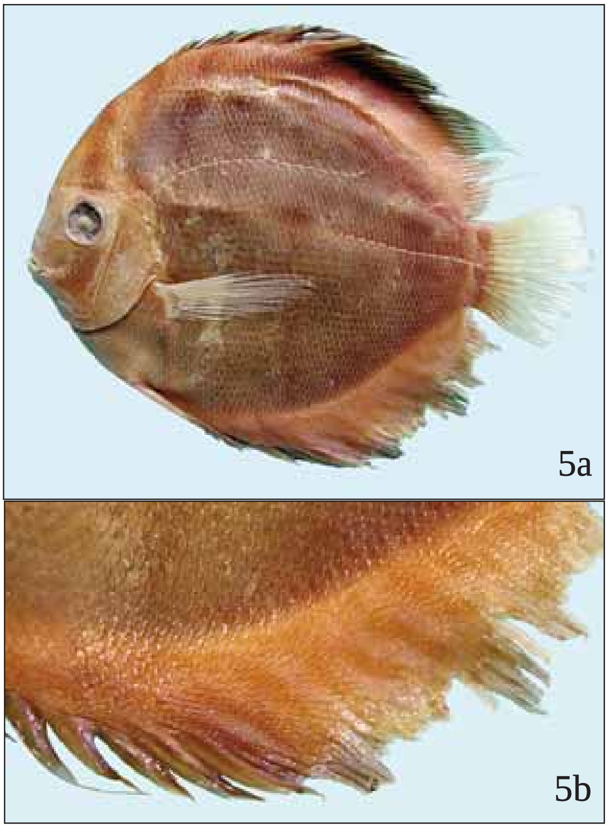


Fig. 3. a. Large syntype (1902/134) of Pellegrin's 1904 *S. discus* var. *aequifasciata* from Teffé, Brésil. Adult fish. – b. Detail of the same specimen, where the red dots can be seen, although the fish is preserved for more than 100 years. This is the typical colour pattern of *S. aequifasciata* (green discus).

Fig. 4. a. Pellegrin's (1904) second syntype (MNHN 1902/135) of *S. discus* var. *aequifasciata,* Teffé, Brésil. Semi-adult fish. – b. Detail of the same specimen where the red dots can hardly be seen, as they develop only later, at the adult stage (see also Fig. 6).

Fig. 5. a. Pellegrin's (1904) third syntype (MNHN 1902/130) of *S. discus* var. *aequifasciata* Santarém, Brésil; an adult specimen. – b. Detail of the same specimen were the red stripes can be clearly seen, after more than 100 years in alcohol. This colour pattern is typical for *S. haraldi* (compare with Figs 8 & 9). Photos by N. Khardina, courtesy of the MNHN.

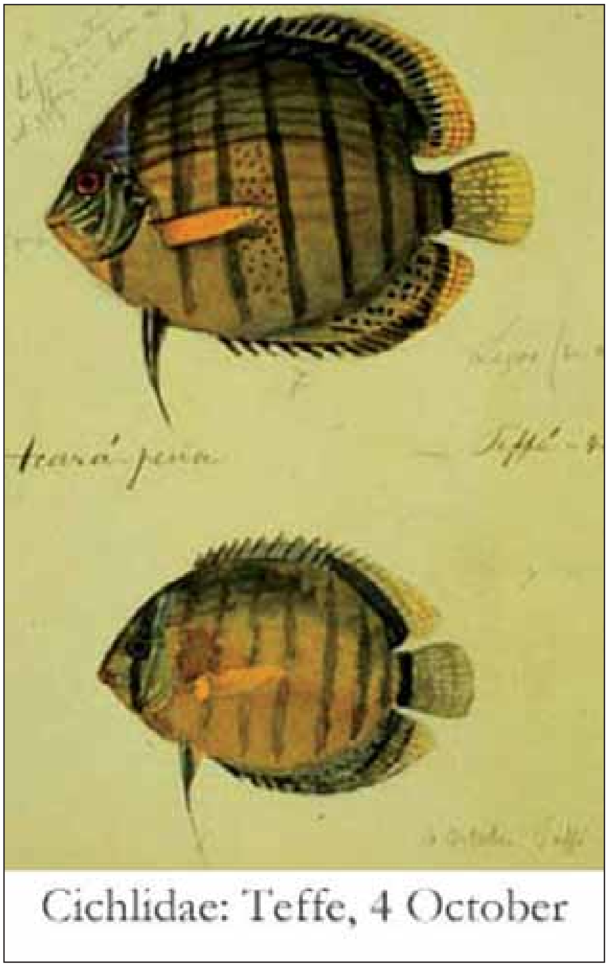


Fig. 6. Painting by Jacques Burkhardt (1808-1867) of the Thayer Expedition (1865-66) made at Lake Tefé depicting two specimens collected there, on 4 October 1865 (= green discus, *S. aequifasciatus).* Painting courtesy of Harvard University.

peut-être ramenée á 2 types…" but unfortunately did not specify the difference. Today we know that there were two species involved (see below). It is interesting to note that the Thayer Expedition (1865-1866) included discus in its extensive collections and some were from Teffé, collected between 14 September and 22 October 1865 and painted in colour by Agassiz' "personal artist" the German Jacques Burkhardt on 4 October 1865 (Fig. 6). The painting shows an adult discus with red spots on the body and anal fin, and a juvenile with hardly any red spots. Figure 7 also shows an adult specimen with red spots, which was collected in the Putumayo Region. The two latter look the same as Pellegrin's specimens (MNHN 1902/134-135). In the larger of the two preserved specimens

Fig. 7. Green discus "red-spotted green", *S. aequifasciatus*, from the Rio Putumayo Region. Photo by Heiko Bleher.

the red spots can still be seen (Fig. 3 b). Burkhardt painted also another discus collected by Agassiz and others on 28 August 1865 near Porto de Moz on the lower Xingú, which is clearly a different species without any spots, but a brown base colour with eight black bars (Fig. 8).

The location of the latter sample is less than 300 km east of Santarém, where Pellegrin's syntype (MNHN 1902/130) comes from, with almost identical water parameters, while the type locality of Pellegrin's taxon (MNHN 1902/134-135) lies more than 1200 km west of Santarém. The discus on Burkhardt's painting from Porto de Moz looks also almost identical to Pellegrin's syntype from Santarém (Fig. 5 a). Even the anal fin stripes in the painting are the same as those in the preserved specimen and Burkhardt noted already in 1865: "Heckel en décrit une autre espècie …" ("Heckel has described another species …", Fig. 8).

There are very few later scientific collections with precise locality data, except for some British collections (Regan 1905) and those by D. Melin and others in 1923. In 1921, the first life discus was brought from the Santarém Region to Hamburg, for the aquarium hobby, but the specimen soon died. The first photograph of a discus from the next successful life collection of some specimens by

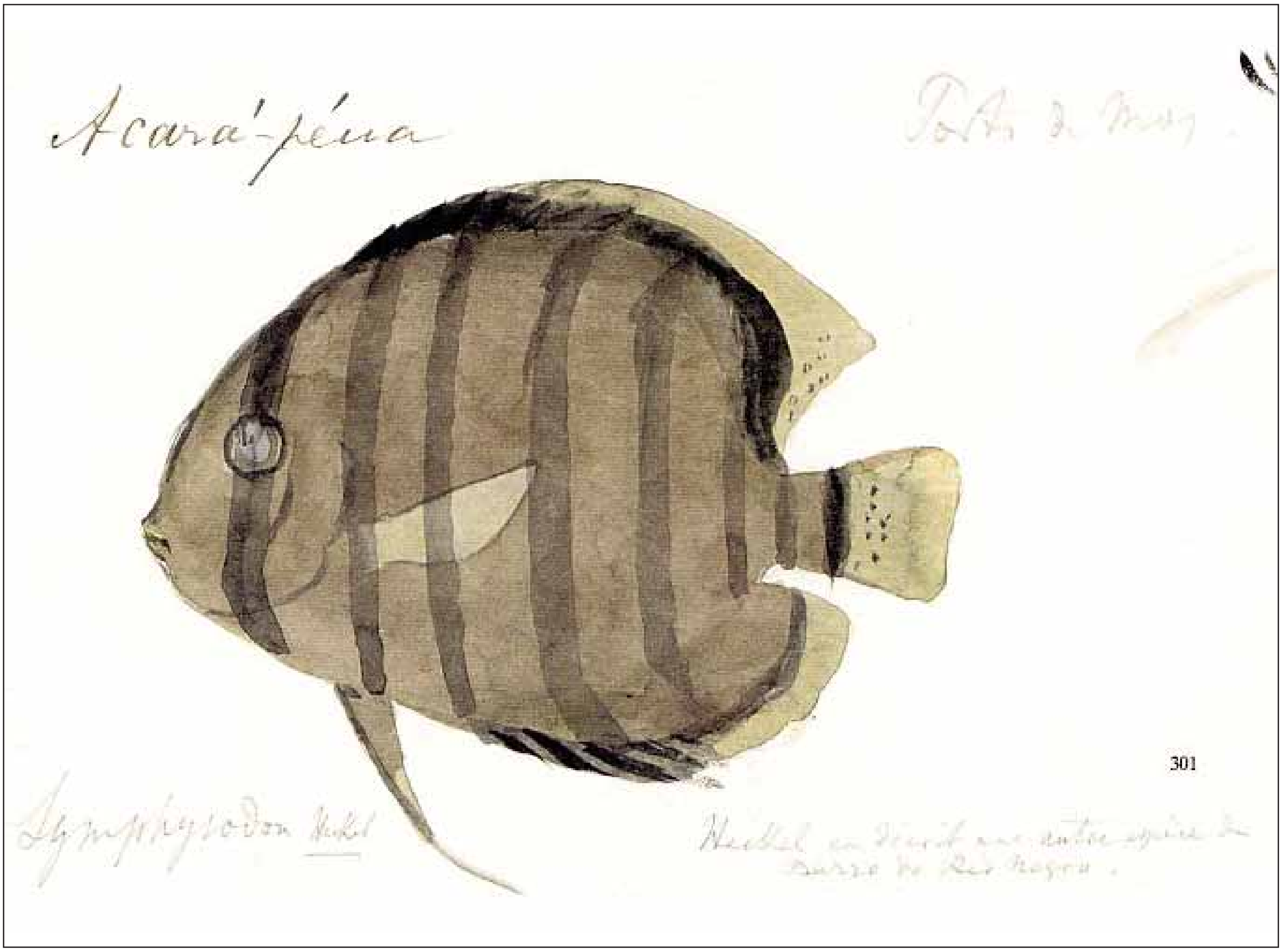


Fig. 8. Jacques Burkhardt's painting of a discus collected by Thayer Expedition (1865-66) members near Porto de Moz, Xingú, in 1865. It has eight bars on the flanks. Pellegrin (1904) noted "Heckel en déscrit une autre espècie...". Courtesy Harvard University.

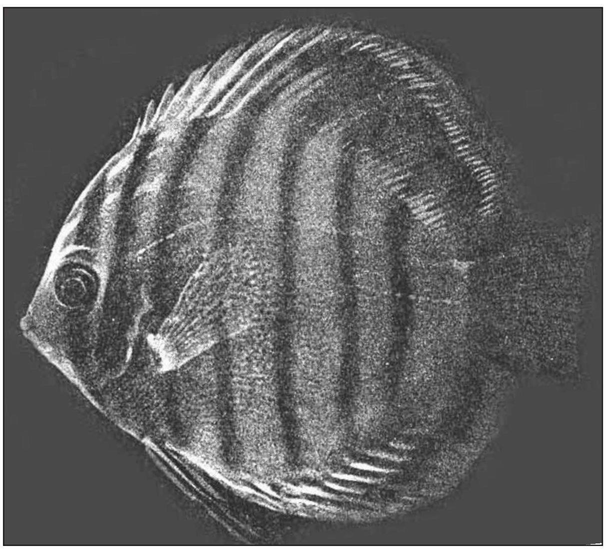


Fig. 9. Photograph by W. T. Innes of the first life discus from Santarém taken in 1934 showing eight bars on the flanks.

Karl Griem, also from the Santarém Region, can be seen in Fig. 9. It is identical with the discus painted by Burkhardt at Porto de Moz and with Pellegrin's syntype (MNHN 1902/130), see Fig. 5. Since than the "Pompadour Fish", as the discus was named by W. T. Innes in 1933, also "The Crested Cichlid", and from 1934 on "King of Aquarium Fishes" (Innes 1934), became one of the most popular aquarium fish and it is still "the King" today. It is the most popular and appreciated ornamental fish world-wide, about 18 million discus being bred every year (Bleher 2006). Only a tiny fraction of ca 30,000 specimens are collected in the wild and only during the low water season between August and November each year. But most of the life and preserved specimens and records just before and after World War II, came from the Santarém and the lower Xingú Region. Only the A. Campos collection of 1944 was made west of Santarém in the lago Ararí (Arary) area. Those discus were all blue and brown varieties, published as *S. discus* until 1960. The real Heckel discus *(S. discus)*, the holotype which had been collected by Natterer, includes some specimens collected by Thayer Expedition members, one collected by Jobert and a few by Melin and others. The first live specimens were imported in the early 1960s.

In 1958 the Brazilian ethnologist Harald Schultz, collected his first "green" discus alive (Fig. 10) in the Lake Tefé area (same area as the greens discus of the Thayer Expedition), in the small Lago de Jurity. It was Schultz who gave this discus with its red spots the name "green", even though he described the spots as "blood-red blotches" (Schultz 1959). Schultz had collected discus before, but never the green discus, and not in that part of the Amazon Region (Tefé).

The same year (1958) the Indian Raffael Wandurraga, who collected ornamental fishes for the aquarium fish trader Fred Cochu of Paramount Aquarium, USA, also found these green discus in the Amazon state of Columbia (pers. comm.). He reported it from the lake areas of the Río Putumayo (which in part forms the border between Colombia and Peru; once the river crosses the Brazilian border it is called Rio Içá, a left, northern Rio Solimões tributary) and one of us (HB) found them later in the Putumayo region as well. Until today, they are exported from Peru as "red-spotted green discus" (Fig. 7). Another ornamental fish exporter and at the time a competitor of Fred Cochu, Mike Tsalikis, who was also a friend of Wandurraga (both lived in the Leticia area), became aware of this "new" discus. Tsalikis ordered his fishermen to collect them and in 1959 the first export of green discus from the Río Putumayo area were flown out from Leticia to Chicago (Lyons 1960). Those fishes arrived alive and were displayed and sold, but none of those specimens were preserved (Fig. 11). It is without doubt that these belong to the same species as Pellegrin's two syntypes from Teffé (MNHN 1902/134-135), as do Schultz' specimens from Lake Jurity and the discus carrying the recently created name "*Symphysodon tarzoo* Lyons, 1959" (Ready et al. 2006). They all show the typical red spots on their body, or at least in their anal fin region; all have nine dark bars, a green or light blue stripe pattern in the upper body portion or at least above the eyes.

In the same year (reportedly on 28 November 1959) Earl Lyons, wrote an article about this discus from the Río Putumayo area (he gave the locality name as "Leticia", but Leticia was just the city of export) in the aquarium fish magazine "Tropicals Magazine" a holiday issue, which was published in 1960.

In 1960 L. P. Schultz published the first review of the genus *Symphysodon*. He arrived at the conclusion that at least four different *Symphysodon* occur in South America. Although he did not actually research Heckel's or Pellegrin's types and much of his material came from dubious sources, except for his green discus, which he identified as *S. aequifasciata aequifasciata* (Géry & Bleher in Bleher 2004). Unfortunately, some of the NMNH catalogue numbers are different from those published in his review (Bleher 2006). Schultz (l. c.) elevated *S. discus* var. *aequifasciata* to species level and described two new subspecies, besides the nominal subspecies:

1. *Symphysodon aequifasciata aequifasciata* (Fig. 10)
   Symphysodon discus aequifasciata Pellegrin, 1903: Mem. Soc. Zool. France, vol. 16, p. 230, type locality, Lago Teffé and Santarém, Brazil.
   Symphysodon discus Steindachner 1875 (in part): Sitzungsber. Akad. Wiss. Wien, vol. 71: 46-47, (Lago Teffé, those were the specimens from the Thayer Expedition from Teffé), and many paintings, drawings and photographs from several publications with various discus recorded until 1955).

The types he designated, USNM 179611, from Lago Teffé, collected by Harald Schultz, which were the same green discus with red spots (L. P. Schultz wrote under color pattern: "… with scattered light spots… base dark green") as those collected by the Thayer Expedition and by Jobert at Teffé.

2. *Symphysoson aequifasciata haraldi* (Fig. 12 a, b)
   S. discus tarzoo Lyons, Tropicals Magazine, Holiday issue 1960, vol. 4, no. 3, pp. 6-10, 4 figs., Nov. 28, 1959 (Amazon in vicinity of Leticia), nomen nudum.
   S. discus Meinken in Holly, Meinken and Rachow, 1943: Die Aquarienfische in Wort und Bild, Lieferung 75-76: 769-773; 41, 6 photographs, colour drawing; and Fowler, 1945: Mus. Hist. Nat. Univ. Nac. Mayor Marcos, Lima p. 253, fig. 87 (Peruvian Amazon).

Schultz established for the holotype (USNM 179829, Fig. 13) the type locality Benjamin Constant (which is surely not correct, as the fishes shown in his review (Fig. 12 a, b) are from Lake Jari, and/or Lake Berurí, both in the lower Purus Region (compare with Fig. 12 c, d, live specimens from that area.)

Fig. 10. The green discus from Teffé (individual from the nearby Lago Jurity), Brazil. This photo was used in L. P. Schultz's review of the genus *Symphysodon* in 1960 to illustrate *Symphysodon a. aequifasciata.* Photo by H. Schultz.

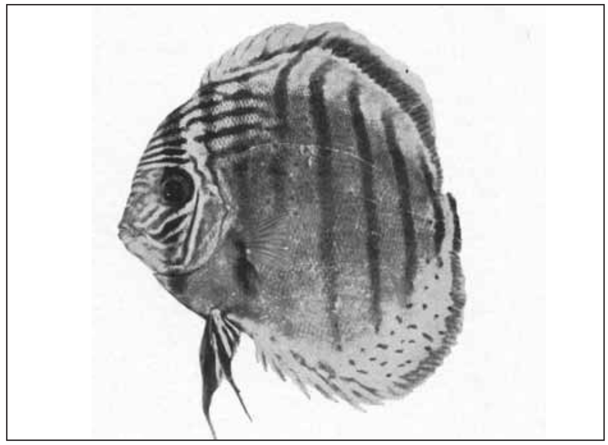


Fig. 11. Photo from Lyons' (1960) article in the popular magazine "Tropical Magazine, Holiday Issue" with regard to "Symphysodon Discus Tarzoo". Photo by Earl Lyons.

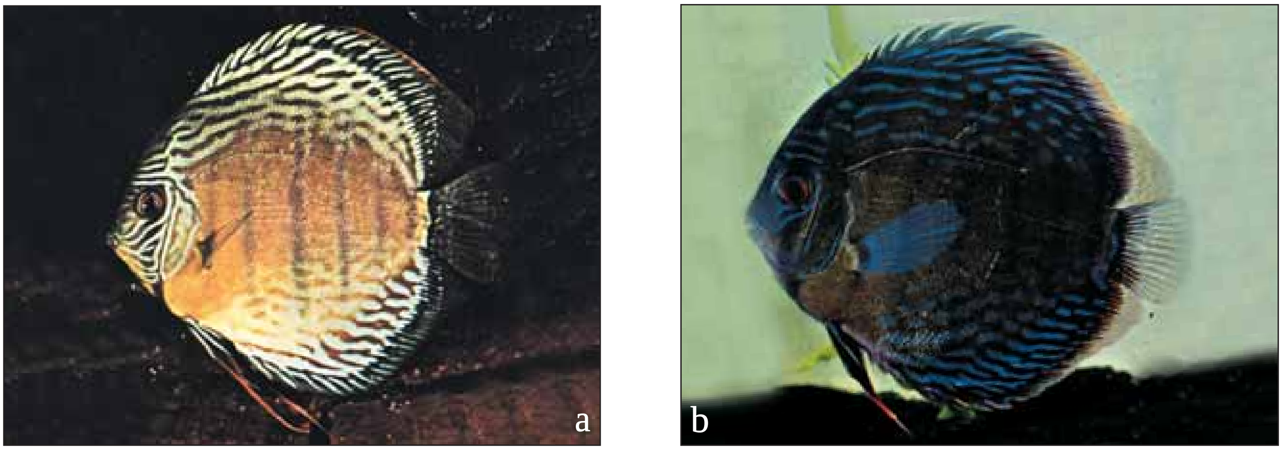


Fig. 12a-b. Captions to the photos by Dr E. Schmidt (a) and H. R. Axelrod (b) from the review by L. P. Schultz (1960), referring to the new subspecies, the blue discus, *Symphysodon aequifasciata haraldi:* "This specimen (a) differs considerably in its shade of colour from the individual shown top on page 8 (b). But the only difference is that the fish on page 8 (b) is in an aquarium with black gravel, while this one (a) was kept in an aquarium with a white substrate." Photos courtesy of T.F.H. Publications.

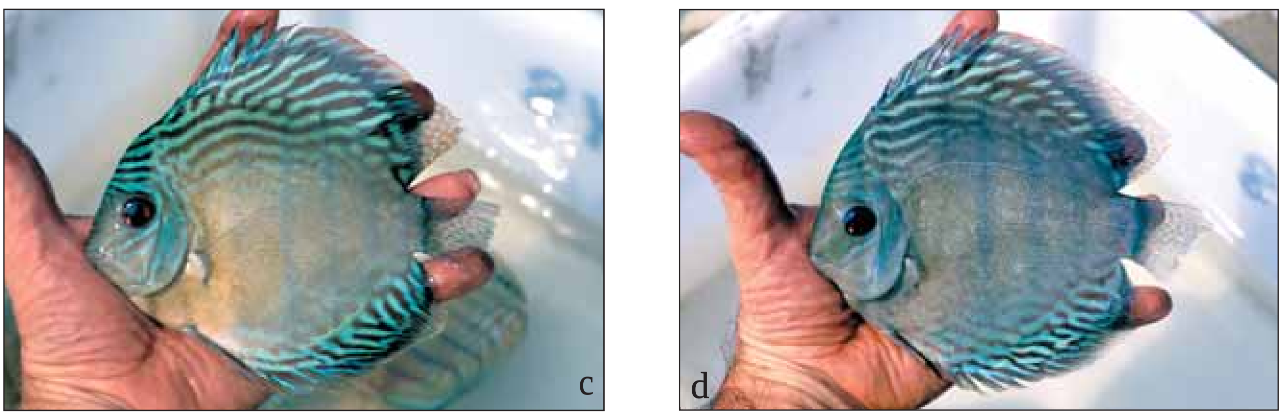


Fig. 12c-d. Blue discus, *Symphysodon haraldi*. Both specimens were collected by H. Bleher, 2004, in Lake Jari near the community Castanha. They have the typical colour pattern of discus from the Rio Purus Region. Photos by H. Bleher.

Schultz justified the nomen nudum status of "*S. discus tarzoo*", explaining that after 1 January 1931, a specific name must have been published with a statement in which the author attempted to indicate differentiating characters or with a summary of characters, which distinguish the species, concluding that the rules of zoological nomenclature adopted at the 1927 Budapest Congress had not been fulfilled.

3. *Symphysodon aequifasciata axelrodi*

No synonyms.

The collecting site of the holotype of *S. a. axelrodi* (USNM 00179831) given as Belém by H. R. Axelrod, is surely not correct. There has never been any record of any discus, not even from the vicinity of Belém. The closest discus habitat is the Rio Tocantins, nearly 300 km southwest from Belém (or rather was, because after the construction of the Tucuruí hydro-electric dam in 1984, no more specimens have been found there). The preserved holotype (Fig. 14) looks very much like the discus collected in the lower Xingú (Fig. 15) and those found in the lower Tapajós (= Santarém) Region.

Following the names he had established in 1958, Schultz (1960) cited the following common names: for *S. a. haraldi* the "blue discus", for *S. a. axelrodi* the "common discus" (which later was also called the "brown discus" in the ornamental fish trade and in the aquarium hobby), and for *S. a. aequifasciata* (from Teffé) the "green discus".

In the following years, the animal trader Hans Willy Schwartz under the name of his newly established Aquario Rio Negro in Manaus, collected and exported *Symphysodon discus* for the first time from the Rio Negro area.

In 1965, a Rio Trombetas Expedition, organised by the Museu Goeldi in Belém and the Museu de Zoologia da Universidade de São Paulo (MZUSP), collected discus specimens in the Lago Jacaré, a left-hand lower Trombetas lake area. Those were identified by Kullander as *S. discus*, but none of those specimens at the São Paulo Museum has a dark fifth bar (Fig. 16), which is a typical pattern for *S. discus*. One of us (HB), during several field trips to the same area (the first one in 1965), was unable to find *S. discus* in the Trombetas Region, nor in the Lago Batata (contrary to what has been published in some fish hobby magazines). It was found only further west, in the Rio Nhamundá system.

The MZUSP holds the specimens collected in 1944 by A. Campos at Lago Ararí (Arary): one specimen collected by H. Schultz in 1953 near Santarém, four specimens collected by R. Best in Lake Amanã (without date), three from Lago Manacapuru collected in 1967 (EPA), one from the Rio Curuá (Alenquer Region) collected in 1969 (EPA), one from the Rio Maicá (Santarém) collected in 1971 (EPA), one from Belo Monte (Xingú) collected in 1982 by M. Goulding, 10 from the Igarapé do Grito (Tocantins) collected in 1970 (EPA). All of these, including the MZUSP specimens from the Lago Jacaré (Trombetas), belong to the group of blue and brown discus *(S. haraldi).*

Warren Burgess (1981) described one more subspecies: *Symphysodon discus willischwartzi* (Fig. 17) and for the first time *S. discus* was recorded from a location south of the Amazon river in Rio Abacaxís (correct holotype location). It is identical with *S. discus* from the Rio Negro area (Fig. 18). In the meantime, *S. discus* specimens were found in several other locations by one of us (HB), but exclusively in waters with extreme chemical parameters (see below).

Kullander (1986) synonymised all of Schultz' subspecies and recognised only two species: *Symphysodon aequifasciatus* Pellegrin, 1904 (synonyms: *S. discus* var. *aequifasciata* Pellegrin, 1904; *S. discus tarzoo* Lyons, 1959; *S. a. axelrodi* Schultz, 1960; and *S. a. haraldi* Schultz, 1960) and *Symphysodon discus* Heckel, 1840 (synonym: *S. d. willischwartzi* Burgess, 1981). Kullander (l. c.) mentioned that he was unable to redo Schultz' scale counts, but still found lower counts in *S. discus* (48-56), than in *S. aequifasciatus* (54-60). Kullander (1996) published another paper on *Symphysodon* where he maintained the two species, *S. discus* and *S. aequifasciatus*, and Kullander in Reis et al. (2003) still recognised the same two species with the same synonyms as in his previous publications.

Géry & Bleher in Bleher (2004) gave the corrected type localities of all *Symphysodon* types and designated one of Pellegrin's two Tefé specimens as lectotype (MNHN 221-68-2-2, 90.5 mm SL) and paralectotype (MNHN 1902/134-135, 122.5 mm SL) of *S. aequifasciatus*. In the same publication they recognised, for the first time, three *Symphysodon* species: *S. discus*, *S. aequifasciatus*, and *S. haraldi* Schultz, 1960. Ready et al. (2006) also recognised three species and revalidated for the third one the name "Tarzoo", which since Schultz' (1960) review had unanimously been accepted as being a nomen nudum or a synonym of *S. haraldi*. They designated a neotype for *S. tarzoo* from Rio Jutaí. Despite the previous lectotype designation by Géry

Fig. 13. *S. aequifasciata haraldi*, holotype (USNM 00179829), supposedly from Benjamin Constant and collected by H. R. Axelrod (no date). Photo courtesy USNM.

Fig. 14. *Symphysodon aequifasciata axelrodi*, holotype (USNM 00179831), 105 mm SL, supposedly from Belém and collected by H. R. Axelrod (no date). Photo courtesy USNM.

Fig. 15. *Symphysodon haraldi* collected south of Vitoria do Xingú. It has the same colour pattern as the one from the Thayer Expedition (Fig. 8). Photo by P. Rüegg.

Fig. 16. *Symphysodon discus.* It is questionable whether the specimens collected 1965 in Lago Jacaré and deposited in the MZUSP are really *S. discus*. Photo by N. Khardina.

Fig. 17. *Symphysodon discus willischwartzi* Burgess, 1981, holotype. Photo by S. O. Kullander.

Fig. 18. *Symphysodon discus* from the Rio Negro used by L. P. Schultz for his 1960 Review. Photo courtesy of T.F.H. Publications.

& Bleher in Bleher (2004), Ready et al. (l. c.) designated Pellegrin's syntype from Santarém as lectotype of *S. aequifasciatus*. Besides the fact that this lectotype designation is invalid, the specimen on which it is based belongs to a different species *(S. haraldi* Schultz, 1960).

INTRODUCTION MOLECULAR STUDIES

Mitochondrial sequences are widely used in phylogenetic and population genetic studies. While being maternally inherited, large copy numbers of mitochondria in the cell and the known genomic architecture greatly facilitate analyses. Mitochondrial control regions, also termed displacement loops (D-loop), are evolving much faster than the average mitochondrial sequence and are therefore well suitable to document comparatively recent evolutionary relationships. The D-loop is a noncoding DNA region and is therefore under no constraint concerning the accumulation of mutations. It is assumed that the accumulation of mutations is a largely clockwise process, and depending on the elapsed time more and more mutations accumulate. Closely related individuals will always share most of these mutations, which suggests a more recent common ancestor than in organisms with higher genetic differentiation.

Interbreeding (of individuals) results in the sharing of genetic material, such that diversity accumulated over time is again removed. Since mitochondrial DNA (mtDNA) is maternally inherited (there is no genetic contribution by the father), the mtDNA genetic composition is entirely defined by the maternal lineage, which limits its utility as genetic marker for hybridization. If discrepancies are found between mtDNA relationships and morphologically distinct lineages (a possible indication of hybridization or the retention of ancient polymorphism) several nuclear DNA markers should be characterised. Highly variable, therefore sensitive nuclear DNA markers such as microsatellite DNA have been used for the study of gene flow, population structure and hybridization (see e.g. Meyer et al. 2006).

The number of haplotypes that can be detected in each lineage is a function of both the absolute number of haplotypes that exist within a group of organisms (species), but also of the number of individuals tested. To optimise this relationship, specimens from as many distinct habitats and localities as possible should be sampled. This is particularly important as distribution ranges of potential taxonomic units can overlap. In case the extremes of such distribution ranges are not tested, two species might be considered genetically distinct units, whereas their genetic composition in reality is a continuum that has not been sampled with sufficient thoroughness. To minimise this issue, samples from all commercially important streams in Central Amazonia, Brazil, have been collected for this study, covering the entire distribution range of *Symphysodon* as completely as possible.

Taxonomists employ clear, largely morphological and formally precise concepts of how a species is defined, which, in case of cichlid fishes, includes the colour pattern and also the geographic distribution of a particular morphotype. Following the biological species concept, a biological species must be reproductively isolated from all other species. Reproductive isolation, will lead to an accumulation of mutations and therefore to genetically more and more distinct groups. These genetic differences are then detectable, and particularly so in fast evolving sequence regions, such as the mitochondrial D-loop. Consequently, if no genetic differentiation can be found, there is no genetic evidence supporting the hypothesis of two or more distinct species, irrespective of the formal taxonomic status. However, mere observation of the degree of differentiation between two or more forms cannot reveal the processes that might lead to loss or accumulation of diversity. Since some very young species will have hardly any diagnostic mutations that separate them, genetic data are always only hypotheses of a distinct species status. Additionally, there are no rules stipulating that a certain percentage of genetic difference requires the erection of new species or even new genera. What matters in the assignment of the species status based on genetic markers are the topology of the phylogenetic tree and the reciprocal monophyly of the tentative taxonomic units.

Abbreviations:

EPA = A Expedição Permanente da Âmazonia São, Paulo, Brazil

ICZN = International Code of Zoological Nomenclature

Fig. 19. Haplotype network analysis of 48 discus specimens based on the analysis of partial D-loop sequences (see Table I for species and locality information and GenBank accession numbers). Each mitochondrial haplotype is indicated, also with the number of specimens that have or share a particular haplotype. Small black dots indicate mutational steps that separate the haplotypes found.

The three *Symphysodon* cluster.
(In parentheses: the next major river or city).

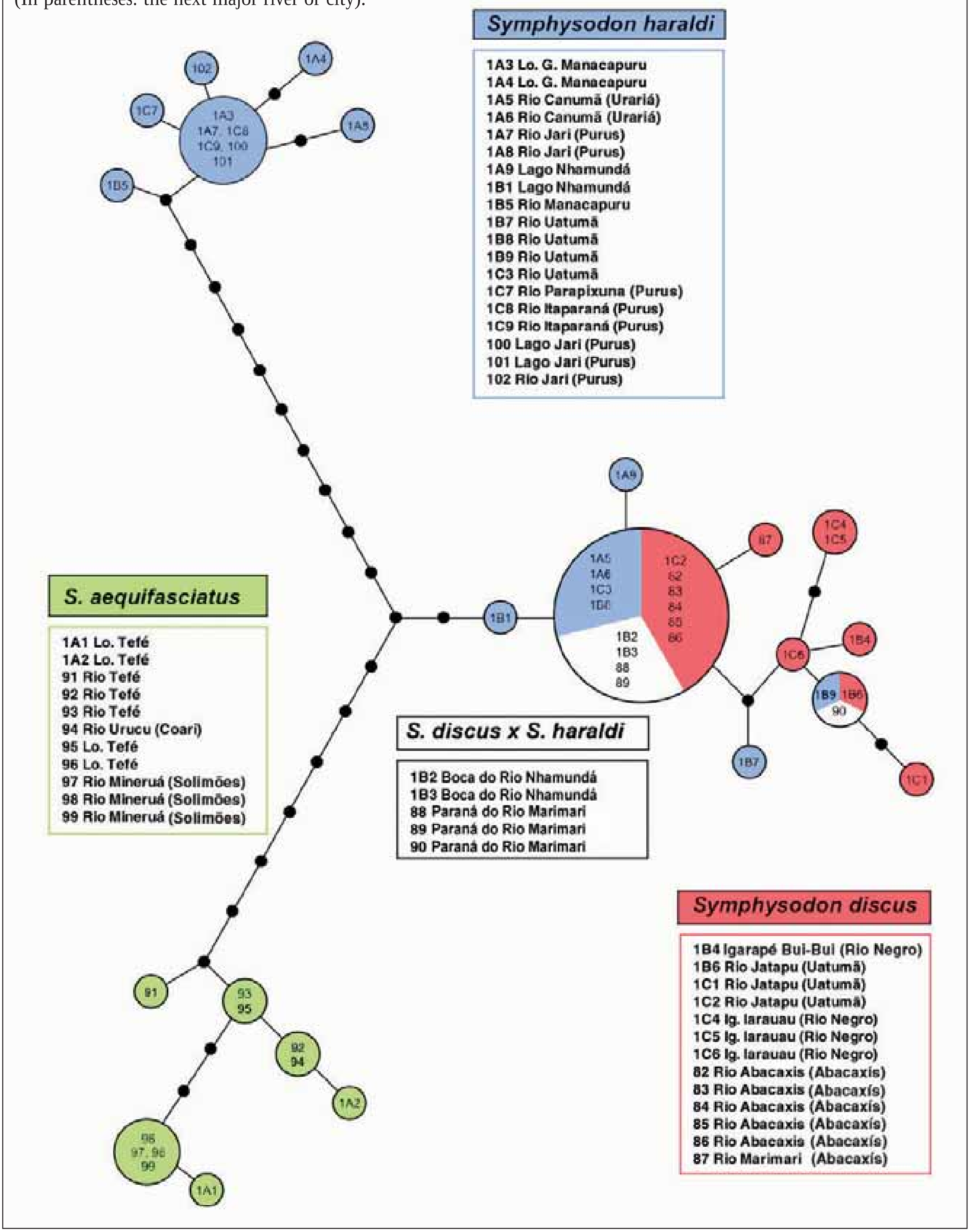
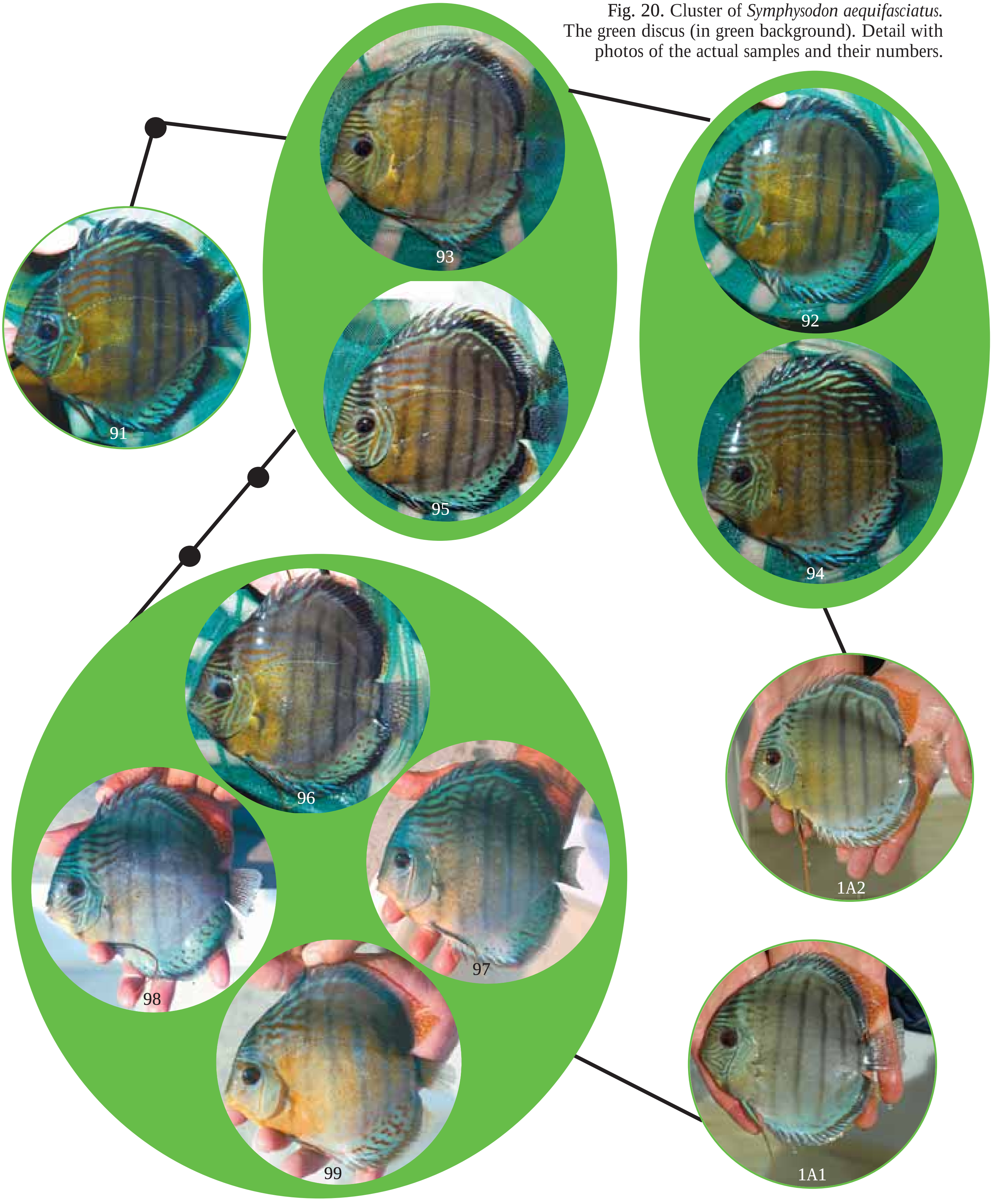


Fig. 20. Cluster of *Symphysodon aequifasciatus*. The green discus (in green background). Detail with photos of the actual samples and their numbers.



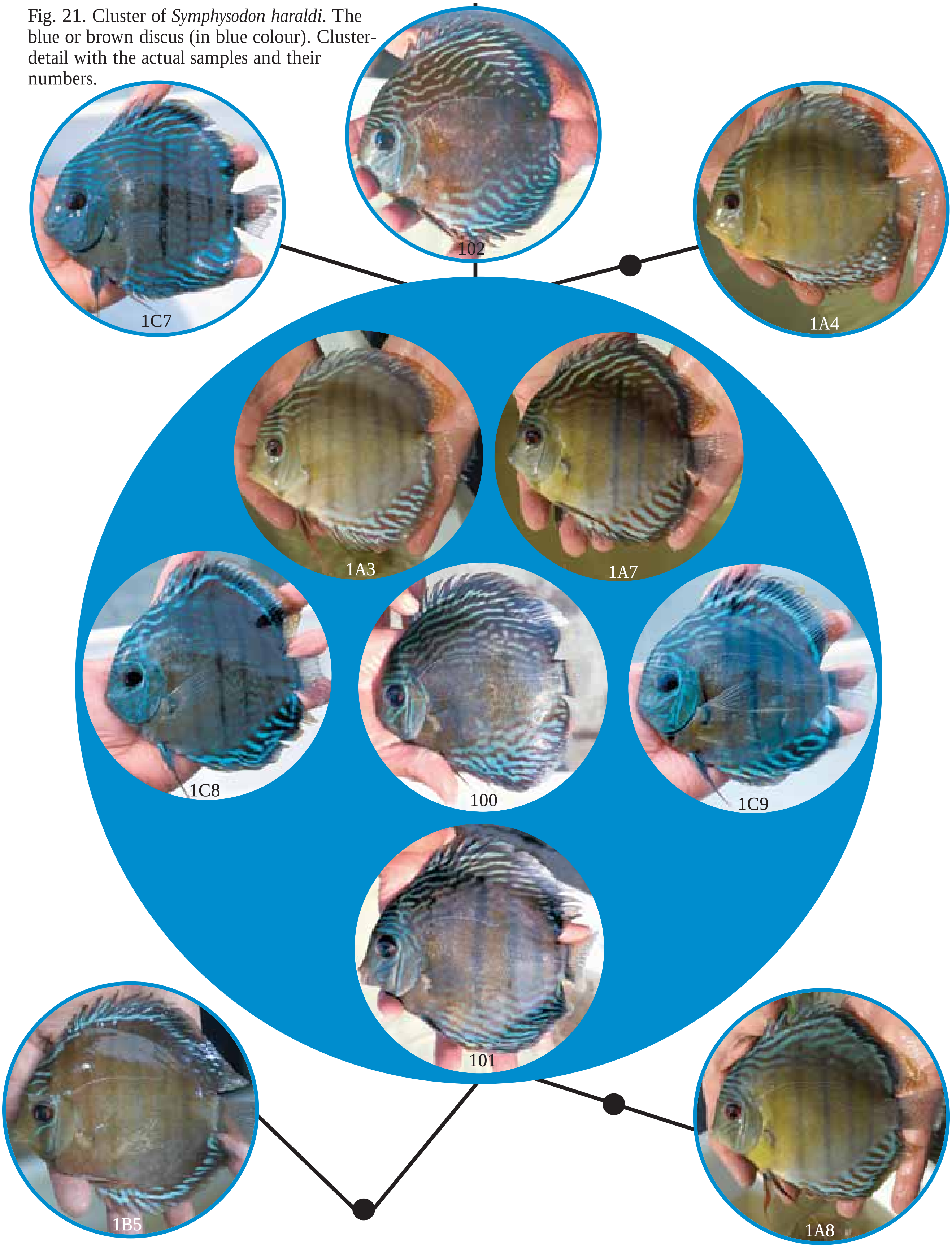
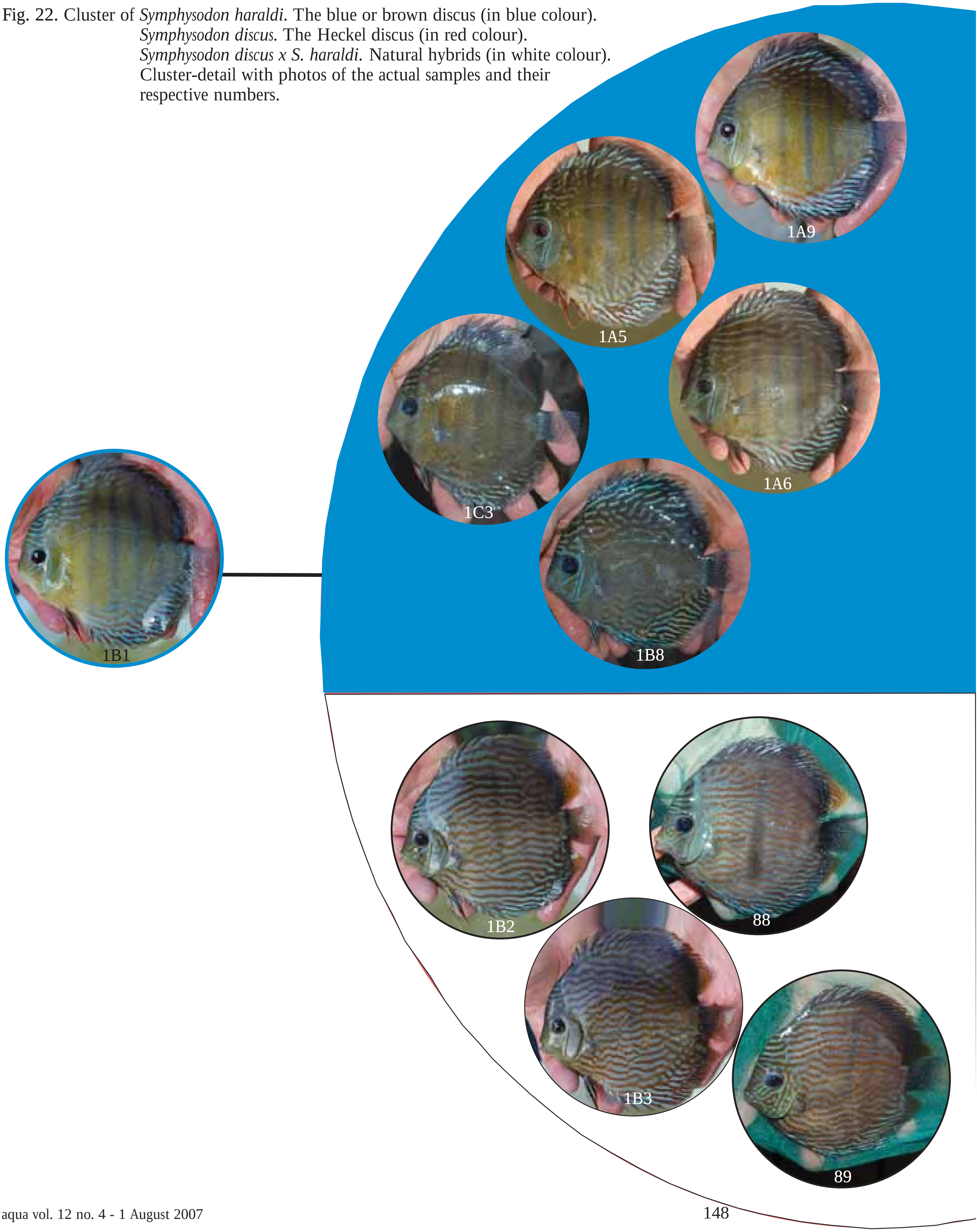


Fig. 21. Cluster of *Symphysodon haraldi*. The blue or brown discus (in blue colour). Cluster-detail with the actual samples and their numbers.

Fig. 22. Cluster of *Symphysodon haraldi*. The blue or brown discus (in blue colour).
Symphysodon discus. The Heckel discus (in red colour).
Symphysodon discus x S. haraldi. Natural hybrids (in white colour).
Cluster-detail with photos of the actual samples and their respective numbers.



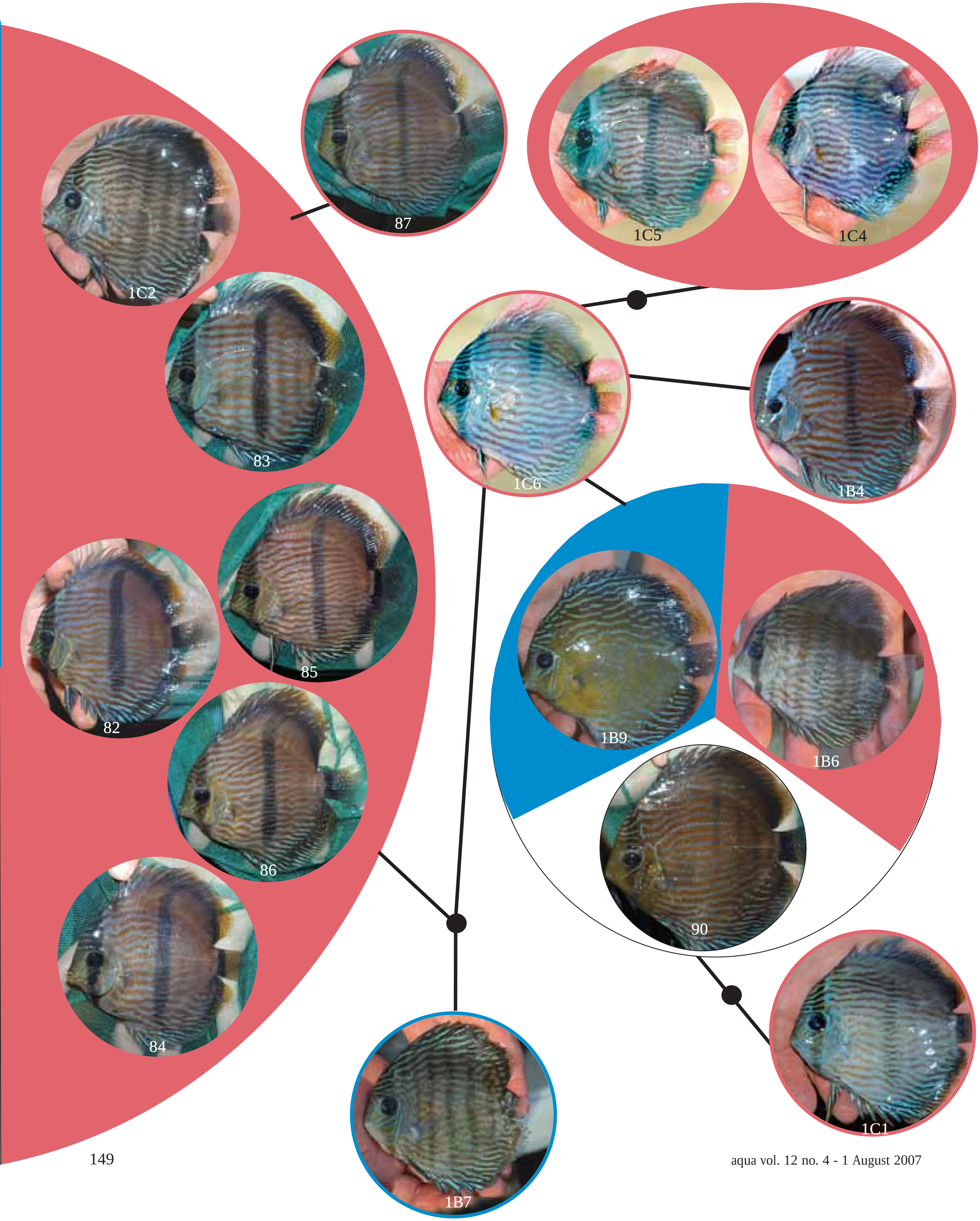
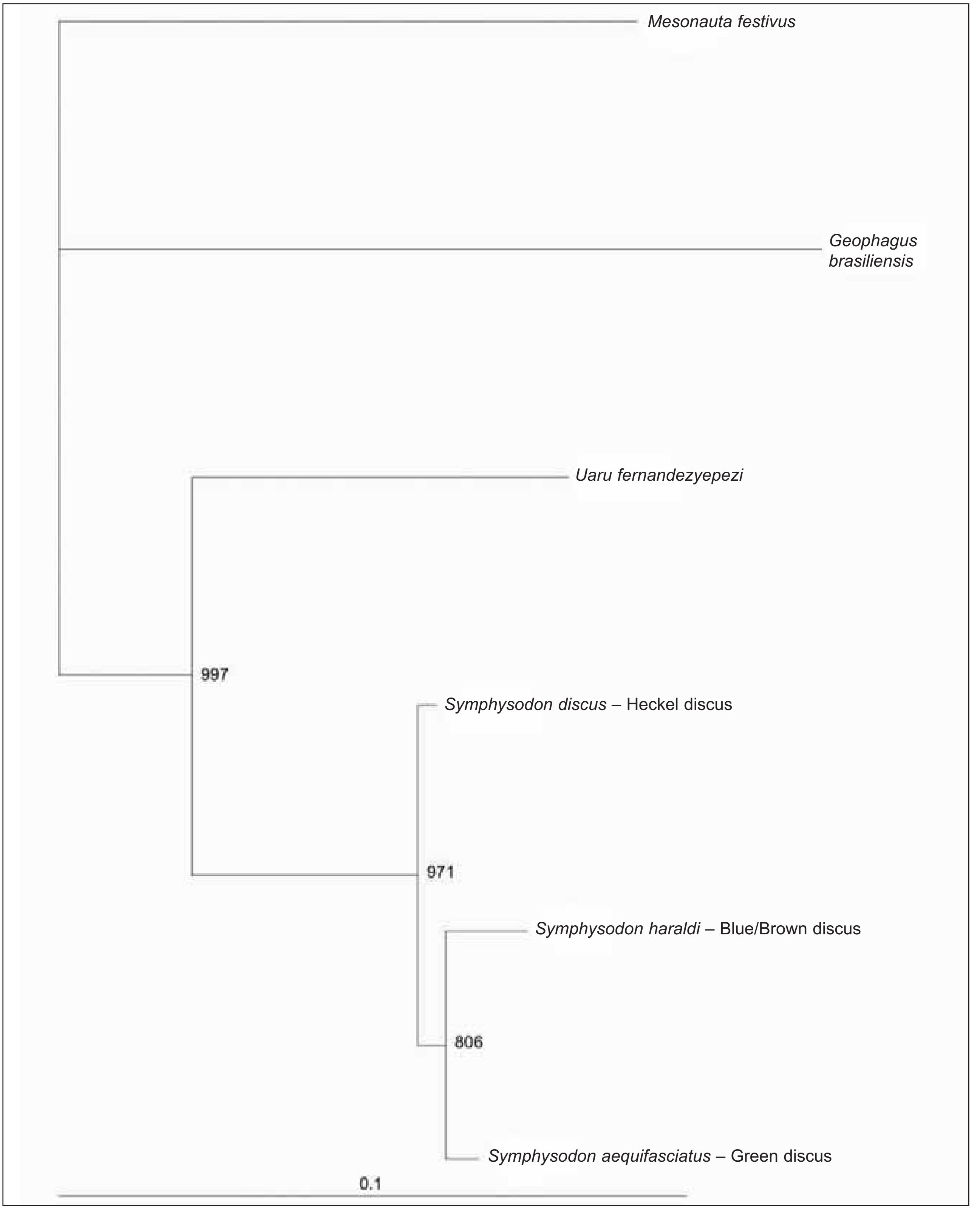

87
1C5
1C4
1C2
83
1C6
1B4
85
82
1B9
1B6
86
90
84
1C1
1B7

Fig. 23. Phylogenetic analysis (maximum likelihood analysis with 1000 bootstrap replicates in PHYML under the GTR model) based on partial D-loop sequences (413 basepairs) using *Mesonauta*, *Geophagus* and *Uaru* as outgroups showed that *Uaru* is the closest realtive to *Symphysodon*, and that *S. discus* is likely to be the deepest branch within *Symphysodon*.



INPA = Instituto Nacional da Pesquisa da Amazonia, Manaus, Brazil

MNHN = Muséum National d'Histoire Naturelle, Paris, France

MZUSP = Museu de Zoologia da Universidade de São Paulo, Brazil

NMW = Naturhistorisches Museum Wien, Austria

NRM = Naturhistorika Riksmuseet Stockholm, Sweden

USNM = United States National Museum, Washington D. C., USA

MATERIALS AND METHODS

Selection of specimens: The sampling for molecular studies comprised 48 *Symphysodon* specimens, representing the three species recognised by us. A total of 11 *S. aequifasciatus,* 20 *S. haraldi*, 12 *S. discus* and five natural hybrids *(S. discus x haraldi*) were included. *Symphysodon aequifasciatus* comprised four specimens from Lago Tefé, three specimens from Rio Tefé and an additional individual from the Rio Urucu (the easternmost distribution area of the green discus). The *S. haraldi* samples consist of two specimens from Lago Grande Manacapuru, one specimen from Rio Manacapuru, two specimens from Rio Jari, two from Lago Nhamundá, four from Rio Uatumã, and two from the Rio Canumã. *Symphysodon discus* is represented with one specimen from Rio Negro, three from Rio Jatapu, and six from Rio Abacaxís. Five naturally occurring hybrids of *S. haraldi x S. discus* were included, two from the Rio Nhamundá Region, the remaining three from Rio Marimari (Figs 19-22). Additionally, three *Mesonauta festivus* and two *Uaru fernandezyepezi* samples from individuals bred in captivity were included as outgroup samples (Fig. 23). Fin clips were taken from living specimens for DNA analysis and obtained sequences were deposited in GenBank. Digital images were taken from all discus specimens to compare individuals (Figs 20-22). More detailed information on the sampling localities of the specimens included in the molecular analyses can be found in Fig. 24.

Laboratory protocols: Total genomic DNA was extracted from fin-clips preserved in 95 % ethanol. Approximately 2 mm^2 of fin clip were dissolved with Proteinase K in a buffered SDS solution and DNA was extracted by High-Salt-Extraction method (Bruford et al. 1998). DNA was precipitated with NaCl and subsequent addition of 70 % ethanol over night. Purified DNA re-eluted in distilled water served as a template for subsequent polymerase chain reactions (PCR).

The PCR were performed in 25 µl reaction volume on whole genomic DNA; 1 µ (approximately 100 ng) of template DNA was used to amplify the entire mitochondrial control region with the LProF (5'-AACTCTCACCCC-TAGCTCCCAAAG-3') and 12Sr.5 (5'-GGCG-GATACTTGCATGT-3') primers (T. Hrbek, pers. comm.). The 35 cycles long PCR protocol consisted of an initial step of template denaturation for 35 seconds at 94 °C, a 35 seconds step of primer annealing at 48 °C and an extension step of 90 seconds at 72 °C. These 35 cycles were followed by a final extension step of 7 min length at 72 °C to ensure full-length elongation of generated PCR fragments. The PCR products were purified according to the QiaQuick protocol (Qiagen). The forward section of the control region was sequenced with the LProF primer and electrophoresed on an automated ABi 3100 sequencer (Applied Biosystems).

Data analysis: All sequences were aligned manually with the BioEdit package version 5.0.9 (Hall 1999). Haplotype networks were reconstructed manually from unrooted maximum parsimony (MP) trees reconstructed with PAUP (Phylogenetic Analysis Using Parsimony) version 4.10b (Swofford 2002). The MP trees were reconstructed with 100 heuristic searches, random sequence addition, and employing the tree bisection and reconnection algorithm. No differential character-weighting regime was used. Resulting trees were bootstrapped 1000 times to estimate confidence. The MP based network was drawn by translating the number of reconstructed changes on each branch into stepwise mutations between sequence haplotypes.

DNA sequencing: Sequencing resulted in 515 base pairs of unambiguously aligned mitochondrial control region DNA for each individual. With 480 invariable and 26 variable and parsimony informative sites only nine variable sites of the alignment were parsimony uninformative. Base frequencies of 31 % adenine, 23 % cytosine, 32 % thymine and only 14 % guanine indicated the clear anti-G-bias of amplified mitochondrial DNA (Zhang & Hewitt 1996). The sequence identity was confirmed by BLASTn searches against other vertebrate nucleotide sequences deposited at GenBank and revealed closest hits with other cichlid fish D-loop sequences.

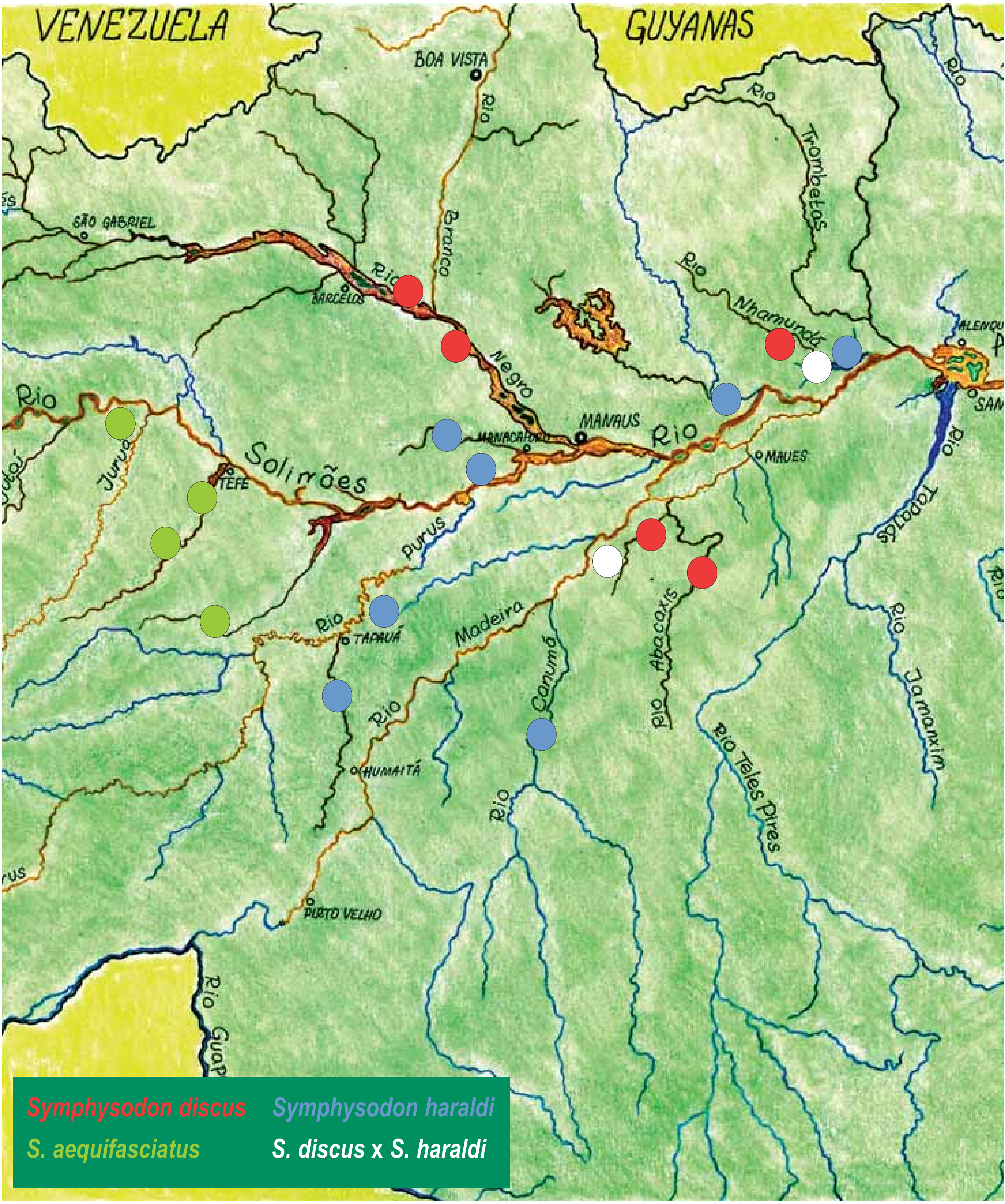

VENEZUELA
GUYANAS
BOA VISTA
Rio Branco
SÃO GABRIEL
BARCELOS
Rio Negro
MANAUS
MANACAPURU
Rio Trombetas
Rio Nhamundá
ALENQU
SAN
Rio Tapajós
MAUES
Rio Solimões
TEFE
Juruá
Purus
Rio TAPAUÁ
Madeira
Rio
Canumã
Rio Abacaxis
Rio Teles Pires
Rio Jamanxim
HUMAITÁ
PORTO VELHO
Rio Guap
Symphysodon discus
Symphysodon haraldi
S. aequifasciatus
S. discus x S. haraldi

Fig. 24. Sampling spots of *Symphysodon* specimens (each spot may represent more than one specimen).
Symphysodon aequifasciatus – green discus
Symphysodon haraldi – blue or brown discus
Symphysodon discus – Heckel's discus
Symphysodon discus x *haraldi* – natural hybrids

Haplotype networks: A haplotype network reconstruction revealed three distinct groups of *Symphysodon* specimens (see Fig. 19). All included *S. aequifasciatus* samples (1A1, 1A2 and 91-99) form a monophyletic group (Fig. 20), but *S. haraldi* are polyphyletic. One distinct group consists of only *S. haraldi* samples (100-102, 1A3, 1A4, 1A7, 1A8, 1B5, 1C7-1C9) (Fig. 21), while the third group consists of all remaining samples of *S. haraldi*, *S. discus* and their natural hybrids (Fig. 22). Some of the *S. discus* individuals do not seem to be genetically very distinct from some phenotypic *S. haraldi* samples in this cluster.

With a maximum number of six mutations within their group, *S. aequifasciatus* samples exhibit a larger diversity than the distinct cluster of *S. haraldi* with four mutations. The agglomerate of *S. discus*, *S. haraldi* and their hybrids shows also a maximum of six mutations. Notable, 17 of the 26 tested individuals (65%) within this group share their haplotypes (i.e. their genetic composition); and the naturally occurring hybrids only occur among those 17 individuals.

Water chemistry: Water parameters were measured with the Multiline-P4 equipment (WTW, Weilheim, Germany). Nonparametric Kruskall-Wallis tests (analysis of variance) compared the ranked measurements between habitats grouped by the three *Symphysodon* species (df=2, significance cutoff set to $p<0.05$) (see Tables III-V).

The habitats of *Symphysodon* species differ significantly in their water chemistry: *S. discus* lives only in extreme acid black waters of an pH almost constantly around 4.6 (except when it rains very strongly and areas get washed and/or water rises suddenly and white waters enter their habitats), with an oxygen concentration of 2.8 mg/l. *Symphysodon discus* lives only in areas of extremely soft waters. Electronically measured, the conductivity averaged below 10 μS/cm (Table III). *Symphysodon aequifasciatus* lives in waters of low pH values around 5.2, where the oxygen concentration averages 2 mg/l, and they require also soft and black water to survive, averaging around 10 μS/cm (Table IV). Only *S. haraldi* can live in clear water (sometimes, especially at the beginning of the rainy season, mixed with white water) and with a pH-value of almost constantly above 6.0 (and often above 7.0), with oxygen concentrations around 4.1 mg/l and fairly high conductivity of normally more than 25 μS/cm (Table V). The latter are rarely found in black water habitats.

RESULTS

Systematic accounts

Symphysodon Heckel, 1840

Symphysodon Heckel, 1840: 332; type species: *Symphysodon discus* Heckel, 1840 (by monotypy)

Symphysodon discus Heckel, 1840

Symphysodon discus Heckel, 1840: 332; type locality: Moreré am Rio-negro (= Moreira = Moreyra, not Barra do Rio Negro). Holotype: NMW 35612 (Fig. 2).

Symphysodon discus willischwartzi Burgess, 1981: 37; type locality: Rio Abacaxís (a southern tributary of the Paraná de Urariá; Rio Abacaxís is not a tributary of Rio Madeira, as often cited). Holotype: MZUSP 15375 (Fig. 17).

Common names: In the nineteenth century in Brazil it was called “peixe de moreré” or just “moreré” as this was the location on the Rio Negro were it was discovered, which today is called Moreira. In the beginning of the twentieth century locals called it “acará-moreré”. Later it was known as “acará-disco” in Brazil, even though the Tupí word “acará”, applied to many cichlid species in Brazil, means “heron” or “white bird”. In Brazil it remained in use until today and refers to all species of discus. By the early 1960s it was called the “red discus” or “Echter Diskus” in German speaking countries and since the late 1960s the common name “Heckel Diskus” or “Heckels Diskus” became established, while in English speaking countries and in the rest of the world “Heckel-discus”, “Heckel’s discus” or simply “Heckel’s” are used.

Description: Meristic counts for *S. discus* have been published by several authors (e.g. Heckel 1840; Kullander 1986, 1996). Several of these counts showed discrepancies, even for one and the same specimen, i.e. for the holotype counts in Kullander (1986: 229) differ from those in Kullander (1996: 13). Studies by all researchers showed clearly, that the average scale count in *S. discus* is lower than in the other two species. Additionally, specimens of *S. discus* are, throughout the species’ range of distribution (Fig. 31), smaller than those of the other two species. Even if the species show slightly different meristic counts and morphometric measurements, these are not truly diagnostic, as suggested by Kullander (1986, 1996) and Ready et al. (2006). The colour pattern offers the best (and probably besides the molecular results the only) distinguishing characters of the three species of *Symphysodon*, as is the case in many other species of the family Cichlidae.

Colour in vivo: Heckel (1840) stated: “Drei dunkelbraune vertikale Binden von der Breite eines Augendiameters umgeben den Körper, die erste geht durch das Auge selbst, die zweite um die Mitte des Körpers von der Basis der ersten weichen Rückenflossen-Strahlen herab, und die letzte umgibt die Schwanzwurzelbasis.” There are three intense bars, one across the eye, one in the middle of the flanks starting from below the first soft dorsal fin ray (which is normally the most prominent and the broadest), and a third one in the caudal peduncle. Heckel did not realise that there are also three very light-coloured bars, each between the first and second, and between the second and third dark bars (Fig. 28). Those are normally not visible in preserved individuals, but they appear quite frequently in live specimens (Bleher 2006: 165-172). Another colour pattern, that distinguishes this species from its congeners was also noted by Heckel (1840): “18 etwas wellenförmige, schmale, rostbraune Längsstreifen, welche eben so schmale Zwischenräume haben, durchziehen den Rumpf in paralleler Richtung...”. All *S. discus* have 16 to 21 stripes running horizontally across the entire body, which are wavy, rarely in strait lines and almost never interrupted. Over the last 40 years, this pat-

Methods

- N = 48 samples
  - – 18 localities
  - – All species, including natural hybrids
- First half of d-lop = ˜ 530bp
- BioEdit, PAUP, PHYML, PowerPoint
  - – hoplotype-network (530bp, MP, PAUP)
  - – Tree I (˜˜330bp aligned, GTR, ML, 1k bootstrap)
  - – Tree II (413 bp aligned, GTR, ML, 1k bootstrap)

Table I. Sampling scheme. Isolate: Sample Identifier. Taxonomy: Traditional species assignment. Molecular result: Species assignment according to molecular results. Locality: Origin of sample. Tributary: Major watersheds to which samples belong. GenBank Accession No.: GenBank Accession Numbers.

Isolate	Taxonomy	Molecular result	Locality	Tributary	GenBank Accession No.
1A1	*Symphysodon aequifasciatus*	*Symphysodon aequifasciatus*	Lago Tefé	Tefé	DQ198094
1A2	*Symphysodon aequifasciatus*	*Symphysodon aequifasciatus*	Lago Tefé	Tefé	DQ198095
1A3	*Symphysodon haraldi*	*Symphysodon haraldi*	Lago Grande Manacapuru	Manacapuru	DQ198122
1A4	*Symphysodon haraldi*	*Symphysodon haraldi*	Lago Grande Manacapuru	Manacapuru	DQ198123
1A5	*Symphysodon haraldi*	*"Symphysodon discus"*	Rio Canumã	Urariá	DQ198124
1A6	*Symphysodon haraldi*	*"Symphysodon discus"*	Rio Canumã	Urariá	DQ198125
1A7	*Symphysodon haraldi*	*Symphysodon haraldi*	Rio Jarí	Purus	DQ198126
1A8	*Symphysodon haraldi*	*Symphysodon haraldi*	Rio Jarí	Purus	DQ198127
1A9	*Symphysodon haraldi*	*"Symphysodon discus"*	Lago Nhamundá	Nhamundá	DQ198128
1B1	*Symphysodon haraldi*	*"Symphysodon discus"*	Lago Nhamundá	Nhamundá	DQ198129
1B2	*S. discus x S. haraldi*	*"Symphysodon discus"*	Boca do Rio Nhamundá	Nhamundá	DQ198117
1B3	*S. discus x S. haraldi*	*"Symphysodon discus"*	Boca do Rio Nhamundá	Nhamundá	DQ198118
1B4	*Symphysodon discus*	*"Symphysodon discus"*	Ig. Bui-Bui	Negro	DQ198105
1B5	*Symphysodon haraldi*	*Symphysodon haraldi*	Rio Manacapuru	Manacapuru	DQ198130
1B6	*Symphysodon discus*	*"Symphysodon discus"*	Rio Jatapu	Uatumã	DQ198106
1B7	*Symphysodon haraldi*	*"Symphysodon discus"*	Rio Uatumã	Uatumã	DQ198131
1B8	*Symphysodon haraldi*	*"Symphysodon discus"*	Rio Uatumã	Uatumã	DQ198132
1B9	*Symphysodon haraldi*	*"Symphysodon discus"*	Rio Uatumã	Uatumã	DQ198133
1C1	*Symphysodon discus*	*"Symphysodon discus"*	Rio Jatapu	Uatumã	DQ198107
1C2	*Symphysodon discus*	*"Symphysodon discus"*	Rio Jatapu	Uatumã	DQ198108
1C3	*Symphysodon haraldi*	*"Symphysodon discus"*	Rio Uatumã	Uatumã	DQ198134
1C4	*Symphysodon discus*	*"Symphysodon discus"*	Ig. Iarauau	Negro	DQ198109
1C5	*Symphysodon discus*	*"Symphysodon discus"*	Ig. Iarauau	Negro	DQ198110
1C6	*Symphysodon discus*	*"Symphysodon discus"*	Ig. Iarauau	Negro	DQ198111
1C7	*Symphysodon haraldi*	*Symphysodon haraldi*	Parapixuna	Purus	DQ198135
1C8	*Symphysodon haraldi*	*Symphysodon haraldi*	Itaparaná	Purus	DQ198136
1C9	*Symphysodon haraldi*	*Symphysodon haraldi*	Itaparaná	Purus	DQ198137
82	*Symphysodon discus*	*"Symphysodon discus"*	Rio Abacaxís	Abacaxís	DQ198112
83	*Symphysodon discus*	*"Symphysodon discus"*	Rio Abacaxís	Abacaxís	DQ198113
84	*Symphysodon discus*	*"Symphysodon discus"*	Rio Abacaxís	Abacaxís	DQ198114
85	*Symphysodon discus*	*"Symphysodon discus"*	Rio Abacaxís	Abacaxís	DQ198115
86	*Symphysodon discus*	*"Symphysodon discus"*	Rio Abacaxís	Abacaxís	DQ198116
87	*Symphysodon discus*	*"Symphysodon discus"*	Rio Marimari	Abacaxís	DQ198138
88	*S. discus x S. haraldi*	*"Symphysodon discus"*	Paraná do Rio Marimari	Abacaxís	DQ198119
89	*S. discus x S. haraldi*	*"Symphysodon discus"*	Paraná do Rio Marimari	Abacaxís	DQ198120
90	*S. discus x S. haraldi*	*"Symphysodon discus"*	paraná do Rio Marimari	Abacaxís	DQ198121
91	*Symphysodon aequifasciatus*	*Symphysodon aequifasciatus*	Rio Tefé	Tefé	DQ198096
92	*Symphysodon aequifasciatus*	*Symphysodon aequifasciatus*	Rio Tefé	Tefé	DQ198097
93	*Symphysodon aequifasciatus*	*Symphysodon aequifasciatus*	Rio Tefé	Tefé	DQ198098
94	*Symphysodon aequifasciatus*	*Symphysodon aequifasciatus*	Rio Urucu	Coari	DQ198099
95	*Symphysodon aequifasciatus*	*Symphysodon aequifasciatus*	Lago Tefé	Tefé	DQ198100
96	*Symphysodon aequifasciatus*	*Symphysodon aequifasciatus*	Lago Tefé	Tefé	DQ198101
97	*Symphysodon aequifasciatus*	*Symphysodon aequifasciatus*	Rio Mineruá	Solimões	DQ198102
98	*Symphysodon aequifasciatus*	*Symphysodon aequifasciatus*	Rio Mineruá	Solimões	DQ198103
99	*Symphysodon aequifasciatus*	*Symphysodon aequifasciatus*	Rio Mineruá	Solimões	DQ198104
100	*Symphysodon haraldi*	*Symphysodon haraldi*	Lago Jari	Purus	DQ198139
101	*Symphysodon haraldi*	*Symphysodon haraldi*	Lago Jari	Purus	DQ198140
102	*Symphysodon haraldi*	*Symphysodon haraldi*	Rio Jari	Purus	DQ198141
1	*Uaru fernandezyepezi*	No revision	Bred in Captivity	-	DQ198142
2	*Uaru fernandezyepezi*	No revision	Bred in Captivity	-	DQ198143
3	*Mesonauta festivus*	No revision	Bred in Captivity	-	DQ198091
4	*Mesonauta festivus*	No revision	Bred in Captivity	-	DQ198092
6	*Mesonauta festivus*	No revision	Bred in Captivity	-	DQ198093

Table II. Sequence alignment. Variable positions in the sequence alignment of 513 basepairs from the mitochondrial control region (Dloop), as required to reconstruct haplotype networks (Fig. 19). Isolate: Sample ID. Dots represent basepairs identical to the ones in the upper most sequence. Slashes represent missing basepairs.

Isolate																																						
1A1	A	C	A	T	C	C	–	C	T	T	A	T	C	C	T	A	T	A	–	G	T	T	C	A	G	A	G	A	C	C	C	A	C	T	T	T	T	T
1A2	G	A	.	.	T	.	–	.	.	.	.	.	.	.	.	G	.	.	–	A	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.
1A3	.	A	.	C	T	.	T	A	A	.	C	C	.	T	C	.	C	.	-	.	C	C	T	.	A	.	-	.	T	.	.	.	.	.	C	.	.	.
1A4	.	A	.	C	T	.	T	A	A	.	C	C	.	T	C	.	C	.	-	.	C	C	T	.	A	.	-	.	T	.	.	.	.	.	.	.	.	C
1A5	.	A	.	.	T	.	-	.	.	C	.	.	.	T	.	.	.	.	-	.	C	.	T	.	A	.	A	.	T	T	.	.	T	C	.	.	C	.
1A6	.	A	.	.	T	.	-	.	.	C	.	.	.	T	.	.	.	.	-	.	C	.	T	.	A	.	A	.	T	T	.	.	T	C	.	.	C	.
1A7	.	A	.	C	T	.	T	A	A	.	C	C	.	T	C	.	C	.	-	.	C	C	T	.	A	.	-	.	T	.	.	.	.	.	C	.	.	.
1A8	.	A	.	C	T	.	T	A	A	.	C	C	.	T	.	.	C	.	-	.	C	C	T	.	A	.	-	.	T	.	T	.	.	.	C	.	.	.
1A9	.	A	.	.	T	.	-	.	.	C	.	.	.	T	.	.	.	.	-	.	C	.	T	.	A	.	A	G	T	T	.	.	T	C	.	.	C	.
1B1	.	A	.	.	T	.	-	.	.	.	.	.	.	T	.	.	.	.	-	.	C	.	T	.	A	.	A	.	T	T	.	.	T	C	.	.	C	.
1B2	.	A	.	.	T	.	-	.	.	C	.	.	.	T	.	.	.	.	-	.	C	.	T	.	A	.	A	.	T	T	.	.	T	C	.	.	C	.
1B3	.	A	.	.	T	.	-	.	.	C	.	.	.	T	.	.	.	.	-	.	C	.	T	.	A	.	A	.	T	T	.	.	T	C	.	.	C	.
1B4	.	A	.	.	T	T	-	.	.	C	.	.	.	T	.	.	.	.	-	.	C	.	T	.	A	.	A	.	T	T	.	G	T	C	C	.	C	.
1B5	.	A	.	C	T	.	T	A	A	.	C	C	.	.	C	.	C	.	-	.	C	C	T	.	A	.	-	.	T	.	.	.	.	.	C	.	C	.
1B6	.	A	.	.	T	.	-	.	.	C	.	.	.	T	C	.	.	.	-	.	C	.	T	.	A	.	A	.	T	T	.	G	T	C	C	.	C	.
1B7	.	A	.	.	T	.	-	.	.	C	.	.	.	T	.	.	.	.	-	A	C	.	T	.	A	.	A	.	T	T	.	.	T	C	C	.	C	.
1B8	.	A	.	.	T	.	-	.	.	C	.	.	.	T	.	.	.	.	-	.	C	.	T	.	A	.	A	.	T	T	.	.	T	C	.	.	C	.
1B9	.	A	.	.	T	.	-	.	.	C	.	.	.	T	C	.	.	.	-	.	C	.	T	.	A	.	A	.	T	T	.	G	T	C	C	.	C	.
1C1	.	A	G	.	T	.	-	.	.	C	.	.	.	T	C	G	.	.	-	.	C	.	T	.	A	.	A	.	T	T	.	G	T	C	C	.	C	.
1C2	.	A	.	.	T	.	-	.	.	C	.	.	.	T	.	.	.	.	-	.	C	.	T	.	A	.	A	.	T	T	.	.	T	C	.	.	C	.
1C3	.	A	.	.	T	.	-	.	.	C	.	.	.	T	.	.	.	.	-	.	C	.	T	.	A	.	A	.	T	T	.	.	T	C	.	.	C	.
1C4	.	A	.	.	T	.	-	.	.	C	.	.	T	T	.	.	.	.	-	.	C	.	T	.	A	.	A	.	T	T	.	G	T	C	C	.	.	.
1C5	.	A	.	.	T	.	-	.	.	C	.	.	T	T	.	.	.	.	-	.	C	.	T	.	A	.	A	.	T	T	.	G	T	C	C	.	.	.
1C6	.	A	.	.	T	.	-	.	.	C	.	.	.	T	.	.	.	.	-	.	C	.	T	.	A	.	A	.	T	T	.	G	T	C	C	.	C	.
1C7	.	A	.	C	T	.	T	A	A	.	C	C	.	T	C	.	C	.	-	.	C	C	T	.	A	.	-	.	T	.	.	.	.	.	C	C	.	.
1C8	.	A	.	C	T	.	T	A	A	.	C	C	.	T	C	.	C	.	-	.	C	C	T	.	A	.	-	.	T	.	.	.	.	.	C	.	.	.
1C9	.	A	.	C	T	.	T	A	A	.	C	C	.	T	C	.	C	.	-	.	C	C	T	.	A	.	-	.	T	.	.	.	.	.	C	.	.	.
82	.	A	.	.	T	.	-	.	.	C	.	.	.	T	.	.	.	.	-	.	C	.	T	.	A	.	A	.	T	T	.	.	T	C	.	.	C	.
83	.	A	.	.	T	.	-	.	.	C	.	.	.	T	.	.	.	.	-	.	C	.	T	.	A	.	A	.	T	T	.	.	T	C	.	.	C	.
84	.	A	.	.	T	.	-	.	.	C	.	.	.	T	.	.	.	.	-	.	C	.	T	.	A	.	A	.	T	T	.	.	T	C	.	.	C	.
85	.	A	.	.	T	.	-	.	.	C	.	.	.	T	.	.	.	.	-	.	C	.	T	.	A	.	A	.	T	T	.	.	T	C	.	.	C	.
86	.	A	.	.	T	.	-	.	.	C	.	.	.	T	.	.	.	.	-	.	C	.	T	.	A	.	A	.	T	T	.	.	T	C	.	.	C	.
87	.	A	.	.	T	.	-	.	.	C	.	.	.	.	.	.	.	.	-	.	C	.	T	.	A	.	A	.	T	T	.	.	T	C	.	.	C	.
88	.	A	.	.	T	.	-	.	.	C	.	.	.	T	.	.	.	.	-	.	C	.	T	.	A	.	A	.	T	T	.	.	T	C	.	.	C	.
89	.	A	.	.	T	.	-	.	.	C	.	.	.	T	.	.	.	.	-	.	C	.	T	.	A	.	A	.	T	T	.	.	T	C	.	.	C	.
90	.	A	.	.	T	.	-	.	.	C	.	.	.	T	C	.	.	.	-	.	C	.	T	.	A	.	A	.	T	T	.	G	T	C	C	.	C	.
91	.	A	.	.	T	.	-	.	.	.	.	.	.	.	.	.	.	.	-	A	.	.	.	G	A	.	.	.	.	.	.	.	.	C	.	.	.	.
92	G	A	.	.	T	.	-	.	.	.	.	.	.	.	.	.	.	.	-	A	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.
93	.	A	.	.	T	.	-	.	.	.	.	.	.	.	.	.	.	.	-	A	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.
94	G	A	.	.	T	.	-	.	.	.	.	.	.	.	.	.	.	.	-	A	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.
95	.	A	.	.	T	.	-	.	.	.	.	.	.	.	.	.	.	.	-	A	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.
96	.	A	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
97	.	A	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
98	.	A	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
99	.	A	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
100	.	A	.	C	T	.	T	A	A	.	C	C	.	T	C	.	C	-	G	.	C	C	T	.	A	.	-	.	T	.	.	.	.	.	C	.	.	.
101	.	A	.	C	T	.	T	A	A	.	C	C	.	T	C	.	C	-	G	.	C	C	T	.	A	.	-	.	T	.	.	.	.	.	C	.	.	.
102	.	A	.	C	T	.	T	A	A	.	C	C	.	T	C	.	C	.	G	.	C	C	T	.	A	C	-	.	T	.	.	.	.	.	C	.	.	.

Table III. Water parameters *Symphysodon discus* (sensu stricto). Tributary: Major watersheds. Biotope: Sample locality. Date: Sampling date. Watertype: major type of river, with 'black'= dark humic-acid stained acidic rivers, 'white'=grayish-white sediment rich rivers; 'clear' = unstained clear rivers. Temp.: temperature in degrees Celsius. Oxy.: Oxygen content in mg/l. Cond.: Conductivity in microsiemens.

Tributary	Biotope	Date	Watertype	pH	Temp.	Oxy.	Cond. μS/cm
Rio Negro	Parana do Rocky	31.01.04	black	4.73	31.7	2.14	9
Abacaxís	Rio Marimari	05.02.04	black	4.85	29.3	3.14	6
Abacaxís	Rio Marimari	05.02.04	black	4.81	29.3	3.09	6
Abacaxís	Rio Abacaxís	05.02.04	black	4.9	28.9	3.4	8
Rio Negro	Rio Ariau	01.02.04	black	4.03	29.8	4.56	9
Amazonas	Rio Urubu	21.01.04	black	4.67	29	4.83	12
Rio Jatapu	Lago Iri	23.01.04	black/clear	5.03	29.8	4.56	9
Nhamundá	Rio Nhamundá	06.12.98	black	4.23	27.5	nk	11
Rio Negro	Anavalinhas	24.09.86	black	4.3	nk	nk	11
Rio Negro	São Gabriel	17.10.86	black	4.42	27.1	7.24	15
Rio Negro	Rio Cuiuni	08.04.82	black	4.3	26.4	3.7	12.5
Rio Negro	Rio Curicuriari	15.04.82	black	4.9	26.5	4.6	8.4
Rio Negro	Rio Xeruiní	20.10.71	black	4.4	27.9	nk	11.8
Rio Negro	Rio Aracá	11.11.83	nk	5.125	nk	3.4	
Rio Negro	Manaus	12.10.72	black	4.7	27.7	nk	8.2
Rio Negro	Rio Jufarís	20.11.67	black	5.126	nk	5.4	
Rio Negro	Rio Padaueri	27.11.67	black	4.927	nk	6.8	
Rio Negro	Bay 20 km N/M	1967/68	black	5.04	nk	nk	8.7
Rio Negro	Mouth Tarumã	1975/76	black	4.46	nk	nk	12.1

Table IV. Water parameters *Symphysodon aequifasciatus*. Tributary: Major watersheds. Biotope: Sample locality. Date: Sampling date. Watertype: major type of river, with 'black'= dark humic-acid stained acidic rivers, 'white'=grayish-white sediment rich rivers; 'clear' = unstained clear rivers. Temp.: temperature in degrees Celsius. Oxy.: Oxygen content in mg/l. Cond.: Conductivity in microsiemens.

Tributary	Biotope	Date	Watertype	pH	Temp.	Oxy.	Cond. μS/cm
Solimões	Rio Amaturá	25.01.04	black	5.3	28.3	2.4	8
Solimões	Rio Jutaí	26.01.04	black	5.02	28.1	0.88	9
Solimões	Paraná Jutaí	26.01.04	black	5.05	27.7	0.92	8
Solimões	Tocantins	26.01.04	black	4.83	27.4	0.56	7
Urucu/Coari	Ig. Serrado	07.02.04	black	5.23	28.4	1.48	11
Urucu/Coari	Ig. Açú	07.02.04	black	5.22	27	1.28	15
Coari	Rio Urucu	07.02.04	black	5.27	29.3	1	9
Tefé	Rio Tefé	06.08.97	black	4.89	29.1	nk	nk
Tefé	Lago Tefé N	10.08.97	black/clear	5.731	nk	6	
Tefé	Lago Tefé S	07.11.85	black	5.9	29	7.47	15
Tefé	Rio Tefé	07.11.85	black	5.4531	nk	15	
Juruá	Lago Juruá	30.07.97	black	5.3	28.5	nk	nk
Iquitos/Peru	Rio Nanay	01.11.87	black	5.26	nk	2.8	6

Table V. Water parameters *Symphysodon haraldi*. Tributary: Major watersheds. Biotope: Sample locality. Date: Sampling date. Watertype: major type of river, with 'black'= dark humic-acid stained acidic rivers, 'white'=grayish-white sediment rich rivers; 'clear' = unstained clear rivers. Temp.: temperature in degrees Celsius. Oxy.: Oxygen content in mg/l. Cond.: Conductivity in microsiemens.

Tributary	Biotope	Date	Watertype	pH	Temp.	Oxy.	Cond. µS/cm
Manaus	Rio Uatumã	22.01.04	clear	6.31	31.8	6.9	8
Manaus	Lago Jaquarequara	22.01.04	clear	6.86	33.6	6.86	7
Tabatinga	Ig. Tacaná	25.01.04	black/clear	6.3	27.2	0.24	62
Tabatinga	Ig. Belém	25.01.04	black/clear	5.84	26.7	0.3	20
Tabatinga	Rio Jandiatuba	25.01.04	clear	5.2	27.3	2.6	9
Solimões	Rio Içá/ Ig. Betania	27.01.04	clear	5.84	28.6	3.81	14
Solimões	Rio Içá/ Bo. Jacupara	27.01.04	black/clear	5.89	28.1	0.24	27
Solimões	Rio Camatia	27.01.04	black	6.48	27.9	nk	73
Manacapuru	Macumeri	02.02.04	clear/white	6.49	29.8	4.33	80
Manacapuru	Doema	02.02.04	clear/white	6.75	30.2	4.54	77
Canumã	Ig. S. Domingo	05.02.04	black/clear	5.4	29.8	3.07	8
Canumã	Rio Canumã	05.02.04	black/clear	5.34	29.3	3.39	7
Solimões	Rio Coari	07.02.04	black/clear	5.4	27.6	1.71	11
Solimões	Ig. Gibian/ Coari	07.02.04	black/clear	5.68	25.1	1.33	18
Solimões	Ig. Gibian/ Coari	07.02.04	black/clear	5.32	27.6	1.62	10
Solimões	Rio Coari Grande	07.02.04	black/clear	5.18	28.4	3.1	9
Amazonas	Lo. Maximo/Parentins	09.02.04	clear	6.59	29.4	2.09	31
Amazonas	Ig. Maximo /Parentins	09.02.04	clear	6.11	29.5	1.51	59
Amazonas	Caburí	11.02.04	clear	6.36	30	2.58	26
Nhamundá	Lago Nhamundá/Tigre	11.02.04	clear	5.75	28.6	2.72	17
Trombetas	Lago Batata	12.02.04	clear	5.95	30.5	1.17	14
Trombetas	Água Fria	12.02.04	black/clear	5.8129	nk	20	
Trombetas	Lago Acarí	12.02.04	black/clear	5.7	30	0.87	12
Trombetas	Lago Curupira/Trombetas	12.02.04	black/clear	5.9	30.1	0.6	23
Trombetas	Rio Trombetas	12.02.04	black/clear	6.36	30	2.58	30
Alenquer	Rio Curuá	14.02.04	clear	6.39	31.4	nk	25
Alenquer	Rio Curuá/ Barra Mansa	15.02.04	clear	6.81	30.7	nk	26
Alenquer	Rio Curuá/ Pacoval	15.02.04	clear	6.37	30.3	nk	26
Alenquer	Lago Grande	16.02.04	clear/white	6.57	26.4	nk	22
Santarem	Rio Tapajós	16.02.04	black/clear	6.38	29.5	nk	17
Santarem	Ig. Tapajós	16.02.04	clear	4.7826	nk	14	
Amazonas	Silves	12.10.86	clear	6.4	31.5	nk	16
Rio Branco	Lago Canaraçí	06.10.86	clear	6.3	30.3	nk	23
Manacapuru	Lago Manacapuru	03.10.86	clear	6.7629	nk	40	
Purus	Lago Beruri	03.10.86	black/clear	7.67	31.3	nk	63
Laeticia	Lago Tarapoto	13.03.87	clear	6.72	nk	3	116
Maues	Rio Maués	12.10.86	clear	6.2	32	6.9	17
Tocantins	Rio Tocantins	25.06.86	clear	6.6	–	nk	40
Tocantins	Ig. Cametá	25.06.86	black/clear	5.78	nk	nk	20
Curua-Una	Curua-Una	xx.07.76	black/clear	5.5	28.4	5.8	25
Tocantins	Rio Paruru	07.10.99	clear	5.98	27.9	nk	24
Tocantins	Rio Tocantins	07.10.99	black/clear	6.55	26.9	nk	43
Breves	Ig. Grande	xx.09.99	black/clear	6.3	27.6	nk	nk
Portel	Rio Taquanaquara	xx.07.02	clear	5.98	27.9	nk	65
Xingú	Victoria do Xingú	24.07.02	clear	6.55	27.9	nk	21
Purus	Lago26/Itaparaná	05.08.02	black/clear	5.828	nk	10	
Purus	Lago Solitario	08.08.02	black/clear	6.2	28.5	nk	48
Purus	Rio Itaparaná	04.08.02	black/clear	6.0728	nk	10	

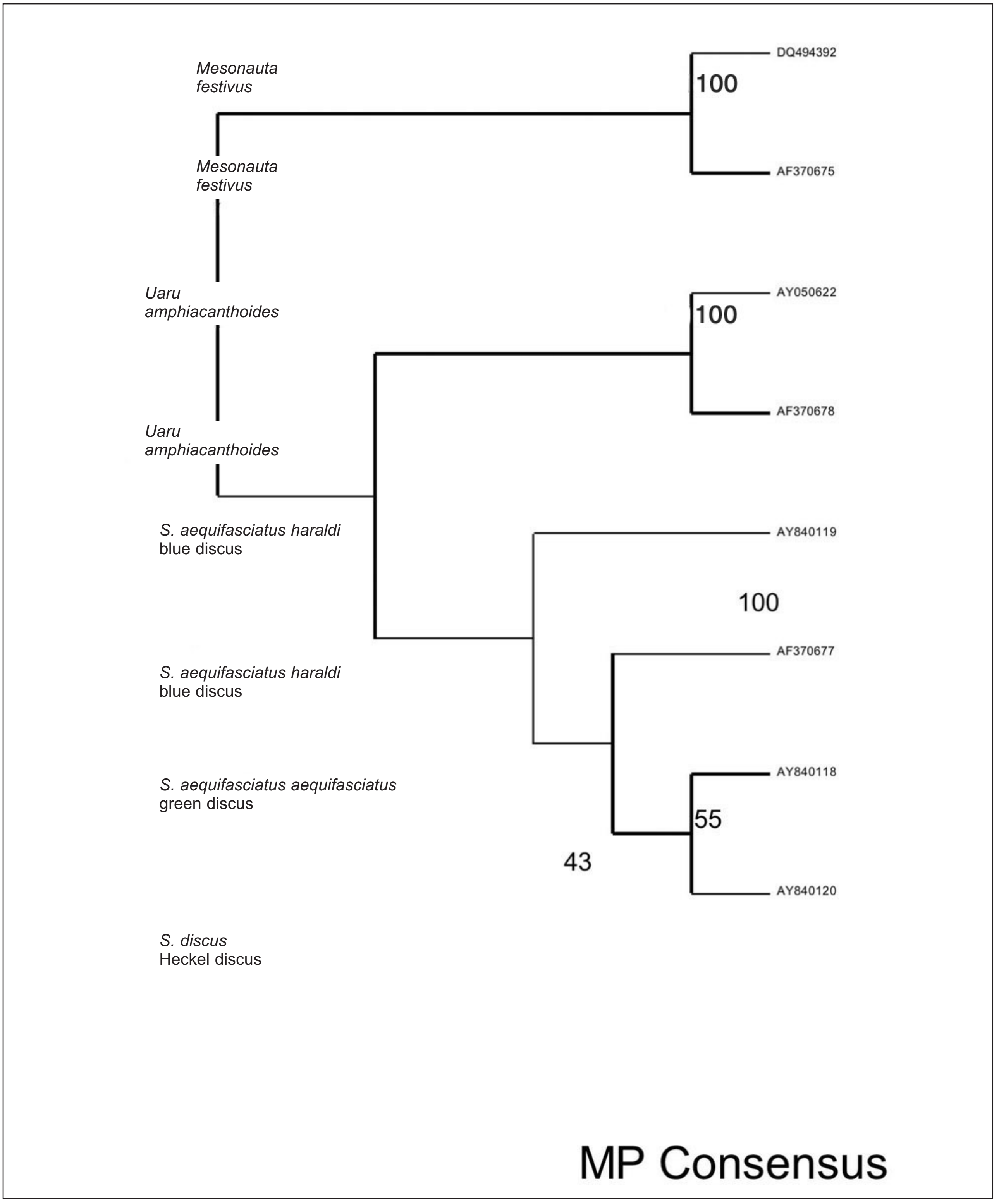
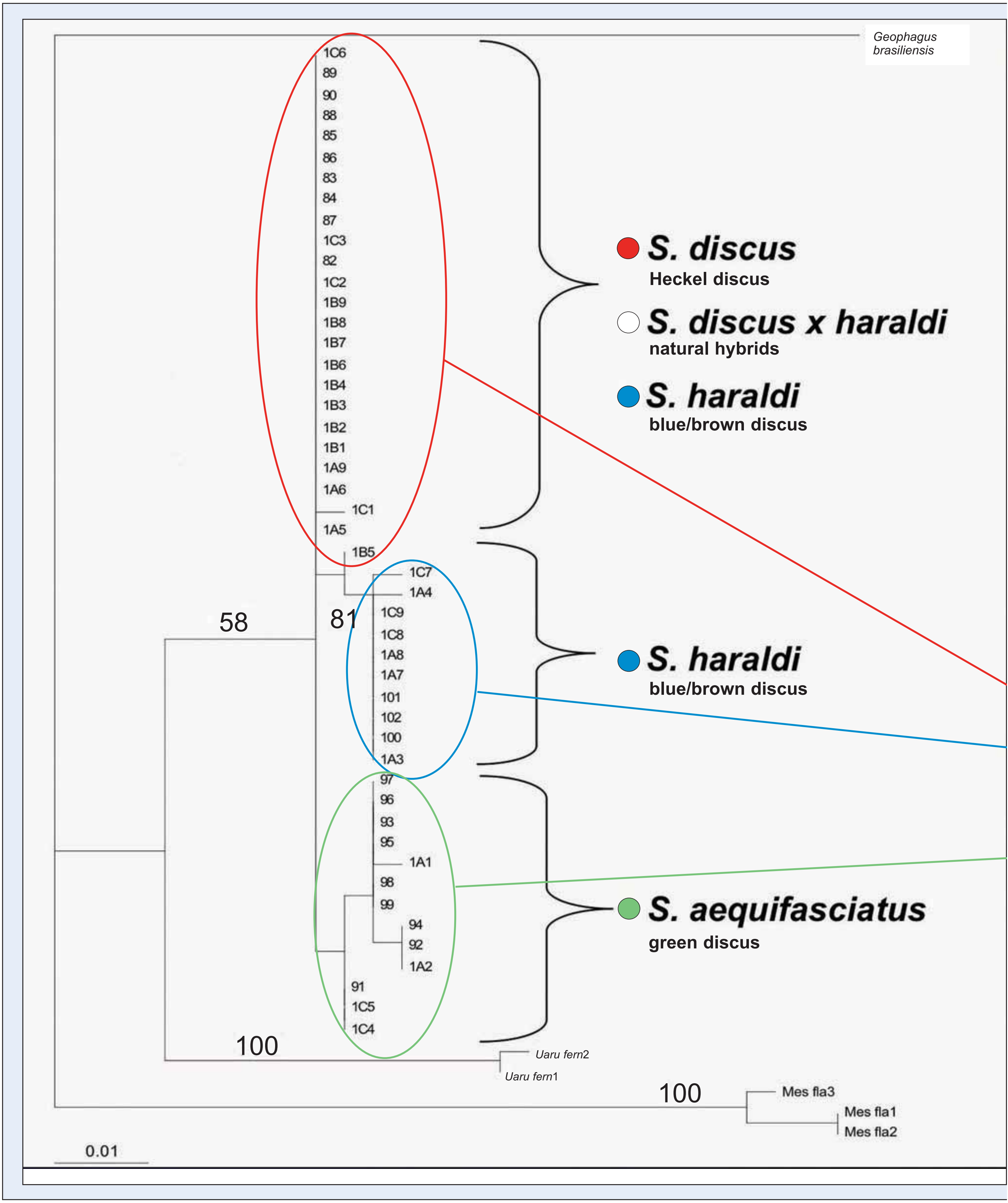


Fig. 25. This figure shows a phylogenetic analysis based on the somewhat more slowly evolving cytochrome b gene of *Mesonauta festivus*, *Mesonauta insignis*, *Uaru amphiacanthoides* and different GenBank entries for *Symphysodon*. Shown are species names and the corresponding GenBank accession numbers. The same phylogenetic pattern emerges as with our new D-loop data (see legend of Table II).


Geophagus brasiliensis
1C6
89
90
88
85
86
83
84
87
1C3
82
1C2
1B9
1B8
1B7
1B6
1B4
1B3
1B2
1B1
1A9
1A6
1C1
1A5
1B5
1C7
1A4
58
81
1C9
1C8
1A8
1A7
101
102
100
1A3
97
96
93
95
1A1
98
99
94
92
1A2
91
1C5
1C4
100
Uaru fern2
Uaru fern1
100
Mes fla3
Mes fla1
Mes fla2
0.01
S. discus
Heckel discus
S. discus x haraldi
natural hybrids
S. haraldi
blue/brown discus
S. haraldi
blue/brown discus
S. aequifasciatus
green discus

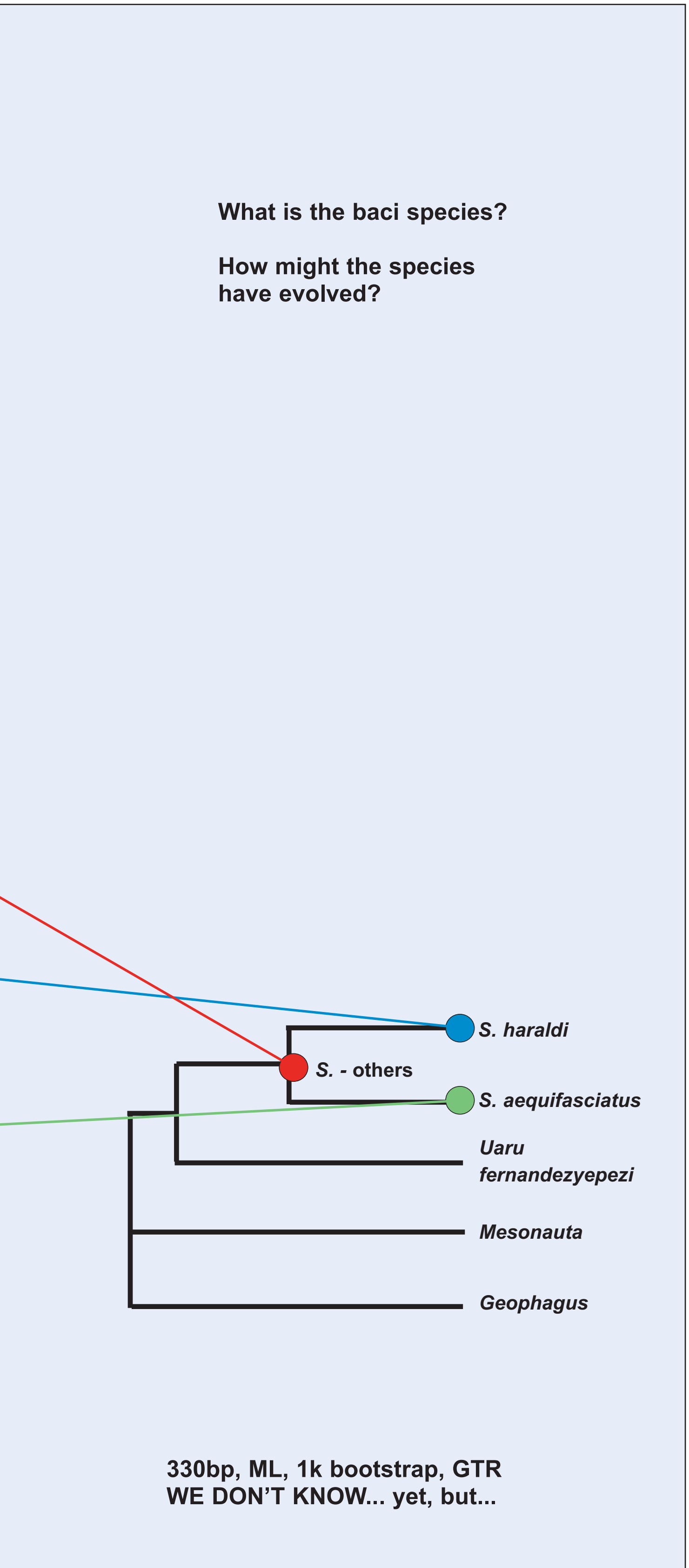


Fig. 26. Maximum likelihood analysis (based on 330bp of D-loop sequences with 1000 bootstrap replicates) with new *Mesonauta*, *Geophagus*, *Uaru* and all *Symphysodon* sequences. The same pattern as in Table II emerges.

tern was observed by one of us (HB) in more than 5,000 specimens from the species' entire range of distribution, including the specimens used for molecular samples. Only in Rio Unini one of us (HB) found specimens, which had only 13 to 14 horizontal stripes, while others with 23 stripes occurred in the same habitat. These are the lowest and highest numbers ever recorded (Bleher 2006: 167, figs 5, 8). Heckel (1840) gave 18 stripes for the holotype. Those colour patterns and the smaller size (the largest specimen on record has a SL of 123 mm) distinguish *S. discus* from its congeners. Additionally, the colour and chemical parameters of the waters they inhabit are different. *Symphysodon discus* does hybridise with *S. haraldi* in nature and in aquaria, while no aquarium hybrids with *S. aequifasciata* have ever been recorded. None of the aquarium hybrids is fertile after the F_3 generation. The base colour of *S. discus* may vary from grey, to light or dark brown, reddish and occasionally red (i.e. we found some specimens with a red base colour in the Rio Negro, see Bleher 2006: 379, lower figure; Abacaxís, Bleher 2006: 175, figs 8-10; Marimari, Bleher 2006: 177, fig. 7; Jatapu, Bleher: 170, fig. 2; Igarapé Tukano and Rio Nhamundá, Bleher: 378 top figure and 172, fig. 5 respectively). *Symphysodon discus* rarely has a yellowish, yellow, orange or violet base colour. The stripes of *S. discus* are almost always and in all varieties light blue, rarely intense blue or grey, but may turn grey when the fish is stressed.

Colour in alcohol: Preserved *S. discus* show the three intense bars while the horizontal stripes are hardly visible (including the holotype).

Distribution (Fig. 31): *Symphysodon discus* is limited to habitats of black waters with extremely low conductivity: in the middle and lower Rio Negro, well below São Gabriel de Cachoeira, and its black water tributaries, where it occurs mainly in their igapós and lagos. The north-western limit of distribution is in the Rio Uneuxí Region, while in the south it extends to the vicinity of Novo Airão. It is no longer found in the Rio Negro mouth area, nor in the Tarumã River near Manaus, due to tremendous human-induced environmental impacts. In the black water region of the middle Rio Urubu they may have become extinct, due to continuous deforestation, cattle farming and agricultural impact. They occur in Rio Jatapu (Uatumã Region), the lower Rio Nhamundá (well below the first rapids). The only *S. discus* known from a black water region to the south of the Amazon occur in the middle and lower Rio Abacaxís and the lower Rio Marimari (also called Mari-Mari) drainage, were they find almost the same habitats and chemical water composition as in the other distribution areas. This is certainly the reason why they have survived south of the Amazon, as hardly anywhere else in the southern part are water conditions that extreme.

The occurrence in Rio Trombetas is doubtful, as the specimens in MZUSP (44141-3) from Lago Jacaré (also called Lago do Jacaré) on the left bank of the lower Rio Trombetas and the specimen in USNM (224865) from the Lago Batata on the right bank of the lower Rio Trombetas (both areas are well below the Cachoeira Porteira) do not belong to *S. discus*, but are *S. haraldi*. The preserved specimens from Lago Jacaré do not have an intense bar (Fig. 16). During 10 field trips, between 1965 and 2004, the first author (HB) never found this species in the Trombetas Region, nor did any of the local commercial discus fishermen. Only *S. haraldi* occurred there.

Natural hybrids: Hybridization occasionally occurs where *S. discus* overlaps in distribution with its congeners, either at times of extremely high water and/or in areas of river confluence, such as those of Rio Nhamundá with Lago Nhamundá (Lago de Faro of some authors; Bleher 2006: 173, fig. 3). Hybrids may be found where Rio Canumã floods and connects with Rio Marimari and/or Paraná Urariá (Bleher 2006: 177, figs 8-10) and at the confluence of Rio Jatapu with Rio Uatumã. None of the wild collected natural hybrids was fertile.

Symphysodon aequifasciatus Pellegrin, 1904

Symphysodon discus var. *aequifasciata* Pellegrin, 1904 in part, syntype MNHN 1902-130, from Santarém (Fig. 5).

Symphysodon discus var. *aequifasciata* Pellegrin, 1904. *Mém. Soc. zool. Fr.* 16: 213-215, Teffé Brésil; Santarém Brésil; syntypes: MNHN 1902-134 (1) (Fig. 3) and MNHN 1902-135 (1) (Fig. 4) Teffé; type locality: Lago Tefé, Brazil; lectotype designated by Géry & Bleher in Bleher 2004: MNHN 1902-135; 90.6 mm SL, Lago Tefé, Brazil (Fig. 4); paralectotype: MNHN 1902-134; 122.5 mm SL, Lago Tefé, Brazil (Fig. 3).

Common names: Harald Schultz was the first to name this species from the western Amazon Region, the Lago Tefé area, stating “...my suggestion would be to call it the ‘Green discus’, because green is its most intense color” (Schultz 1959); and L. P. Schultz, in his 1960 review, followed H. Schultz calling it the “Green Discus from Lago Teffé”. The illustrations in both publications show the same red spotted specimens as *S. tarzoo* of Ready et al. (2006).

Description: Meristic counts and morphometric mesurements of *S. aequifasciatus* overlap with those of the other two species: Schultz (1960) gave 50 to 61 vertical scale rows while Kullander (1986) found 54 to 60. We have to bear in mind that L. P. Schultz (1960) examined numerous specimens from dubious and/or unknown locations (except for the *S. a. aequifasciata* specimens from Lago Tefé). The majority of the material reviewed by him came from an exporter in Manaus (Kullander 1996, Bleher 2006). Kullander (1986) worked in part with specimens from ornamental fish exporters in Iquitos and part of the material he examined in his 1996 study originated from unknown collecting stations (many museum specimens of *Symphysodon* have been deposited with incorrect data or no collecting data at all; see Bleher 2004, 2006). Studies by these authors and the one by Ready et al. (2006) leave uncertainty or doubt about the correct locality data of part of the specimens they examined, especially their *S. tarzoo* (green) specimens from the Rio Madeira. In addition, Kullander (1996) stated: “Die einzigen bekannten Merkmale, um *S. discus* sicher von *S. aequifasciatus* zu unterscheiden, bietet das Zeichenmuster.” Besides its distinct colour pattern, the genetic results show that *S. aequifasciatus* forms a monophyletic assemblage.

Colour in vivo: Pellegrin (1904) already noted that *S. aequifasciatus* has nine almost uniformly intense dark bars: “...les 9 barres transversales sont minces et égales entre elles...”, and all specimens we have examined exhibit precisely this pattern. Over 10,000 specimens have been checked over the last 40 years by one of us (HB), and no variation to this pattern was found. The excellent drawing by Burkhardt (1865) of a specimen from Tefé confirms this pattern (Fig. 6). Pellegrin also noted “...il n’y a pas de lignes longitudinales parallèles”, and that distinguishes this species from its congeners: *S. aequifasciatus* does never show horizontal stripes over its entire body.

It has very few, mostly wavy stripes (up to six) dorsally in the head region and upper front body portion, very rarely extending beyond the fifth bar. The most prominent colour pattern is the red spots of one to three millimetres in diameter. They are always present in the anal-fin region, which may, in rare cases, include short red dotted lines (Fig. 20, no. 93). Adult individuals always have these red dots, sometimes only a few, but other times (often in alpha individuals) they are spread all over the body (see cover photo of this issue; Bleher 2006: 183 and 184, figs. 8 and 4 respectively). To date, this pattern has not been found in any other species. Pellegrin (1904) in the original description did not note these red dots, even though they are still visible in the larger of the two specimens from Teffe (Fig. 3b). He noted: "La coloration de ces spécimens peut-être ramenée a 2 types." He was aware that among the three specimens there were possibly two varieties. The name, *aequifasciata*, comes from "aequus", a Latin adjective meaning "equal", and "fasciatus" meaning "band", "bar" or "belt", referred to the series of equal bars on the body of the species, which are found in all individuals, but never in any specimen of *S. haraldi*, nor in Pellegrin's syntype from Santarém (MNHN 1902-130). *Symphysodon aequifasciatus* frequently has a light yellowish, yellow and sometimes even orange base colour (Bleher 2006: 184, fig. 3), while the base colour is rarely grey, brown or violet (Bleher 2006: 188, fig. 4). There is always a green, emerald green, turquoise or bluish area along the entire anal-fin region, with red dots and in rare cases red dotted lines.

Colour in alcohol: The nine bars on the flanks are normally visible in preserved specimens, and all alcohol-preserved adult individuals show red (or rust coloured) dots. If not in the body, those dots can always be seen in the anal-fin region (Fig. 3).

Distribution: (Fig. 31) Like the previous species, *S. aequifasciatus* has a limited distribution, but differs in that its area of occurrence is almost continuous, rather than fragmented. Its most easterly distribution begins in the Coari Region, in Rio Urucu and extends south of the Solimões to Rio Jandiatuba, where it ends abruptly at the last black water (lagos and igapós). Also north of the white water Rio Solimões its distribution encompasses black water regions only and it is found south of Rio Japurá in the lake region of its mouth, in the Reserva de Mamirauá, north of the Solimões in Rio Tonantins, all the way north-west in lagos east of Rio Içá, in the region of the lower Rio Putumayo, its southern tributaries and lagos. The record by Ready et al. (2006) from Rio Içá cannot be confirmed. It was not found during several field trips to the area not by one of us (HB), nor by the only other collector in the Rio Içá Region, T. Hongslo. Hongslo deposited his specimen from lagos near Cuiavá in the NRM (Kullander 1986: 419), but none of those are *S. aequifasciatus* (nor the *S. tarzoo* of Ready et al. 2006), as red dots are absent in life as well as in all preserved specimens. Also the photos taken on the spot by Hongslo (Fig. 27 a, b) show that all collected discus were brown. The specimens collected by HB (not deposited) are identical, only one was reddish and became know in the hobby trade as the "Red Içá" (Bleher 2006: 477). In other words: all discus collected from the Içá Region were *S. haraldi*. Hongslo was able to see *S. aequifasciatus* only in Leticia, probably collected in the Rio Amaturá or Putumayo Region (pers. comm.).

There are no further natural distribution areas known to date (2007), and *S. aequifasciatus* like *S. discus* are exclusively found in black water areas, except when the water rises and white water penetrates into its habitat. This was already noted by H. Schultz (1959). In Amazonia, not all black water habitats are identical and there is a difference between the chemical compositions of waters inhabited by either species (see Tables III-IV).

The occurrence in Rio Nanay was by incidence: more than 25 years ago some exporter has brought *S. aequifasciatus* from the Tefé area and introduced them in the black waters of the Nanay (Bustamante, pers. comm.) Some people claim they came from the Putumayo. In that river they breed very well, as its chemical water composition is almost identical to that of Lake Tefé.

Natural hybrids: Hybrids are unknown, but might occur in the area of the lower Rio Urucu at the confluence with Lago de Coari, were the Rio Grande Coari merges nearby. They might be found in the lake and in Rio Grande Coari and its tributaries, where *S. haraldi* has been recorded by one of us (HB). Natural hybridisation might also take place in the Rio Içá Region, along the upper and middle left banks of lagos and igapós, if those water bodies connect during floods with the habitat of *S. aequifasciatus* in the Rio Tonantins, although we were thus far unable to find any, nor did Hongslo record them (pers. comm.).

Symphysodon haraldi Schultz, 1960

Symphysodon aequifasciata haraldi Schultz, 1960. Tropical Fish Hobbyist 8 (10): 11 (Fig. 13); Benjamin Constant, Brazil in the Amazon; correct type locality: Lagoa Berurí, lower Purus Region (Géry & Bleher in Bleher 2004). Holotype: USNM 00179829.

Symphysodon Discus Tarzoo (sic) Lyons, 1960. Tropicals Magazine Holiday Issue, 1960 (for 1959): 6-10 (Fig. 11); Leticia, Colombia; correct collecting area: Rio Putumayo. No type known.

Symphysodon aequifasciata axelrodi Schultz, 1960. Tropical Fish Hobbyist 8 (10): 14, Belém, Brazil, Amazon River; correct type locality: lower Rio Urubu (Géry & Bleher in Bleher 2004). Holotype USNM 00179831 (Fig. 14).

Common names: The "Pompadour Fish" was the first one to become available in the ornamental fish hobby in the 1930s and was than called the "The Aristocrat of the Aquarium" (Innes 1933, 1934). Than many other names followed, such as "brown scalare", "pancake fish", "blue scalare", "buffalo fish", "the King" and many more. In the 1950s the first colour variants appeared and by the 1960s, after Schultz's review, the name "blue" discus became established world-wide, besides "brown" or "common discus". The names "blue" and "brown" discus are still used today for *S. haraldi*.

Description: Meristic counts and morphometric measurements for *S. haraldi* have been given by L. P. Schultz (1960), but in all characters an overlap with the other two species occurs (e.g. Kullander 1996, even though only two species, *S. discus* and *S. aequifasciatus*, were compared in that study). The only exception is possibly the lower vertical scale count, which differs between *S. discus* and *S. haraldi*. The fact remains, that also this species can only be distinguished externally by its colour pattern, and *S. haraldi* has the most varied colour pattern of the three discus species, being distinct from the two others. The species also differ in size, with *S. haraldi* ranging between 100 to 200 mm SL (L. P. Schultz' largest specimen had a SL of 139 mm). Larger specimens are not uncommon, the largest one found by one of us (HB) originated from Rio Arapiuns (Bleher 2006: 196, fig. 3) with a SL of 220 mm. Generally, *S. haraldi* is the largest of the three species.

Colours in vivo: *Symphysodon haraldi* shows the greatest range of colours and colour patterns, probably because of its wide range of distribution (Fig. 31) and its adaptation to mostly clear water habitats. The species may have eight (Figs 8, 9), nine, 10, or more bars, in some cases up to 15 or 16 (a feature never recorded in either of the two other species). The bars may be of equal width or some are very narrow (Fig. 21). One or more bars may be wider in their central part. Specimens with such a pattern can be found throughout the species' distributional range, even though they are rare (Bleher 2006: 213 and 310, figs 2 and 4 respectively). In some specimens only the fifth, sixth, seventh and ninth bars are widened centrally (Bleher 2006: 194, fig. 4), or the fifth to ninth bar (Fig. 21 no. 1C7). Some specimens have white bars or no visible bars at all (Bleher 2006: 192, figs 2, 3). Some specimens of *S. haraldi* have no stripe pattern, or only very few stripes, but stripes are always present on anal-fin rays. There is a series of stripes on the forehead region and/or on the lower ventral. Fractions of stripes may be dispersed over the entire body or half the body, a pattern which in the aquarium fish hobby is called "semi-royal blue" (Fig. 21, no. 100). Some specimens (mostly alpha animals) have stripes over their entire flanks (Bleher 2006: 69, figs 5-7), which aquarists call "royal-blue". In some rare cases they may even form strait horizontal lines (Bleher 2006: 205, fig. 12), but most *S. haraldi* have irregular or wavy stripes. In various locations some individuals may have only fragmented stripes and/or a pattern, which looks like series of pearls known as "pearl discus" in the ornamental fish hobby (Bleher 2006: 204, fig. 1). In other cases, a pattern of very fine stripes may be found (called "snake-skin" in the ornamental fish trade; Bleher 2006: 206, figs 1, 2) and in some specimens the stripes may even run vertically (Bleher 2006: 201, fig. 5). The base colour ranges from grey to yellowish, to pure yellow, but no individual of this species has the striking colour pattern of the other two species.

Individuals of *S. haraldi* of completely different appearance and origin readily interbreed, another reason why new variants are continuously being discovered, often in the same biotope. Interbreeding occurs in the tanks of commercial breeding establishments worldwide and even in aquaria of hobby breeders, with the offspring always being fertile. It is relatively easy to line-breed the most unusual colour forms of *S. haraldi*, with numbers of unusual offspring continuing to increase almost on a monthly basis. Crossing *S. haraldi* with *S. aequifasciatus* in captivity is extremely difficult and hybrids between *S. haraldi* and *S. discus* are always sterile (at least after the F_3 generation).

Colours in alcohol: Bars are visible, normally all of them being equally prominent, except for the ones through the eye and in the caudal peduncle, which are darker. Stripes present in live animals remain visible, if preserved immediately after collection in formaldehyde and than transferred to alcohol (Fig. 16). If specimens are preserved with delay, the stripes are likely to disappear (Fig. 12b, compare with Fig. 13, which is the same specimen before and after preservation). However, stripes in the anal-fin region (Fig. 5) always remain visible.

Notably, Pellegrin's specimen from Santarém still clearly shows its anal-fin stripes, even after more than 100 years in alcohol (Fig. 5b). Burkhardt's (1865) paintings of a specimen from Porto de Moz and the two specimens he painted at Teffé are another indication that Pellegrin had received two different species from Jobert.

Distribution: *Symphysodon haraldi* is generally restricted to clear water lakes and rivers and its "blue" and "brown" colour variants are found syntopically almost everywhere throughout their range of distribution. They also commonly interbreed. *Symphysodon haraldi* has the largest distribution area of the three species, which is almost continuous. It is found from the extreme east of the Amazon basin (in the Tocantins) to across the border with Colombia and Peru in the west, and to the north as far as the Apaporis. Throughout this area the various colour forms are mixed at random.

In the western Amazon, north of Rio Solimões, its distribution area ends north of Tabatinga, which is north of Calderón. In the Igarapé Tacaná, Igarapé do Belém and along the lower Rio Içá Region it occurs in lagos and igapós. Towards the east it is found in the northern Solimões Region from Lake Piorini all the way to Manacapuru. This area of distribution is interrupted by black water of Rio Negro and the Urubu. *Symphysodon haraldi* is then again found at the mouth of the Urubu, in Lago Saracá and Lago Canaraçí, all the way east to Lago Grande de Monte Alegre, where its distribution area reaches the northern Rio Amazon Region. It does not occur in the extremely acid waters of the Rio Jatapu and Rio Nhamundá Regions, where only *S. discus* is found. South of the Amazon, its easternmost distribution is in Rio Tocantins (and not in Belém, contrary to what is written on the labels of some museum specimens and elsewhere; no discus was ever found east of the Tocantins). *Symphysodon haraldi* is found in most parts of the southern Rio Amazonas and Rio Solimões, in clear water tributaries and lake regions, all the way to Rio Grande Coari in the west, but not in the white water rivers, such as Rio Purus or Rio Madeira (any such data in the literature is erroneous). Not even for a few hours would they survive in those sediment-loaded, turbid, white waters. *Symphysodon haraldi* is also absent from the extreme black waters of Rio Abacaxís and Marimari (which are confined to *S. discus).* It is absent from the Rio Negro system (Fig. 31), except for specimens collected by one of us (HB) in the Lago Cueru (also spelled Cureru), a clear water lake, which lies to the left of the Rio Branco mouth region.

In most of these locations brown specimens, intensely coloured ones and all intermediate stages occur syntopically. However, they have never been found together with either *S. aequifasciatus* or *S. discus.* Although *S. haraldi* lives almost exclusively in clear water zones, it tolerates mixed water to a greater extent than do the two other species. The Amazonian mixed waters occur mostly during the rainy season and have a substantially increased pH values (up to neutral or even above pH 7) and increased conductivity (30 to 70 μS/cm, and higher). This is quite different from the water parameters of habitats where *S. discus* and *S. aequifasciatus* occur (Tables III, IV).

DISCUSSION

1. Molecular studies: Mitochondrial data suggest the existence of three clearly distinct clusters of individuals, which are not entirely in agreement with the traditional taxonomic view of the species of *Symphysodon*. While *S. aequifasciatus* is recovered as a genetically clearly distinct lineage, separated by at least nine mutations from the next clade, *S. haraldi* does not form a monophyletic group. Some phenotypic *S. haraldi* samples seem to group genetically with or near the phenotypic *S. discus* specimens. Also specimens, which are considered to be naturally occurring hybrids between *S. discus* and *S. haraldi* group in this genetic *discus-haraldi* cluster (Fig. 22). However, a distinct cluster of *S. haraldi* samples remains, separated by at least 12 mutations from the possibly interbreeding *S. discus* and *S. haraldi*. The *haraldi* cluster occurs only upstream of the conflux of Rio Negro and Rio Solimões. Downstream of this conflux these fish seem to hybridise, and we can only hypothesise that this might be due to the mixing of

the waters at extremely high waters (floods), which would then become a contact zone for individuals of the two species.

If one wanted to assign names to the molecular clusters discovered they could be chosen such that *Symphysodon aequifasciatus* should unambiguously be kept at its present taxonomical status, while *Symphysodon haraldi* should be assigned only to samples from the Purus (lagos Berurí, Jari, rios Itaparaná, Tapauá, etc.) and the Manacapuru Region (Lago Manacapuru, Lago Grande de Manacapuru and both rios with the same name). This possibility has to be supported by studies following traditional taxonomic approaches. However, the detected haplotype diversities within each cluster (four to six mutations) as compared to at least twice this number of mutations observable between the three clusters of individuals suggests that the three clusters can be considered to be three different species of *Symphysodon*.

Even though mitochondrial data clearly suggest a separation of three distinct molecular lineages within the genus *Symphysodon* that can be termed three species, it remains unclear what the finding of the third cluster, with *S. discus* and also some *S. haraldi* phenotypes in that same cluster, implies. The assignment of phenotypic *S. haraldi* individuals to this phenotypically heterogeneous cluster might indicate either introgression between *S. haraldi* and *S. discus* or phenotypic convergence to the *S. haraldi* phenotype of some populations of the *S. discus* genetic cluster. To clarify this situation an approach including nuclear molecular markers such as sequences or microsatellites should be employed in a future study.

2. Nomenclature: Ready et al. (2006) considered the epithet Tarzoo to be available, selected a neotype from the Rio Jutaí (INPA 25960) and diagnosed the taxon as "having distinct red spots on its anal fin and body".

We dispute this approach and reject it entirely, because:

a) "Tarzoo" is unavailable. Lyons' article explicitly states: "The name used in this story – Symphysodon Discus Tarzoo is unofficial". "Tarzoo" is the original spelling in Lyons (1960), the "specific" and "subspecific" epithets are capitalised and meant to be popular names. Lyon's article was published in 1960, not 1959 contrary to what has been stated elsewhere. No material was deposited of this putative "taxon". Although Kullander (1996) discussed the name "tarzoo", he did not make it available (according to article 11.6 of the ICZN) and Ready et al. (2006) did not make it available either. The ICZN code states: "All names: intention of authors to establish new nominal taxa to be explicit. Every new name published after 1999, including new replacement names (nomina nova), must be explicitly indicated as intentionally new". Ready et al. (2006) intended to "validate" a name that is not available, but they have not described it as new species either. In conclusion, *Symphysodon tarzoo* is not available and the selection of a neotype does not change this fact.

b) Ready et al. (2006) distinguished *S. tarzoo* from all other *Symphysodon* species by "having distinct red spots on its anal fin and body". However, these distinct red spots of a *Symphysodon* have already been recorded by members of the Thayer Expedition in 1865 (Fig. 6), and since Pellegrin (1904) this species carries the name *aequifasciata*. However, Pellegrin (l. c.) lumped the two Teffé specimens (the red spotted discus) and the single Santarém specimen (with stripes in the anal fin, Fig. 5 b) in one series of syntypes. He listed the specimens from Teffé first, and the specimen from Santarém subsequently. In his description of *S. aequifasciata*, Pellegrin referred to the equal bars on the body, which are found in the syntypes from Teffé (Figs 3, 4), but not in the specimen from Santarém (which is *S. haraldi).* The distinct red spots in the anal fin are still visible in the larger specimen from Teffé (Fig. 3b).

c) Harald Schultz in 1958 also collected specimens with distinct red spots in the Lake Tefé Region (Lago Jurity, Schultz 1959) and provided the first detailed description.

d) In 1960, L. P. Schultz, acting as the first reviser of the genus, raised *S. aequifasciata* to specific rank, and selected Teffé as the type locality by indication, because he had not seen the types. He based his redescription on 104 specimens from Lago Teffé, ignoring the second locality mentioned by Pellegrin (1904), Santarém. He listed the name *Symphysodon discus tarzoo* in the synonomy of his newly described *S. aequifasciata haraldi,* stating that according to the ICZN "tarzoo" was a nomen nudum. He mentioned "...nine dark brown vertical bars all of about the same intensity..." for *S. a. aequifasciata*, as Pellegrin (1904) had done, and stated that "...fins are light olive green with scattered light spots basally...", which also applies to Pellegrin's two syntypes from Teffé (and to what Ready et al. considered as *S. tarzoo*).

e) In the 1980s and 1990s Bleher published several papers and books about red spotted green discus, *S. aequifasciatus*. In 1983 he reported the discovery of a red spotted discus from Lago Caiambé and the Lago Tefé, both western Amazon Region (Bleher in Bleher & Göbel 1992). Bleher (1984 a) published Japanese and Chinese editions of his discus book mentioning specifically the red spots of *Symphysodon aequifasciatus*. In another publication that appeared in same year he wrote about the red spotted *S. aequifasciatus* from the Tefé Region (Bleher, 1984 b); in 1985 and 1986 Bleher recorded them from Río Putumayo (Peru), and in 1987 from the Lago Coari region (Rio Urucu) and the Japurá region (Bleher in Bleher & Göbel, 1992).

f) Géry & Bleher (2004: 135-139) recognised three species: *Symphysodon discus*, *Symphysodon aequifasciatus*, and *Symphysodon haraldi* Schultz, 1960.

3. Lectotype and type-locality: Ready et al. (2006) selected Pellegrin's syntype from Santarém (MNHN 1902-130) as the lectotype for *Symphysodon aequifasciatus*, Pellegrin, 1904, which we challenge for the following reasons:

a) Géry & Bleher (2004) had designated a lectotype. The relevant passage reads: "Das Typenmaterial, das im Muséum National d'Histoire Naturelle (MNHN) hinterlegt wurde, besteht aus drei Exemplaren: die zwei mit der Nr. 221-68-2-2, 90,6 u. 122,5 mm SL, die mit „Brésil“ (Brasilien) beschriftet wurden, dürften diejenigen sein, für die Pellegrin die Herkunft Teffé angegeben hat (nach den Katalognummern zu urteilen). Das dritte Exemplar, Nr. 221-68-2-1, ohne mm Angabe, ebenfalls mit „Brésil“ beschriftet, stammt von Santarém. Es ist zwingend geboten (im Blick auf die taxonomische Stabilität und wegen der Bedeutung dieser Form für die Aquarianer, von denen sie „Grüner Discus“ genannt wird), die Auffassungen von L. P. Schultz (die Burgess bestätigt hat) dadurch zu ergänzen, dass die beiden Tefé-Exemplare Pellegrins zum Lectotypus und Paralectotypus erklärt und als Typenlokalität der Lago Tefé, am rechten Ufer des Rio Solimões etwa 64°50' W und 3°20'S bestätigt wird." Hence the two specimens from Tefé are lectotype and paralectotype since 2004, rendering any subsequent lectotype designation invalid.

b) Géry & Bleher (2004) also established, for the first time since Schultz (1960) the correct type locality.

4. Distribution: We dispute several of the distribution records cited in Ready et al. (2006) and the locations where some of their discus specimens reportedly come from. These authors claim that the "Species of the genus *Symphysodon* are restricted to areas where seasonal flooding occurs and are therefore found only near the mainstream of the Amazon River itself ...", which is incorrect. We have found discus in isolated lakes far away from the main stream. In the southern tributaries of the Solimões as far south as to the Tapauá and Rio Cuniuá (tributaries of the middle Rio Purus), and in the northern tributaries of the Paraná do Marium and Paraná do Calado (tributaries of the Rio Demini, a left hand Rio Negro tributary), just to mention a few examples (see map, Fig. 31). The erroneous distribution records by Ready et al. (l. c.) may be attributed to the incorrect information provided to them and mislabelled museum speci-

Fig. 27 a-b. Specimens collected in the Rio Içá near Cuiavá, Amazonas, Brazil, 1972. Photos by T. Hongslo.

Symphysodon discus Heckel, 1840

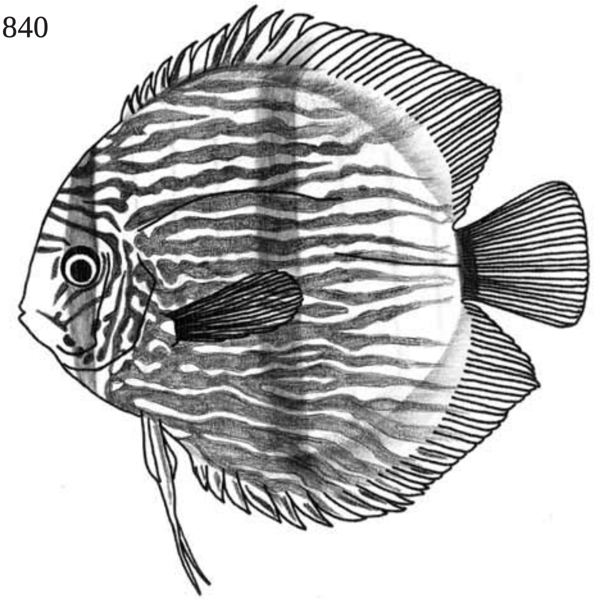


Fig. 28. All *S. discus* have nine bars, only three of which are intense: the first, the fifth and the ninth one in the caudal peduncle, the fifth one being almost always the broadest, the dominant and most intense one. Also, all *S. discus* have stripes running horizontally, most of the times over their entire body, rarely in strait lines. Between 13 and 21 such lines may be present with an average of 18. Drawing by N. Khardina.

Symphysodon aequifasciatus Pellegrin, 1904

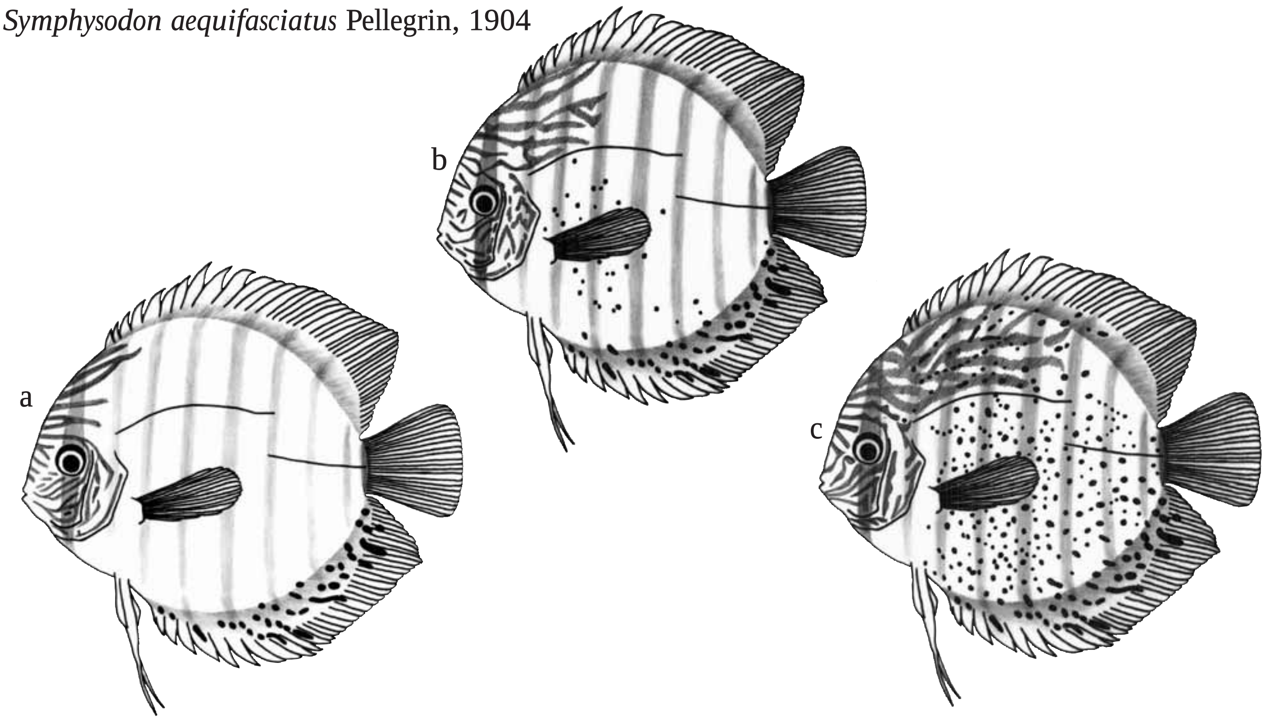


Fig. 29a-c. The main pattern characters for the green discus, *Symphysodon aequifasciatus*, are: a. nine almost uniform, intense bars and presence of spots (normally red or of rust colour), although sometimes the spots can only be seen in the anal-fin region, and stripes in its forehead; b. sporadically distributed ventral spots; c. spots spread over the entire body. Drawings by N. Khardina.

Symphysodon haraldi Schultz, 1960

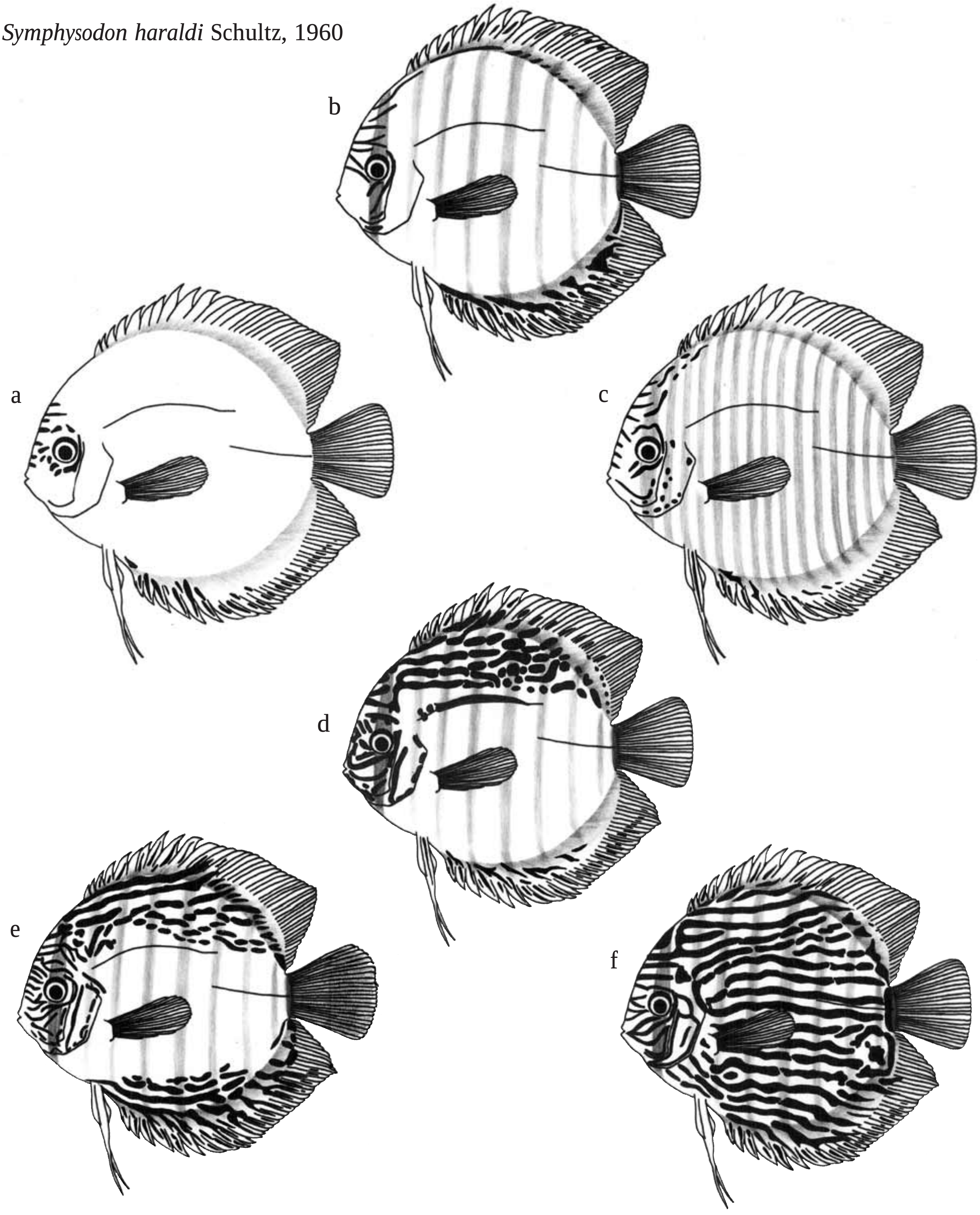
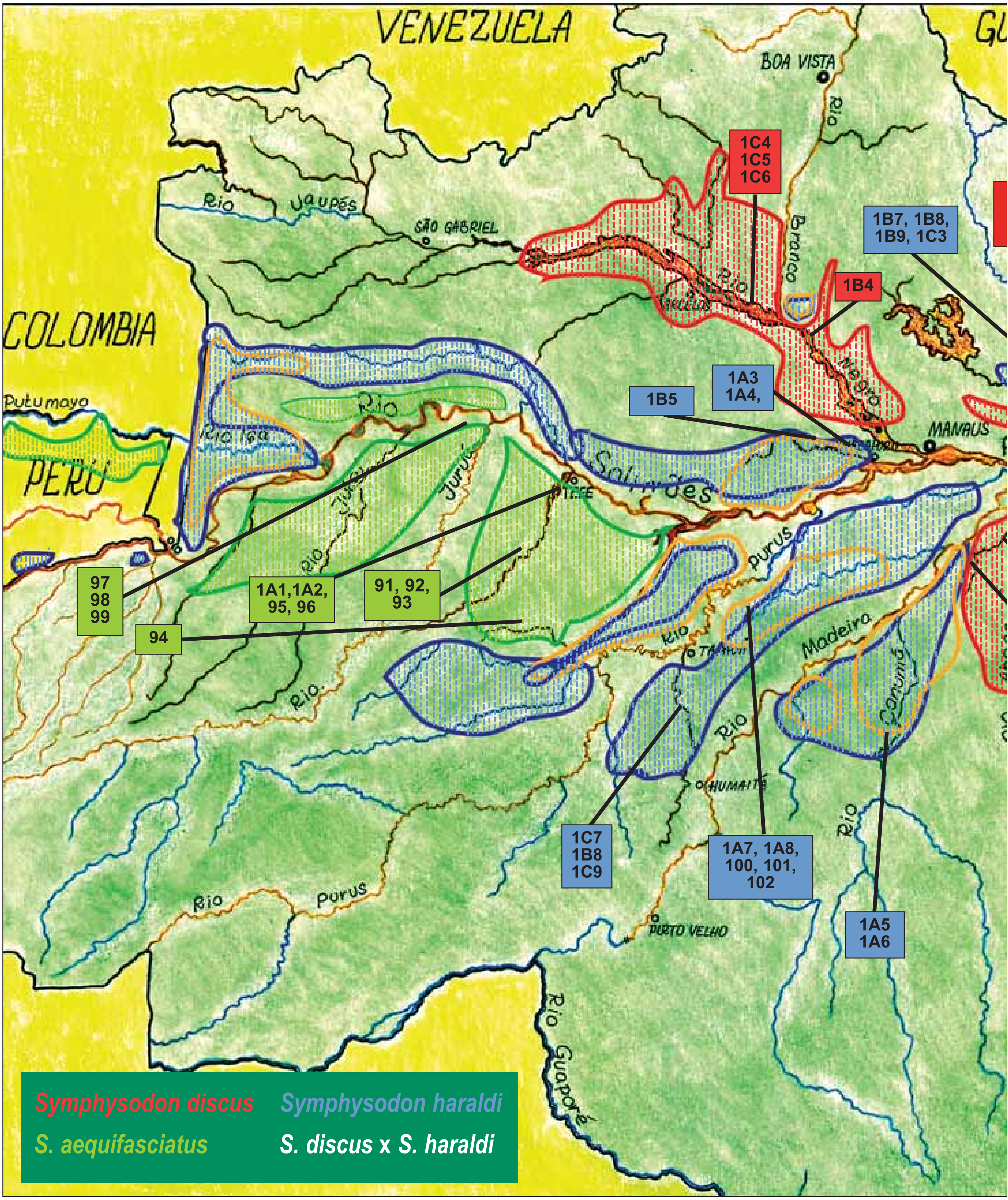


Fig. 30a-f. The main characters which distinguish *S. haraldi* from the other species: a. some specimens may have none or tiny stripes (or fractions thereof) in their head region; spots are always present in the anal fin rays; b. eight or nine bars may be present; c. up to 16 bars; d. stripe pattern may be present in the forehead region; e. forehead and lower ventral region; f. stripes across entire body, either broad or fine, wavy or strait, or only fractions of stripes. Drawings by N. Khardina.


VENEZUELA
BOA VISTA
COLOMBIA
PERU
Rio Uaupés
SÃO GABRIEL
Rio Branco
Rio Negro
MANAUS
Putumayo
Rio Iça
Rio Solimões
Rio Juruá
TEFE
Rio Purus
Rio Madeira
HUMAITÁ
PORTO VELHO
Rio Guaporé
1C4
1C5
1C6
1B7, 1B8,
1B9, 1C3
1B4
1A3
1A4,
1B5
97
98
99
1A1,1A2,
95, 96
91, 92,
93
94
1C7
1B8
1C9
1A7, 1A8,
100, 101,
102
1A5
1A6
Symphysodon discus
Symphysodon haraldi
S. aequifasciatus
S. discus x S. haraldi

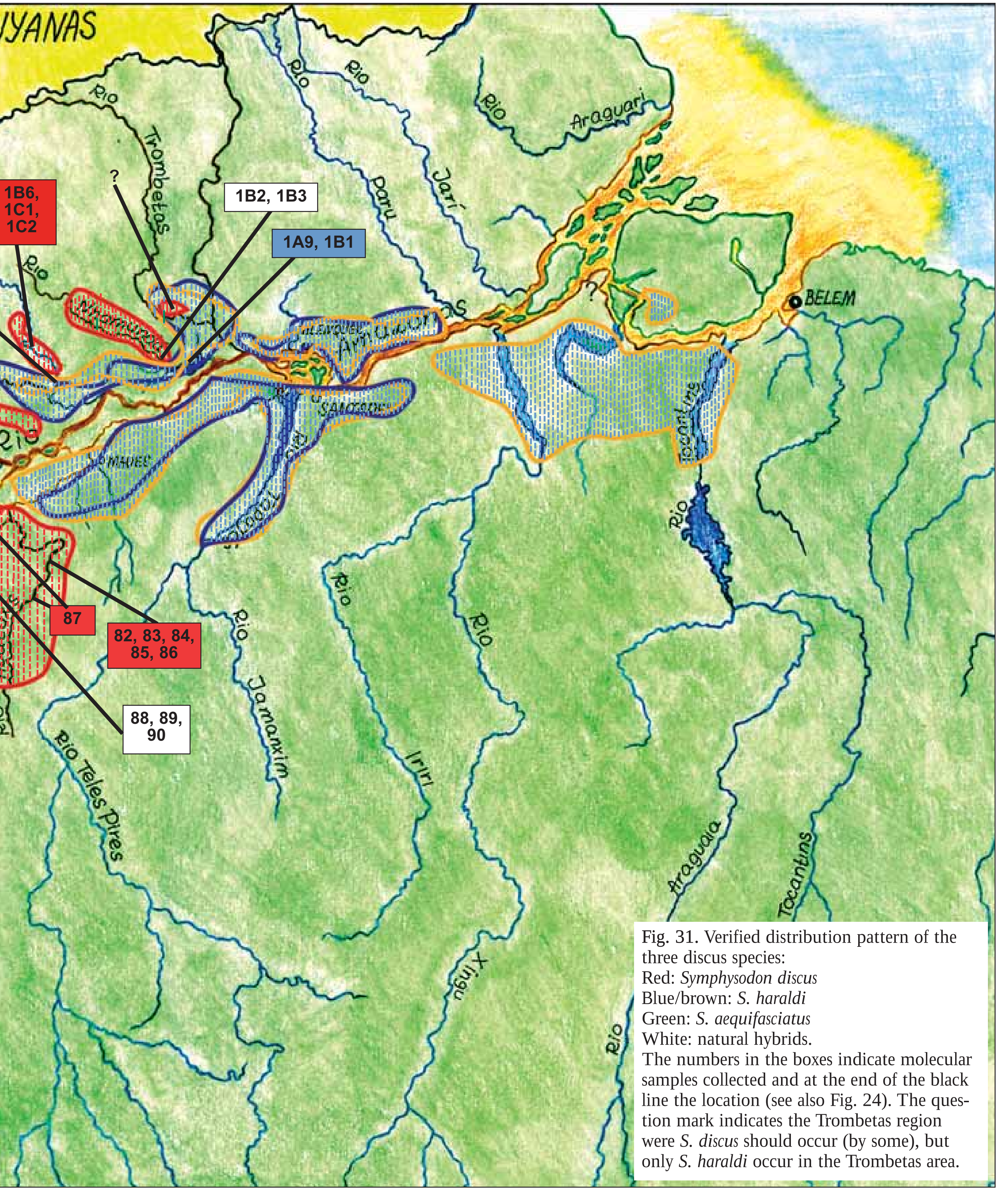


Fig. 31. Verified distribution pattern of the three discus species:
Red: *Symphysodon discus*
Blue/brown: *S. haraldi*
Green: *S. aequifasciatus*
White: natural hybrids.
The numbers in the boxes indicate molecular samples collected and at the end of the black line the location (see also Fig. 24). The question mark indicates the Trombetas region were *S. discus* should occur (by some), but only *S. haraldi* occur in the Trombetas area.

mens. Because of the commercial value of these fishes, fishermen often refuse making their collecting sites known. Inadequate sampling and the availability of insufficient numbers of specimens added up to the inadequacy of their distribution records.

In addition, their statement, that the genus has one large continuous range also contradicts our data, which one of us (HB) collated during more than 300 field trips in over 40 years of research in Amazonia. The hypothetical Amazon barrier, which according to these authors isolates the "western species" *(S. "tarzoo")* cannot stand, because we (and others), have collected in the western Amazon region at least two different species: *S. aequifasciatus* (their *S. "tarzoo")*, and *S. haraldi* (their *S. aequifasciatus)*. The fact that the latter species is not restricted to the eastern Amazon Region was published by Bleher (1983, 1985, 1986, 1987, 1992, 2004, 2006), supported by specimen collections from throughout the Amazon Region (Fig. 24).

Some of the discus samples of Ready et al. (2006) do not bear correct locality data, such as:

– Never ever has a single discus been found in Rio Madeira. They would not be able to survive, because of high predation and because no discus can permanently live in white water. This was confirmed not only by one of us (HB), but also by hundreds of fisherman, specialised in discus collecting (e.g. Manuel Torres, who collected discus for more than 30 years in most Amazonian habitats and lived on the Madeira for many years; pers. comm.). There are discus shipped from Nova Olinda (on the Madeira), but they come from the rivers Canumã, Maués, from several lake areas in that region, from the Abacaxís and Marimari, and all are transported over land to Nova Olinda, as the mentioned rivers and lakes do not connect with the Madeira river. Their record from the "Madeira" is incorrect and no discus was ever found in that region, the nearest occurrence of "red spotted" discus being in Rio Urucu, about 1000 km west of the Madeira.
– *Symphysodon aequifasciatus* was never found in the Rio Içá, neither by the first author (HB), nor by the only other collector working in that region (T. Hongslo, pers. comm.). The assumption, that this species lives in the Rio Içá, is at best doubtful. Bleher (2006: 477-478) shows specimens from that river system and also Hongslo provided additional photographs (Fig. 27a, b), which depict clearly *S. haraldi*.

SUMMARY AND CONCLUSIONS

1) *Symphysodon tarzoo* Lyons, 1960 is a nomen nudum and not available under the rules of the ICZN. The neotype designation by Ready et al. (2006) is invalid. Specimens assigned to *S. tarzoo* by these authors are *S. aequifasciatus* Pellegrin, 1904.

2) Géry & Bleher (2004) designated a lectotype for *Symphysodon aequifasciatus* Pellegrin, 1904 (MNHN 1902/135, 122.5 mm SL) and through this action determined the type locality, which is Lago Tefé, Amazonas, Brazil. The subsequent designation of a lectotype from Santarém by Ready et al. (2006) is invalid.

3) The name *Symphysodon haraldi* Schultz, 1960 remains valid, because *S. tarzoo* Lyons, 1960 is not available. Additionally, retaining the well-established name *S. haraldi* is in the interest of stability of zoological nomenclature. This taxon, which had originally been described as a subspecies of *S. aequifasciata*, was elevated to species level by Géry & Bleher (2004). The type locality is Lagoa Berurí, lower Purus Region. Holotype: USNM 00179829.

4) The Purus arch has not separated species from one another, with *S. haraldi* being found on both sides. Only physical and chemical parameters of the water body may be considered as effective barriers to the dispersal of discus. *Symphysodon aequifasciatus* and particularly *S. discus* require black water with very low conductivity and very low pH values. This is considered the reason for continuing separation during the changes in drainage patterns caused by tectonic processes in the Amazon. The areas where *S. discus* occurs today, are the only ones in the Amazon basin with adequate chemical water composition, as required by this species. Particularly *S. discus* is isolated because of its ecological requirements, which result in distinct reproductive barriers. *Symphysodon aequifasciatus*, with its relatively restricted distribution, limited to the upper part of the Amazon basin, is in a process of increasing isolation. No hybrids between the two species have yet been found in nature.

5) *Symphysodon haraldi* is adapted to more "normal" water conditions, with pH values around 7 and much higher conductivity than is tolerated by its congeners. Such water conditions are found at many sites throughout the Amazon basin. In nature *S. haraldi* interbreeds with its different colour variants, which are found syntopically almost everywhere, throughout its large range of distribution.

6) Discus occur in the region with the highest freshwater fish species diversity anywhere in the world, the central Amazon basin. They occur in large schools protected during the day by deep water (minimum one metre), by bushes, roots and fallen trees along the steep banks of rivers. They have highly specialised ecological requirements. They are difficult to observe and hence our knowledge of their life history in nature is still limited.

7) The systematics of discus, a distinctive genus with comparatively few taxa, is mainly based on their life coloration, as is the case for many members of the family Cichlidae.

8) In *S. aequifasciatus* a congruence of genetic and coloration characters has been found, whereas some specimens that would phenotypically be considered *S. haraldi*, genetically group with the *S. discus* clade. Only future studies using nuclear DNA markers will allow untangling the evolutionary history of the phenotypcially heterogeneous *S. discus* clade.

9) For easy identification, Natasha Khardina compiled the important morphological characters of the three *Symphysodon* species, recognised here as valid, from living specimens:
Symphysodon discus Heckel 1840 (Fig. 28)
Symphysodon aequifasciatus Pellegrin 1904 (Fig. 29)
Symphysodon haraldi Schultz 1940 (Fig. 30)

10) A detailed map (Fig. 31) shows the distribution of *S. discus* in red, *S. aequifasciatus* in green, and *S. haraldi* in blue and brown. It also shows zones of overlap during high floods, which are potential areas of hybridization.

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