El Colegio de la Frontera Sur

Variación e integración morfológica de las mojarras de agua dulce del género *Thorichthys* Meek 1904 (Cichliformes: Cichlidae)

Tesis

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Por:

Limber Sigarroa Gómez

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RESUMEN

El estudio de la diversidad biológica involucra el análisis de los cambios morfológicos. Los cambios en la variación morfológica no siempre tienen el mismo origen y función; existen estructuras que presentan relaciones estrechas y cambian de manera integrada, mientras que otras llegan a ser independientes entre sí, modificándose a través de módulos. Con la finalidad de conocer si la variación morfológica del género *Thorichthys* es resultado de patrones de integridad morfológica, se evaluó la organización modular utilizando seis modelos modulares, mediante el análisis de morfometría geométrica en 246 especímenes pertenecientes a las nueve especies del género. Se emplearon 20 hitos o marcas y cinco semimarcas alineadas a través del método de superimposición Procrustes. Así mismo, para descartar el efecto alométrico causado por las diferencias en el tamaño, se obtuvieron los residuales de la regresión entre el tamaño del centroide y las variables de deformación. Los valores residuales se usaron en los análisis de componentes principales y en el análisis de integración. Se encontró que la variación interespecífica en la forma del cuerpo se acentúa en el perfil de la cabeza, parte media del cuerpo y el pedúnculo caudal. El análisis de integración morfológica reveló que el cuerpo está organizado en módulos. Los modelos más robustos están integrados por dos módulos: uno anterior representado por la cabeza y otro posterior que incluye la parte media del cuerpo y el pedúnculo caudal; el segundo modelo consta de cabeza y parte media del cuerpo como el primer módulo y el pedúnculo caudal como el segundo. Los cambios en el patrón de modularidad corresponden con los eventos de diversificación dentro del grupo. La organización modular de este grupo de peces está relacionada con la filogenia del grupo.

Palabras clave: cíclidos centroamericanos, morfometría geométrica, hipotesis, análisis de modularidad

CAPÍTULO 1. INTRODUCCIÓN

La variación morfológica puede reflejar diferencias a nivel ecológico y de comportamiento (Webb 1984); brinda ventajas ecológicas y evolutivas, como la capacidad de usar una mayor variedad de recursos y favorece el éxito reproductivo (Klingenberg y Ekau 1996; Price et al. 2011; Rüber y Adams 2001). La evolución de un nuevo rasgo es precedida de un cambio en la forma del cuerpo causado por presiones ambientales, mutaciones o por la combinación de fuerzas evolutivas (West-Eberhard 2005). El estudio de estos cambios morfológicos es crucial para comprender la diversidad biológica (Wagner y Altenberg 1996).

Los cambios en la forma de los seres vivos se pueden cuantificar por medio de la morfometría geométrica (MG); esta herramienta permite analizar la forma de los organismos en un espacio geométrico, mediante fórmulas matemáticas y análisis estadísticos multivariados (Bookstein 1982). La MG ha sido utilizada en prácticamente todos los grupos biológicos, en plantas (Klingenberg y Marugán-Lobón 2013), invertebrados (Rosenberg 2002; Aytekin et al. 2007), peces (Trapani 2003; Soria-Barreto et al. 2011), anfibios (Adams y Rohlf 2000), reptiles (Felice et al. 2019), aves (Klingenberg y Marugán-Lobón 2013) y mamíferos, incluido el ser humano (O'Higgins y Jones 1998; González et al. 2009). El concepto principal de esta herramienta es la homología, es decir, estructuras con el mismo origen evolutivo (por ejemplo, aletas de peces o antenas de invertebrados); en la MG estas estructuras deben ser reconocidas en todos los organismos analizados. Dentro del espacio geométrico se ubican las marcas anatómicas en estructuras similares (homologías) y semimarcas, estas últimas se emplean para delimitar estructuras no lineales (como el perfil de la cabeza o forma de aletas) (Bookstein 1991).

El Análisis General de Procrustes (GPA) (Rohlf y Slice 1990) corrige y redimensiona las configuraciones generadas por las marcas a un mismo tamaño, considerando los efectos de posición y desviaciones entre las marcas (Mardia y Dryden 1989). Después de realizar esta corrección, la variación de la forma restante provee una descripción objetiva y eficaz que puede ser utilizada para los análisis multivariados

(Klingenberg 2002). En la MG se utiliza el análisis de componentes principales (ACP), que reordena las configuraciones dentro del plano cartesiano; es un análisis exploratorio, sin grupos predefinidos, con el que se pueden reconocer patrones morfológicos y hacer predicciones futuras (Slice 2007). El análisis de variables canónicas (AVC), en cambio, maximiza las diferencias morfológicas entre los grupos definidos *a priori* especies analizadas. La MG sirve de base para los estudios de biología evolutiva y del desarrollo, debido a que la variación morfológica permite analizar los niveles de integración morfológica (Klingenberg 2010).

Se ha postulado que los mecanismos que limitan o facilitan la variación morfológica son la integración morfológica y la modularidad, respectivamente (Wagner y Altenberg 1996; Wainwright 1996; Hendrikse et al. 2007; Larouche et al. 2015). La integración morfológica se define como la tendencia de los rasgos morfológicos a cambiar de forma coordinada a través de las estructuras o partes de los organismos (Olson y Miller 1958; Klingenberg 2008). En cambio, la modularidad hace referencia a la organización de rasgos fenotípicos en unidades estructurales o funcionales denominadas módulos. Estos módulos se caracterizan por tener alto grado de integración y coordinación en su interior y poca integración con otros módulos. Ambos conceptos están relacionados, ya que si existe integración morfológica existirá nula o poca presencia de módulos (Klingenberg 2008; Klingenberg 2009).

La modularidad es producto de diversos factores, entre ellos está la pleiotropía, donde el efecto de un gen o mutación afecta varios rasgos morfológicos (Cowley y Atchley, 1990; Stearns, 2010). Por otro lado, la modularidad también surge como una respuesta adaptativa a la presión selectiva de variables ambientales (Wagner y Altenberg, 1996). Según el contexto estudiado, se pueden diferenciar cuatro tipos de módulos: de desarrollo, genéticos, funcionales y evolutivos (Klingenberg 2008). Los módulos de desarrollo se relacionan entre sí desde las primeras etapas de la ontogenia, antes de que la estructura este formada. Estos módulos pueden influenciarse unos a otros, por lo que pueden lograr un desarrollo coordinado de tejidos y órganos. Los módulos genéticos, se originan por la acción de un gen sobre varias estructuras (pleiotropía) (Nadeau et al. 2003; Klingenberg 2008; Klingenberg 2010), o por la acción

de varios genes sobre una estructura (epistasis) (Wolf et al. 2006; He et al. 2010). Los módulos funcionales se originan por las interacciones al realizar una o más funciones biomecánicas similares (por ejemplo, la fuerza durante la captura y masticación de alimento, respiración y vocalización, en las cuales varias regiones del cráneo y mandíbula están involucradas) (Nadeau et al. 2003; Klingenberg 2008; Kupczik et al. 2009). Los módulos evolutivos, se forman debido a la acción de la divergencia evolutiva sobre un conjunto de rasgos (Klingenberg 2008; Klingenberg 2010).

En los peces, por ejemplo, los estudios de modularidad se han enfocado en estructuras relacionadas al nado, como las aletas y el pedúnculo caudal (Peres-Neto y Magnan 2004; Larouche et al. 2015; Aguilar-Medrano et al. 2016; Du et al. 2019; Ornelas-García et al. 2017) y en estructuras cefálicas relacionadas con hábitos alimentarios (Ornelas-García et al. 2017). En los cíclidos, un grupo de peces dulceacuícolas altamente diversificado (Miller et al. 2009; McMahan et al. 2013; Říčan et al. 2016), los estudios de integración morfológica y modularidad se han enfocado en las especies de los lagos africanos (Cooper et al. 2010; Parsons et al. 2011; Parsons et al. 2012; Hu et al. 2014), debido a que durante su diversificación han manifestado modificaciones en las estructuras relacionadas con el reparto de recursos (alimento y espacio), como las mandíbulas orales y faríngeas, así como la morfología del cuerpo (Parsons et al. 2011; Friedman et al. 2013; McMahan et al. 2013; Říčan et al. 2013; Burress 2014). Los cíclidos africanos muestran patrones de modularidad en la región preorbital del cráneo, como resultado de la diferenciación en el uso del hábitat (Parsons et al. 2011). Se han determinado dos tipos de módulos asociados a la alimentación, las especies succionadoras (por ejemplo, *Metriaclima zebra*) presentan módulos de tipo funcionales y las especies ramoneadoras (por ejemplo, *Labeotropheus fuelleborni)* con módulos del tipo desarrollo (Parsons et al. 2012). Ambos patrones de modularidad se mantienen en la descendencia, por lo que la integración morfológica en los cíclidos tiene una base genética (Hu et al. 2014).

Los estudios de modularidad en los cíclidos centroamericanos se han enfocado en el aparato faríngeo (Hulsey et al. 2006; Hulsey et al. 2010; Burres et al. 2020). Sin embargo, a pesar de su gran variación morfológica y alta diversidad no se han

desarrollado trabajos que analicen la forma entera del cuerpo. Las especies del género *Thorichthys* (Miller et al. 2009; Fricke et al. 2020) presentan un patrón morfológico único, que combina la morfología craneal especializada para capturar, manipular y seleccionar su alimento en sustratos blandos (filtradoras del sustrato), así como una morfología postcraneal asociada con ambientes con poca o nula velocidad de corriente (lénticos) (Říčan et al. 2016).

El género *Thorichthys* es monofilético (Roe et al. 1997; López-Fernández et al. 2010; Říčan et al. 2016). Está integrado por nueve especies, *Thorichthys affinis*, *Thorichthys aureus*, *Thorichthys callolepis*, *Thorichthys helleri*, *Thorichthys maculipinnis*, *Thorichthys meeki*, *Thorichthys panchovillai*, *Thorichthys pasionis* y *Thorichthys socolofi* (Fricke et al. 2020), cuya distribución incluye Belice, Guatemala, Honduras y México. La mayoría de las especies muestran un patrón de distribución alopátrica, aunque es frecuente encontrar especies en simpatría. A pesar que todas las especies de *Thorichthys* son parecidas morfológicamente, las diferencias se encuentran principalmente en la región cefálica y en la forma del cuerpo (Miller y Taylor 1984; Del Moral-Flores et al. 2017).

En esta tesis se estudió si la diversidad morfológica de las especies del género *Thorichthys* puede ser explicada mediante patrones de integración o modularidad morfológica. Los objetivos fueron: 1) Conocer cómo varía la forma del cuerpo en el género *Thorichthys* y al interior de cada una de las especies, 2) Evaluar los patrones de integración morfológica del género y las especies a través de modelos de organización modular, y 3) analizar la importancia de la integración y la modularidad en la diversidad morfológica del grupo.

De acuerdo con la hipótesis de no independencia evolutiva, se espera encontrar que el parecido morfológico y los patrones de modularidad entre las especies corresponda con sus relaciones de parentesco (Felsenstein, 1985). Además, a partir de las evidencias ecomorfológicas, se espera confirmar que la forma del cuerpo está integrada por dos módulos (craneal y postcraneal) (Říčan et al.*,* 2016).

Los resultados mostraron que la variedad de formas de los cíclidos centroamericanos puede deberse a la organización modular. Estos resultados parecen concordar con la hipótesis de que la modularidad es un factor importante en la diversificación morfológica de los seres vivos (Larouche et al. 2015). De este modo, este trabajo es el primero que se enfoca en la evolución morfológica del cuerpo entero de los cíclidos de Centroamérica. La presencia de un cuerpo constituido por módulos se puede traducir como un mejor aprovechamiento de los recursos alimentarios sin interferir en los patrones de nado en la columna de agua.

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CAPÍTULO 2. Artículo. "Variation and morphological integration in species of *Thorichthys* (Cichliformes: Cichlidae)"

Modularity in *Thorichthys* species

Limber Sigarroa[1], Alfonso A. González-Díaz[1], Miriam Soria-Barreto[2], Rocío Rodiles-Hernández[1]

[1]Departamento de Conservación de la Biodiversidad, El Colegio de la Frontera Sur (ECOSUR), Carretera Panamericana y Periférico Sur s/n, Barrio María Auxiliadora, San Cristóbal de Las Casas, Chiapas, 29290, Mexico.

[2]Centro de Investigación de Ciencias Ambientales, Facultad de Ciencias Naturales. Universidad Autónoma del Carmen. Calle Laguna de Términos s/n, Col. Renovación 2ª Sección, Ciudad del Carmen, Campeche, 24155, Mexico.

Correspondence author: Departamento de la Biodiversidad. El Colegio de la Frontera Sur (ECOSUR), Carretera Panamericana y Periférico Sur s/n, Barrio María Auxiliadora, San Cristóbal de Las Casas, Chiapas, 29290, Mexico. Email: limber.sigarroag@gmail.com

ABSTRACT

Modularity is considered to be one of the most crucial factors when it comes to explaining morphological differences. The bodies of fishes in family Cichlidae represent the integration of two modules related to feeding and locomotion. *Thorichthys* species present a unique morphological pattern, which combines specialized cranial morphology used to process food with a postcranial morphology associated with lentic environments. We analyzed the differences of morphological variations in body shape of species of *Thorichthys* and evaluated whether morphological integration acts like a precursor of constraint or a promoter of morphological variation. For this study, we examined 246 specimens of the nine valid species of *Thorichthys* using geometric morphometric analysis; including 20 landmarks and five semilandmarks in the complete body shape description, in order to correct the data by allometry, we used the residuals from the shape and centroid size (CS) regression. In order to explore the morphospace across the *Thorichthys* species we carried out a principal componente analysis (PCA). Six modularity hypotheses were tested in the allometry-corrected data. Our results showed that major morphological differences in body shape were present in the profile of the head, the middle part of the body, and the caudal peduncle. Integration analyses revealed that the body shape of the *Thorichthys* species were integrated by modules. We recovered two hypotheses as the best-supported models consisting of two modules. The first hypothesis considered the head as a single module, meanwhile the rest of the body as a second one. The second hypothesis recovered the head and middle part of the body as one module, while the caudal peduncle was another one. The results suggest that low body shape covariation may result from phylogenetic relashionships.

Keywords: Middle American cichlids, body shape, morphometric analysis, modularity

Research highlights

The morphological variation of the *Thorichthys* species was more evident in the profile of the head, the depth of the middle part of the body, and the length of caudal the peduncle. The body of *Thorichthys* was integrated by two modular configurations.

Graphical abstract
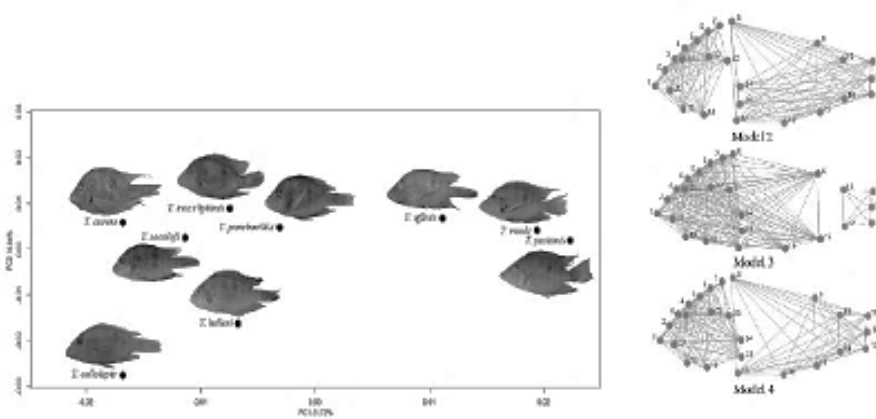
Morphological variation shown differences in the profile of the head, depth of the body and length of caudal peduncule. Meanwhile models 2, 3 and 4 were the best-supported at interspecific and intraspecific levels in species of *Thorichthys*.

1 INTRODUCTION

The study of biological diversity involves the analysis of morphological variation (Wagner & Altenberg, 1996). Morphological integration and modularity can explain the morphological diversification (Hendrikse, Parsons, & Hallgrímsson, 2007; Larouche, Cloutier, & Zelditch, 2015; Wagner & Altenberg, 1996; Wainwright, 1996). Morphological integration describes the tendency of phenotypic traits to change in a coordinated way (Klingenberg, 2008; Olson & Miller, 1958). In contrast, modularity is related to the levels of organization between sets of phenotypic traits (Klingenberg, 2008; Wagner & Altenberg, 1996).

By analyzing the morphological variation patterns it is possible to propose the levels of morphological integration and recognize the existence of modules (Cheverud, Routman, & Irschick, 1997; Klingenberg, 2008). Modularity is the result of various factors; one of the most important is pleiotropy, in which one gene or mutation affects multiple traits (Cowley & Atchley, 1990; Stearns, 2010). In this way, morphological traits show covariation because they share the same genetic origin (Cowley & Atchley, 1990). Additionally, modularity can also be a result of an adaptive response to environmental pressure (Wagner & Altenberg, 1996).

Morphological data have been useful to detect four types of modules: developmental, genetic, functional, and evolutionary (Klingenberg, 2008, 2010). Developmental modules represent developmental interactions from the early stages of ontogeny before the biological structure was formed. These traits can mutually influence each other and lead the coordinated development of tissues and organs. Genetic modules are the result of the

action of one gene over multiple traits (pleitropy) (Klingenberg, 2008, 2010; Nadeau et al., 2003), or the effect of multiple genes over a single trait (epistasis) (He, Qian, Wang, Li, & Zhang, 2010; Wolf, Pomp, Eisen, Cheverud, & Leamy, 2006). Functional modules originate from the interactions among structures that perform one or more similar biomechanical functions, for example, the mechanical forces used in chewing and processing food, breathing, and vocalization (Klingenberg, 2008; Kupczik et al., 2009; Nadeau et al., 2003). Evolutionary modules are the result of evolutionary changes in a set of traits (Klingenberg, 2008, 2010).

Cichlids are one of the most diverse group of freshwater fishes (McMahan, Chakrabarty, Sparks, Smith, & Davis, 2013). Some of the major radiation processes in cichlids are related to the diversification of the food resourses exploitation. In African cichlids, the studies of morphological integration and modularity have focused on the head, and oral and pharyngeal jaws (Burress, 2014; Cooper et al., 2010; Friedman et al., 2013; Hu, Parsons, & Albertson, 2014; McMahan et al., 2013; Parsons, Cooper, & Albertson, 2011; Parsons, Márquez, & Albertson, 2012).

Patterns of modularity in African cichlids are linked to differential habitat use (Parsons et al., 2011). Two types of modules associated with feeding have been identified: the suction-feeding species (e.g. *Metriaclima zebra*) present functional modules and the biting species (e.g *Labeotropheus fuelleborni*) developmental modules (Parsons et al., 2012). Both patterns of modularity are conserved in the offspring; analysis demonstrated that morphological integration in the African cichlids has a genetic basis (Hu et al., 2014).

In Middle American cichlids the modularity studies have been performed in the pharyngeal jaws (Burress, Martinez, & Wainwright, 2020; Hulsey, García de León, & Rodiles-Hernández, 2006; Hulsey, Mims, Parnell, & Streelman, 2010). The *Thorichthys* genus (Fricke, Eschmeyer, & Van der Laan, 2020; Miller, Minckley, & Norris, 2009) presents a unique morphological pattern, which combines specialized cranial morphology used to select, capture, and process their food in soft substrates (substratum-sifting) with a postcranial morphology associated with lentic environments (Říčan, Piálek, Dragová, & Novák, 2016). The unusual morphology suggests a modular evolution. Under these assumptions, *Thorichthys* is an ideal group to explain morphological evolution within the group.

Thorichthys is a monophyletic group (López-Fernández, Winemiller, & Honeycutt, 2010; Říčan et al., 2016; Roe, Conkel, & Lydeard, 1997) that includes nine valid species (*Thorichthys affinis*, *Thorichthys aureus*, *Thorichthys callolepis*, *Thorichthys helleri*, *Thorichthys maculipinnis*, *Thorichthys meeki*, *Thorichthys panchovillai*, *Thorichthys pasionis* and *Thorichthys socolofi*) (Fricke et al., 2020). This group of fishes is widely distributed throughout hydrological basins in Belize, Guatemala, Honduras, and Mexico. Most species are allopatric, although it is common to observe some species in sympatry, for example, *T. meeki*, *T. pasionis*, and *T. helleri*. Even though all species of *Thorichthys* are morphologically similar, it is possible to identify a few differences between some species, for example, *T. helleri* and *T. pasionis* present differences mainly in the cephalic region, as well as in body shape (Del Moral-Flores, López-Segovia, & Hernández-Arellano, 2017; Miller & Taylor, 1984).

This study aims to analyze the variation in body shapes of the *Thorichthys* genus and evaluate whether morphological integration acts like a precursor of constraint or a promoter of morphological variation. Our objectives were 1) to analyze the changes in the shape of the body at interspecific and intraspecific levels, 2) to evaluate the patterns of morphological integration at interspecific and intraspecific levels, and 3) to assess the role of morphological integration/modularity on the morphological diversification processes of the group.

According to the idea of evolutionary non-independence, we expect to find that morphological variation and patterns of modularity between species correspond to their phylogenetic relationships (Felsenstein, 1985). Furthermore, based on ecomorphological evidence, we expect to find that the body is integrated by two modules (cranial and postcranial modules) (Říčan et al., 2016).

2 MATERIALS AND METHODS

2.1 Data collections

A total of 246 specimens were analyzed, representing the nine valid species of the genus *Thorichthys*. All the *Thorichthys* specimens were obtained from three ichthyological collections (Table 1): El Colegio de la Frontera Sur, campi San Cristóbal and Chetumal (ECOSC and ECOCH) and Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Unidad Oaxaca (CIDOAX) in Mexico, and the Fish Collection from the Universidad de San Carlos in Guatemala (USAC). The selected female and male specimens were of similar sizes, approximately 55 mm of standard length (SL).

2.2 Analysis of morphological variation

Fishes were photographed on their left side using a SONY SLT-A37 digital camera. The camera was mounted on a tripod to standardize the distance from the object. A scale of one centimeter was placed on each photograph. The digitizations of the photographs were made in the program tpsUtil (Rohlf, 2018). We used geometric morphometric analysis to capture the whole body shape variation, placing a configuration of 20 landmarks (LM) on the body contour, and five semilandmarks (SMLs) on the cephalic profile (Figure 1; Table 2) using the programs tpsDig (Rohlf, 2017) version 2.32 and MakeFan8, (Sheets, 2010), respectively. To eliminate the elements not related to shape (scale, orientation, and position), the digital coordinates were superimposed using a General Procrustes Analysis (Rohlf & Slice, 1990). To reduce the effect of differences in the sizes of specimens, allometric variation was removed by calculating residuals from the regression of shape on centroid size (CS) using the program Regress8 of the IMP series (Webster & Sheets, 2010). The residual values obtained from the regression were used in the principal component analysis (PCA). We used PCA to visualize the major sources of variation to define the modular models (hypotheses). The PCA was performed in the program PCAGen8, also from the IMP series (Webster & Sheets, 2010).

2.3 Analysis of modularity

Analysis of modularity was performed in the program Mint (Márquez, 2008). We used the residuals from the regression of shape on CS. The patterns of variation obtained from the PCA were used to propose six modular models (M1-M6), including a null model (M6)

which stated that the whole body is highly integrated (i.e. demonstrates no modularity) (Figure 2; Table 3). Each model was tested at interspecific and intraspecific levels.

Each model was evaluated using the goodness of fit or gamma value (γ) (Márquez, 2008). This statistical test was used to determine which model explains better the covariance of the data. A value of $\gamma < 0$ indicates modularity, whereas $\gamma > 0$ indicates morphological integration. Thus, the best-fitting model is the one with the lowest γ value. The significance of γ for the six models was obtained using the parametric Monte Carlo approximation. A low P value (< 0.05) corresponds to large values of γ, suggesting large differences between the data and models analyzed. Confidence intervals (CI) for the γ value were obtained using the Jackknife resampling method. Finally, the Jackknife support was computed, calculating the proportion in which a model ranks first (the best-support model) in 1000 resamples.

3 RESULTS

3.1 Morphological variation

The PCA showed variation in the body shape, and the first two components explained 35.76% of the variance. In the first component (PC1) (21.72%), *T. affinis*, *T. meeki*, and *T. pasionis* were located in the positive axis, separated from the rest of the species, which were found in the negative axis. In component two (PC2) (14.04%), *T. callolepis* and *T. helleri* were found in the negative axis, while the remaining species were located in the positive axis.

Morphological variation in PC1 was accentuated mainly in the head, the position of the pectoral fin, and the length of the caudal peduncle. *Thorichthys affinis*, *T. meeki*, and *T.*

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pasionis display the longest heads with straight cephalic profiles, pectoral fins located in the most posterior position of the body, and shortened caudal peduncles, whereas in *T. panchovillai*, *T. helleri*, *T maculipinnis*, *T. socolofi*, *T. callolepis*, and *T. aureus* the head is shorter, the cephalic profile is round, the pectoral fins are located in the most anterior position of the body, and the caudal peduncle is longer. The variation in PC2 was mainly observed in the depht of the body. *Thorichthys pasionis*, *T. socolofi*, *T. meeki*, *T. panchovillai*, *T. aureus*, *T. affinis*, and *T. maculipinnis* have deep bodies compared to *T. callolepis* and *T. helleri*, which have elongated bodies (Figure 4).

3.2 Modularity in *Thorichthys*

The modularity is present in the body shape of the species of *Thorichthys*. Based on γ values, the best-supported models were found to be M2, M3, and M4, which indicated that the body shape is composed of two modules. At genus level the M4 model was the best-supported. M3 was the best-supported model in *T. helleri*, *T. callolepis*, *T. pasionis*, *T. socolofi*, *T. meeki*, *T. panchovillai*, and *T. affinis* with more than 68% Jackknife support. In *T. aureus* and *T. maculipinnis*, the best-supported models were M2 and M4, with Jackknife support values of 100% and 89.2%, respectively (Table 4). The null model of morphological integration (absence of modularity) was not statistically supported in any species. Monte Carlo probability values suggested models that were very similar. Therefore, the analysis focused on γ and Jackknife support values.

At the interspecific level, the model M4 was the best-supported, with a Jackknife support value of 70.2%, followed by the M3 and M2 models. Both, the M4 and M3 models

considered the presence of two modules, meanwhile the M1 model indicated the presence of three modules.

According to the γ value, in seven species, a single model was recognized as most strongly supported. The M3 model was the best supported in seven species, *T. affinis* (70.2%), *T. callolepis* (92%), *T. helleri* (92%), *T. socolofi* (93.2%), *T. meeki* (85.2%), *T. panchovillai* (99.9%), *T. pasionis* (100%). The second and third models varied among the species (see Table 4).

In *T. aureus* the best-supported model was M2, with a Jackknife support value of 100%. The M4 and M5 models ranked second and third. The M2 and M4 models considered two modules, one anterior and one posterior, while the M5 model considered ventral and dorsal modules.

In *T. maculipinnis* the best-supported model was M4, with a Jackknife support value of 89.2%. The M3 and M2 models ranked second and third. The three models considered the presence of two modules, one anterior and one posterior, the modular configuration depending on the structures that integrated the modules.

4 DISCUSSION

The morphological analysis demonstrated that morphological variation in *Thorichthys* species was accentuated in the head, in the middle part of the body, and the caudal peduncle. However, the modularity analysis indicated that the body was composed of two modules: cranial and post-cranial. These results supported the hypothesis regarding the morphological differentiation between the skull and the rest of the body being primarily attributed to trophic segregation and feeding.

The species of the genus *Thorichthys* showed morphological variations mainly in the profile and length of the head, body depth, caudal peduncle length. Based on this variation, two groups were recognized: one integrated by *T. aureus*, *T. callolepis*, *T. helleri*, *T. maculipinnis*, *T. socolofi*, and *T. panchovillai*, the other formed by *T. affinis*, *T. meeki*, and *T. pasionis*. This morphological differentiation corresponds to the groups proposed by Taylor & Miller (1984): the "helleri group" (*T. aureus*, *T. callolepis*, *T. helleri*, *T. maculipinnis*, and *T. socolofi*) and the "meeki group" (*T. affinis*, *T. meeki*, and *T. pasionis*). Furthermore, this pattern of morphological variation coincides with the current phylogeny of the genus (Říčan et al., 2016).

The observed morphological variation can be attributed to the differential use of resources, such as the type of habitat and feeding (Barrientos-Villalobos, Schmitter-Soto, & de los Monteros, 2018; Langerhans, Layman, Langerhans, & Dewitt, 2003; Pease, Mendoza-Carranza, & Winemiller, 2018; Soria-Barreto, Rodiles-Hernández, & Winemiller, 2019). External morphological differences typically have internal origins; for example, in other Middle American cichlids like *Vieja bifasciata*, *Vieja breidohri*, and *Vieja hartwegi*, changes in body depth and length were observed, changes which were mainly explained by the increase in the number of vertebrae (Gómez-González et al., 2018). The coexistence of sympatric species (for example, *T. helleri*, *T. meeki*, and *T. pasionis*) has been related to external structures involved in the capture and processing of food (Říčan et al., 2016; Roe et al., 1997).

Body shape at the interspecific level showed two modules: the anterior (cranial) module related to food, and the postcranial module related to habitat use (Říčan et al., 2016). The

modules can be classified based on the functions or processes in which they are involved (Larouche et al., 2015; Wagner, Pavlicev, & Cheverud, 2007). In functional modules, for example, two or more modules are related, the phenotypic traits of each module are strongly integrated, and they become semi-independent from each other (Wagner & Altenberg, 1996). This modular configuration, differentiation of the head and rest of the body found in *Thorichthys* is similar to that previously reported in sympatric morphospecies of *Astyanax* genus. In that study the authors suggested that two modules could be associated to the trophic specialization (head length) and locomotion differentiation (Ornelas-García, Bautista, Herder, & Doadrio, 2017). Therefore, they suggested modularity as a triggering factor of the ecological divergence in this system.

In seven species of *Thorichthys* (*T. affinis*, *T. callolepis*, *T. helleri*, *T. meeki*, *T. pasionis*, *T. panchovillai*, and *T. socolofi*), the best-supported model was M3, which differentiated two modules, the first including the head and the middle part of the body, with the second representing the caudal peduncle. This modular configuration is the most common in Actinopterygian fish (Larouche, Zelditch, & Cloutier, 2018), and is related to locomotion; the integration of the head, paired fins, dorsal fin, and anal fin provides more speed and stability to the body of the fish (Webb, 1982). This modular configuration allows improved maneuverability and the posibility to occupy to different types of habitats and different food resources (Pease et al., 2018; Říčan et al., 2016; Soria-Barreto et al., 2019). The caudal peduncle belongs to a different module; its function is primarily related to movement mechanisms (Lauder & Drucker, 2004; Webb, 1982; Webb & Weihs, 1986). The modular configuration in these seven species is related to a functional type and can be observed in fishes' swimming performance; the caudal peduncle provides the initial thrust, while the

dorsal and anal fins operate as stabilizers. Meanwhile, the pectoral fins are used for lateral movements (Feilich, 2016; Webb, 1982).

The best-supported models in *T. aureus* and *T. maculipinnis* were M1 and M4, respectively. The modular configuration of both species was similar; the difference resided in the addition of pectoral fins to the anterior module. The integration of the anterior module has been observed in species of Pomacentridae (Aguilar-Medrano, Frédérich, Balart, & de Luna, 2013). The posterior module was also reported in Cyprinodontiformes, and is related to movement patterns (Larouche et al., 2015; Plaut & Gordon, 1994). The differentiation of the two modules can be attributed to the fact that anterior modules evolve faster than posterior modules due to enviromental pressure, genetics and developmental factors; this feature is clearer in early stages of ontogeny (Lauder, 2000; Lauder & Tytell, 2005; Parsons et al., 2011; Sfakiotakis, Lane, & Davies, 1999).

The two modular configurations seems to be related to the actual phylogeny of the group (Říčan et al., 2016). The relationship between modular configuration and phylogenetic relationships was discovered in the shape of mammals cranium, as well (Márquez, 2008). Meanwhile in the frogs cranium, the modular changes are highly related to feeding (Bardua et al., 2020). Genetic variation has an important role in the evolutionary process (Klingenberg, 2008), in this way, to understand evolution it is necessary to know the factors that shape genetic variation, i.e. pleiotropy, epistasis or epigenetic effects. Thus, genetic modules play an importante role in evolutionary modularity (Felsenstein, 1985). However, the modules found in *Thorichthys* were of the type functional, this type of modules has a key role in modularity evolution, connecting the modular structure with the

body performance (Klingenberg, 2008). The relashionship between modules is not exclusive to genetic and funcional modules; functional modules can influence developmetal modules by processes like bone remodeling, tissue growth or muscles tension (Herring, 1993; Klingenberg, 2008).

As it's previously mentioned, modularity has been linked to evolvability (Cheverud, 1996; Wagner & Altenberg, 1996). This does not mean that species with highly-integrated bodies do not display morphological diversification, but rather in species with high modularity levels, diversification occurs more quickly (Jablonski, 2017; West-Eberhard, 2005). As observed in species of the *Astyanax* complex, with more than 40 species (Schmitter-Soto, 2017), we can observed modularity as a mechanisms in the diversification process (Ornelas-García et al., 2017). Similarly, species of *Amphiprion* show different patterns of morphological variation due to high levels of modularity (Aguilar-Medrano, Frédérich, & Barber, 2016). On the other hand, in Acanthomorph fish, morphological integration seems to positively contribute to diversification (Du, Tissandier, & Larsson, 2019). Therefore, there is no clear rule regarding the impact of morphological integration and modularity on fish diversification (Claverie & Patek, 2013; Gerber, 2013).

A body integrated by modules confers the possibility of exploiting resources, occupying different habitats, and exhibiting certain behavioral patterns. In species of *Thorichthys*, their modular configuration could facilitate their distribution throughout different habitats and even become sympatric species (Miller et al., 2009). Similarly, it presents the possibility to exploit a wide range of food in the column water (López-Fernández et al.,

2010; Pease et al., 2018; Roe et al., 1997; Soria-Barreto et al., 2019) without having alterations in their swimming patterns.

5 CONCLUSIONS

The morphological variation of *Thorichthys* species was found in the profile of the head, the depth of the middle part of the body, and the of lenght of caudal peduncle. It was posible to recognize two body shapes. The interespecific modularity analysis indicated the presence of two modules, anterior (head and pectoral fins) and posterior (middle part of the body and caudal peduncle). At intraspecific level we could recognize two modular configurations. The modular organization observed in the *Thorichthys* species did not vary much, because in nine species, only two modules were detected, differing only by the position of the structures integrating the modules. This modular configuration corresponded with the phylogeny of the group, indicating that modularity in *Thorichthys* has a phylogenetic origin.

With this in mind, evaluation of the origins of each module proposed here and analysis of new modular configurations are recommended. It is also important to evaluate the entire shape of the body and map modules onto the phylogeny. An assesment of the morphological integration and modularity using minimun deviance and graphical methods (i.e. distance-matrix, Escoufier's RV and covariaion ratio methods) is recommended. It is also necessary to map patterns of modularity over ontogeny and through generations as well. Studies that allow measuring the expression quantitative trait loci (eQTL) are imperative, as they will help identify the basis of the modularity in the body of Middle American cichlids.

This study examined morphological variation and discovered evidence of modular evolution. Further studies involving more Middle American cichlids may shed light on the origins of their great morphological diversity.

AUTHORS' CONTRIBUTIONS

Conceived and performed the experiment, analyzed the data, revised data, edited figures, and tables: LS, AAGD, MSB, RRH.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Table 1. List of *Thorichthys* species included in the analysis of modularity

Species	*n*	Collection	Specimen number	Reference
T. affiinis	20	USAC	CDM-481	Günther (1862)
T. aureus	32	USAC	USAC-2104, 0789	Günther (1862)
T. callolepis	18	CIDOAX	CIDOAX-103, 149, 151, 221, 224, 225, 228, 528	Regan (1904)
T. helleri	29	ECOSC, USAC	ECOSC-308, 2894, 4307, 6590, 13209, 12212, USAC-1407, 1564, 1666, 1749	Steindachner (1864)
T. maculipinnis	32	CIDOAX	CIDOAX-151, 156, 157, 158, 172, 174, 175, 227, 231, 233, 318, 559	Steindachner (1864)
T. meeki	33	ECOCH, ECOSC, USAC	ECOCH-1003, 1653, 1653, 1705, 2739, 3126, 3751, 6225, 11951, 13213	Brind (1918)
T. panchovillai	23	CIDOAX	CIDOAX-224, 227, 228, 229, 523	Del Moral et al. (2017)

			ECOSC-023, 2859, 3149, 3343, 3735, 4610,	
T. pasionis	35	ECOSC, USAC	4618, 6511, 7525, 7661, 7835, 11945,	Rivas (1962)
			11955, USAC-1747	
T. socolofi	24	ECOSC	ECOSC-5649, 12750, 12764	Miller & Taylor (1984)

Institutional abbreviations: USAC = Fish Collection of the Universidad de San Carlos (Guatemala); CIDOAX = Fish

Collection of the Centro Interdisciplinario para el Desarrollo Integral Regional, Unidad Oaxaca (México); ECOCH = Fish

Collection of El Colegio de la Frontera Sur, Unidad Chetumal (México); ECOSC = Fish Collection of El Colegio de la

Frontera Sur, Unidad San Cristóbal de las Casas (México).

Table 2. Description of landmarks and semi-landmarks for the geometric morphometric analysis

Landmark/ semilandmark	Landmarks and semilandmarks description
LM1	Anterior tip of the snout
SLMs 2-6	Supracephalic profile
LM7	End of the supraoccipital bone
LM8	Start of the dorsal fin
LM9	Last spine of the dorsal fin
LM10	End of the dorsal fin
LM11	Upper boundary of the caudal fin
LM12	Center of the caudal fin
LM13	Base of the caudal fin
LM14	End of the anal fin
LM15	Last spine of the anal fin
LM16	Origin of the anal fin
LM17	Origin of the pelvic fin
LM18	Anterior operculum insertion at base of isthmus
LM19	Cleithrum
LM20	Posterior end of the upper lip
LM21	Anterior extent of the sphenotic orbit
LM22	Posterior extent of the sphenotic orbit
LM23	Upper end of the operculum

LM24	Dorsal insertion of the pectoral fin
LM25	Origin of the pectoral fin

Table 3. Description of the six models of modularity in the *Thorichthys* species.

Models	Description of modular partitions
1	Module 1: Head (1:7, 18:23)
	Module 2: Pectoral fin (24:25) + dorsal fin (8:9) + anal fin (15:16) + pelvic fin (17) + caudal fin (10:14)
2	Module 1: Head (1:7, 18:23)
	Module 2: Dorsal fin (8:9) + anal fin (15:16) + pelvic fin (17) + pectoral fin (24:25)
	Module 3: caudal fin (10:14)
3	Module 1: Head (1:7) + dorsal fin (8:9) + anal fin (15 y 16) + pelvic fin (17) + pectoral fin (24 & 25),
	Module 2: caudal fin (10:14)
4	Module 1: Head (1:7, 18:23) + pectoral fin (24:25)
	Module 2: + dorsal fin (8:9) + anal fin (15:16) + pelvic fin (17) + caudal fin (10:14)
5	Module 1: Dorsal part of the body (2:12, 21:23)
	Module 2: Ventral part of the body (1, 13:20, 24.25)
6	Null model: body completely integrated

The numbers in parentheses refer to the landmarks and semi-landmarks included in each partition

Table 4. Results of analysis of modularity in *Thorichthys*.

Species	Models	No. Modules	Rank	*P* Monte Carlo	γ	95% CI	Jacknife support (%)
T. affinis	M1	3	4	0.523	-0.14	[-0.149, -0.104]	41
	M2	2	2	0.951	-0.173	[-0.191, -0.140]	70.6
	M3	2	1	1	-0.177	[-0.201, -0.103]	68.9
	M4	2	3	0.961	-0.167	[-0.232, -0.122]	60.4
	M5	2	5	0.907	-0.138	[-0.156, -0.046]	26.3
	M6	1	6	0.004	0	[0,0]	100
T. aureus	M1	3	4	0.023	-0.126	[-0.138, -0.108]	43.2
	M2	2	1	0.705	-0.205	[-0.219, -0.181]	100
	M3	2	5	0.888	-0.101	[-0.117, -0.082]	96
	M4	2	2	0.624	-0.16	[-0.178, -0.132]	95.8
	M5	2	3	0.368	-0.126	[-0.141, -0.098]	21.6
	M6	1	6	0	0	[0,0]	100
T. callolepis	M1	3	2	0.643	-0.153	[-0.163, -0.122]	66.1
	M2	2	3	0.964	-0.146	[-0.159, -0.116]	47.2

	M3	2	1	1	-0.183	-0.210, -0.139]	92.7
	M4	2	4	0.973	-0.144	[-0.163, -0.114]	57.7
	M5	2	5	0.895	-0.077	[-0.090, -0.057]	100
	M6	1	6	0	0	[0,0]	100
T. helleri	M1	3	2	0.176	-0.153	[-0.166, -0.130]	78.4
	M2	2	3	0.665	-0.147	[-0.156, -0.128]	73.5
	M3	2	1	0.999	-0.172	[-0.195, -0.148]	97.8
	M4	2	4	0.729	-0.139	[-0.151, -0.119]	85.3
	M5	2	5	0.437	-0.087	[-0.104, -0.077]	100
	M6	1	6	0	0	[0,0]	100
T. maculipinnis	M1	3	4	0.033	-0.124	[-0.131, -0.100]	91.3
	M2	2	3	0.27	-0.133	[-0.142, -0.114]	91.3
	M3	2	2	0.982	-0.197	[-0.213,-0.185]	89.2
	M4	2	1	0.647	-0.216	[-0.234, -0.183]	89.2
	M5	2	5	0.07	-0.065	[-0.076, -0.050]	100
	M6	1	6	0	0	[0,0]	100

T. meeki	M1	3	3	0.039	-0.101	[-0.112, -0.090]	45.5
	M2	2	5	0.295	-0.091	[-0.110, -0.071]	79.3
	M3	2	1	0.992	-0.149	[-0.165, -0.132]	85.2
	M4	2	2	0.578	-0.14	[-0.155, -0.121]	83
	M5	2	4	0.297	-0.101	[-0.124, -0.074]	29.5
	M6	1	6	0	0	[0,0]	100
T. panchovillai	M1	3	3	0.404	-0.18	[-0.200, -0.126]	39
	M2	2	4	0.801	-0.163	[-0.177, -0.139]	88.6
	M3	2	1	1	-0.226	[-0.248, -0.195]	99.9
	M4	2	2	0.883	-0.18	[-0.194, -0.161]	46.3
	M5	2	5	0.53	-0.058	[-0.070, -0.047]	100
	M6	1	6	0	0	[0,0]	100
T. pasionis	M1	3	3	0.055	-0.142	[-0.157, -0.116]	79.8
	M2	2	4	0.374	-0.127	[-0.141, -0.105]	80.8
	M3	2	1	0.999	-0.197	[-0.210, -0.180]	100
	M4	2	2	0.586	-0.156	[-0.171, -0.126]	82.8

	M5	2	5	0.277	-0.114	[-0.132, -0.089]	80.9
	M6	1	6	0	0	[0,0]	100
T. socolofi	M1	3	3	0.128	-0.086	[-0.103, -0.069]	68.7
	M2	2	5	0.453	-0.082	[-0.143, -0.040]	39.5
	M3	2	1	0.993	-0.186	[-0.204, -0.133]	93.2
	M4	2	4	0.499	-0.082	[-0.110, -0.064]	28.2
	M5	2	2	0.367	-0.124	[-0.131, -0.110]	90.9
	M6	1	6	0	0	[0,0]	100
Genus	M1	3	3	1	-0.158	[-0.165, -0.149]	99.9
Thorichthys	M2	2	4	1	-0.149	[-0.157, -0.142]	99.9
	M3	2	2	1	-0.201	[-0.208, -0.193]	70.2
	M4	2	1	1	-0.205	[-0.214, -0.190]	70.2
	M5	2	5	1	-0.131	[-0.137, -0.123]	100
	M6	1	6	1	0	[0,0]	100

The best-supported model (lowest gamma value) is indicated in gray

Figure legends

Figure 1. Landmarks (black dots) and semilandmarks (red dots) configuration used in *Thorichthys* in the geometric morphometric analysis.

Figure 2. Graphical representation of the six models used in the modularity analysis in *Thorichthys.* Each color represents a module.

Figure 3. Principal Components Analysis (PC1 vs PC2) derived from geometric morphometric analysis in *Thorichthys.* Circles represent means for species.

Figure 4. Graphical description of the morphological variation in the species of *Thorichthys.* The black lines represent the shape change in each direction of the PC score and the gray lines represents average shape.
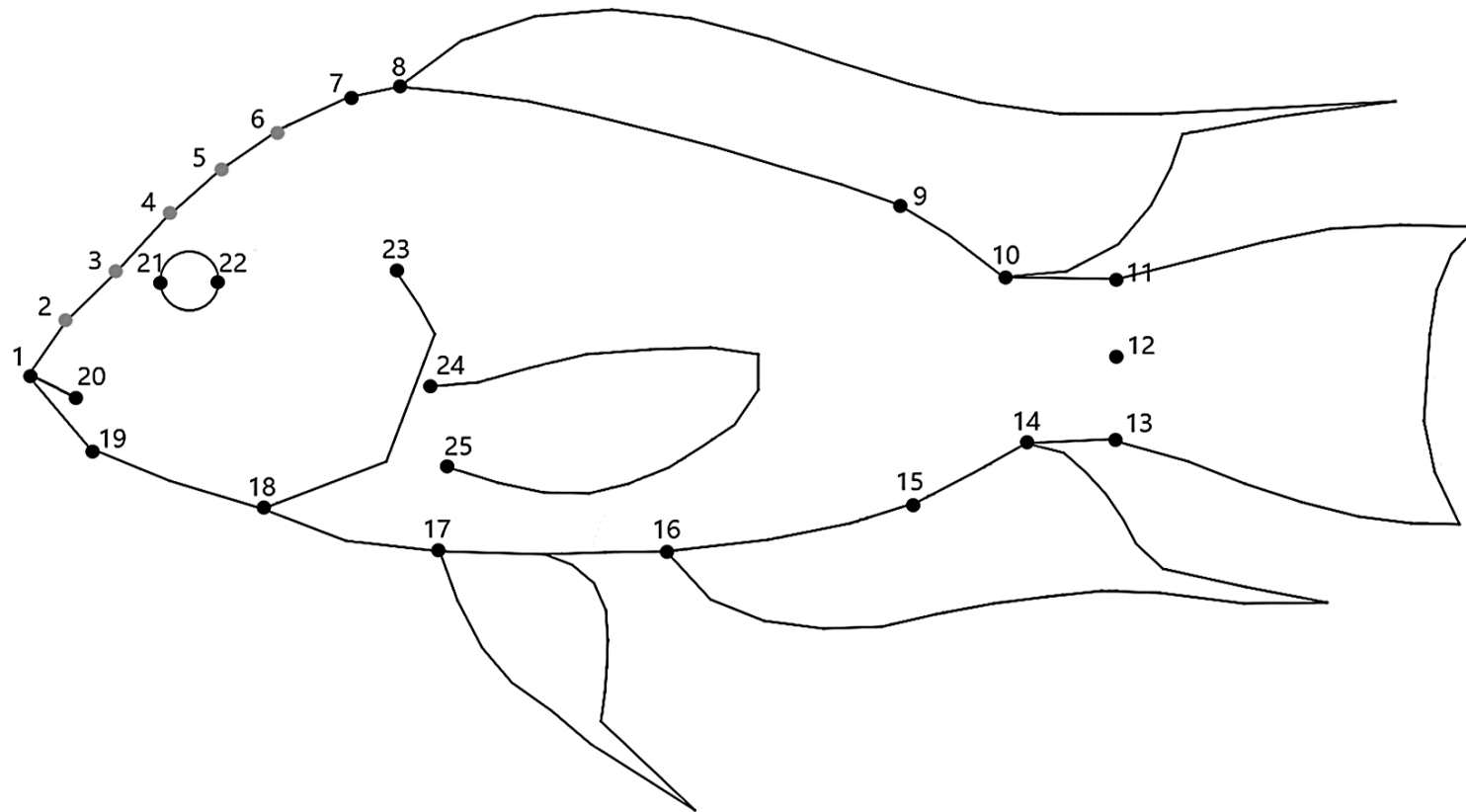
Figure 1 Landmarks (black dots) and semilandmarks (gray dots) configuration used in *Thorichthys* in the geometric morphometric analysis.
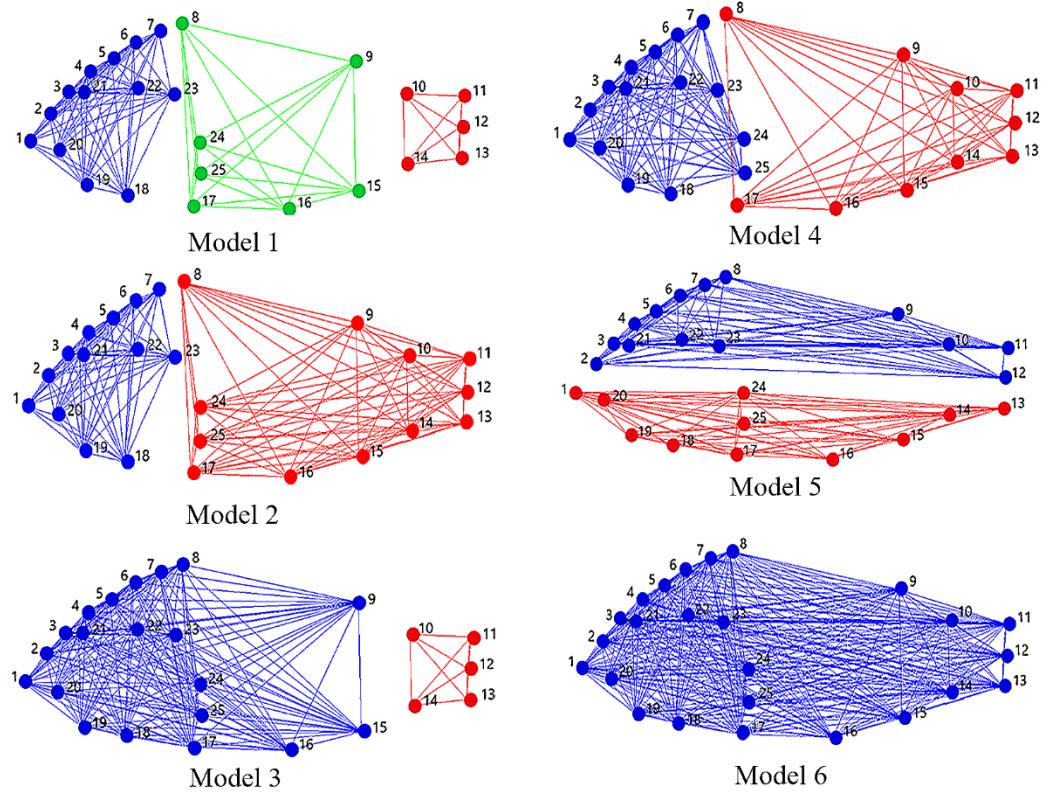
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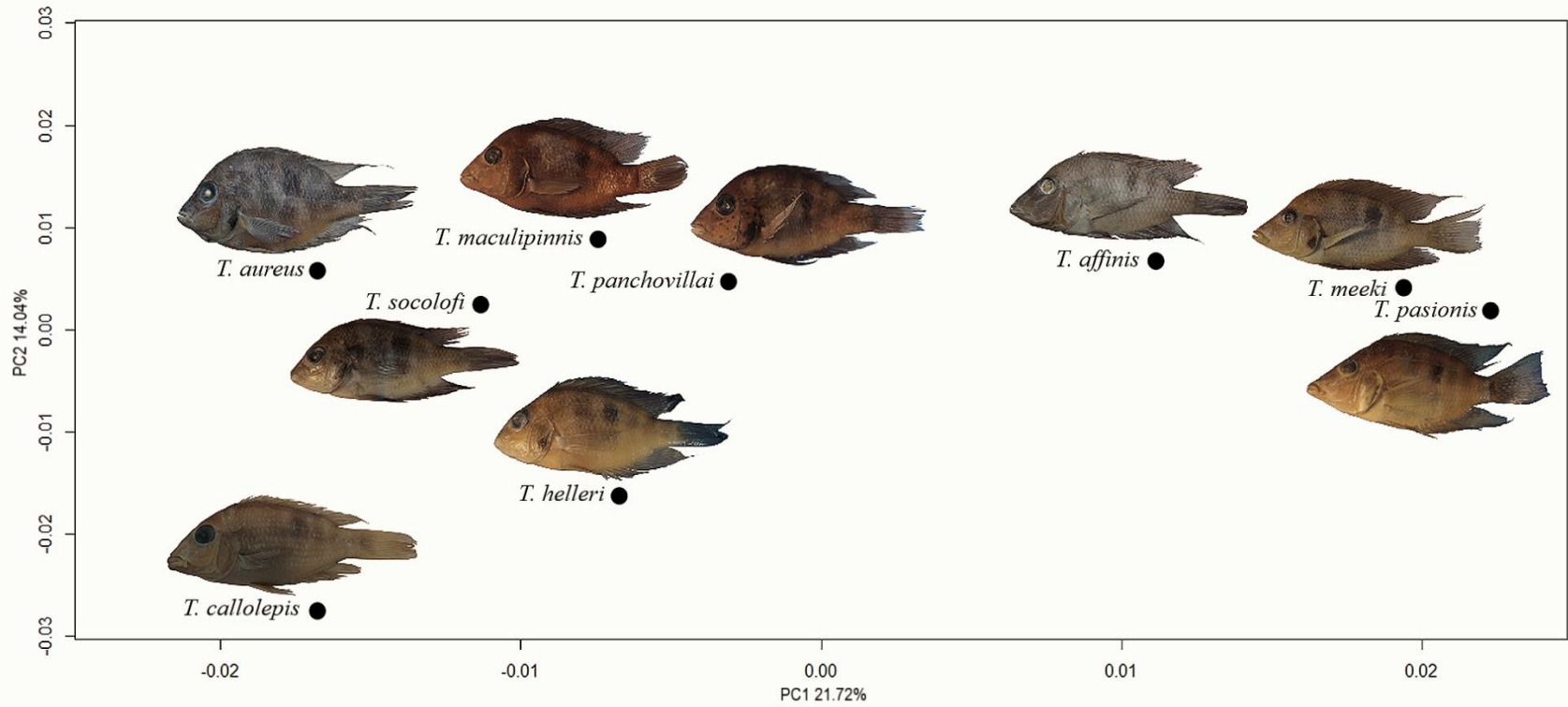
Figure 3 Principal Components Analysis (PC1 vs PC2) derived from geometric morphometric analysis in *Thorichthys*.
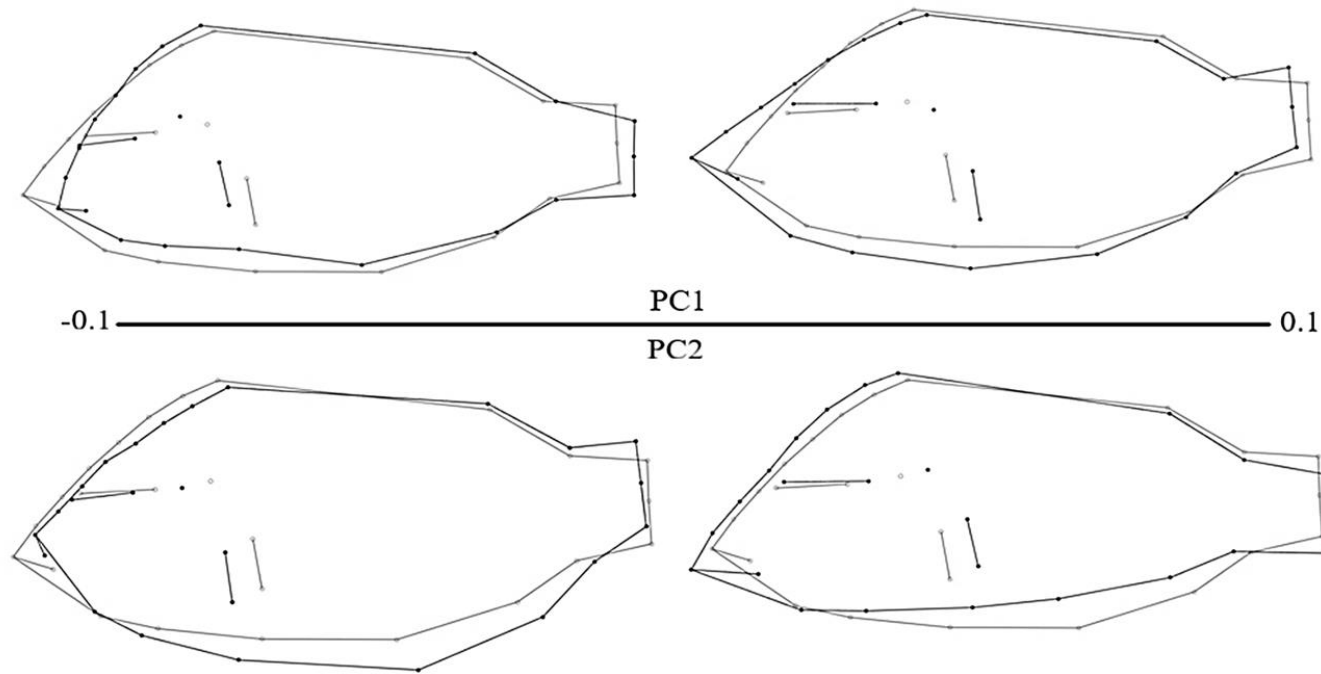
Circles represent means for species.

Figure 4 Graphical description of the morphological variation in the species of *Thorichthys*. The black lines represent the shape change in each direction of the PC score and the gray lines represents average shape.

CAPÍTULO 3. CONCLUSIONES

La variación morfológica de las especies se acentúa principalmente en el perfil dorsal de la cabeza, la altura de la parte media del cuerpo y en la longitud del pedúnculo caudal. Fue posible indentificar dos tipos de forma de cuerpo. Sin embargo, a nivel interespecífico, el análisis de modularidad indicó la presencia de dos módulos, el primero compuesto por la cabeza y las aletas pectorales, mientras que el segundo se compone de la aleta dorsal, anal, pélvicas y el pedúnculo caudal. A nivel intraespecífico, los patrones de modularidad se organizan en dos configuraciones: en siete de las nueve especies el primer módulo agrupo la cabeza y la parte media del cuerpo, mientras que el pedúnculo caudal formó el segundo módulo. En *T. aureus* y *T. affinis* la configuración modular constó de un módulo anterior y otro posterior; aunque en *T. aureus* el primer módulo está representado solo por la cabeza, mientras que en *T. maculipinnis* el módulo anterior agrupa la cabeza y las aletas pectorales. Los patrones de modularidad encontrados en las especies de *Thorichthys* no varían demasiado, puesto que en las nueve especies se encontraron únicamente dos módulos; cambiando únicamente las estructuras que componen a los módulos. De igual manera, la configuración modular correspondió con la filogenia actual del grupo, sugiriendo que la modularidad en *Thorichthys* tiene una base filogenética.

En este sentido, se recomienda evaluar el origen de cada módulo de los propuestos e incluso trabajar con nuevas configuraciones modulares, también es importante evaluar la forma total del cuerpo y por módulos dentro de la filogenia. De la misma manera es necesario evaluar la integración morfológica y modularidad de este grupo utilizando otros métodos de variación mínima y métodos gráficos (por ejemplo, método de matriz de distancia, método de Escoufier y el método de covarianza). Son necesarios los estudios que permitan medir la expresión cuantitativa de rasgos en los loci (eQTL), que ayuden desentrañar las bases de la modularidad en el cuerpo de los cíclidos Centroamericanos.

Este estudio analizó la variación morfológica de las especies del género *Thorichthys* y se encontró evidencia de una posible evolución morfológica por medio de módulos. Estudios posteriores que involucren otros grupos de cíclidos centroamericanos pueden ayudar a conocer el origen de la gran diversidad morfológica de este grupo de peces.

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