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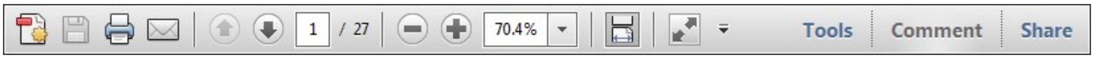

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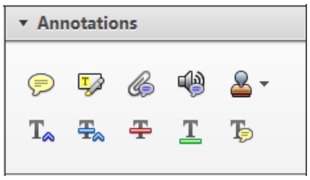

1. Replace (Ins) Tool – for replacing text.

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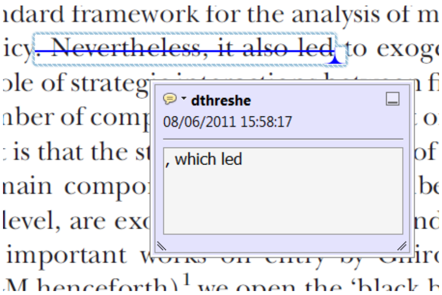
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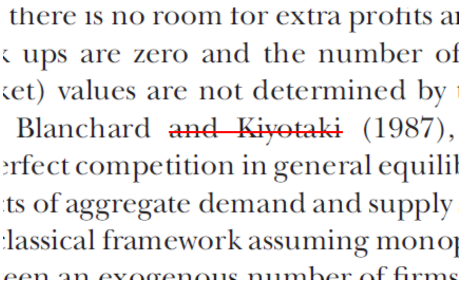

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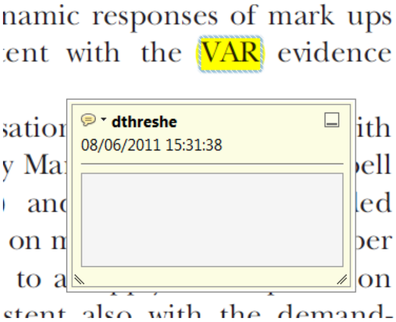
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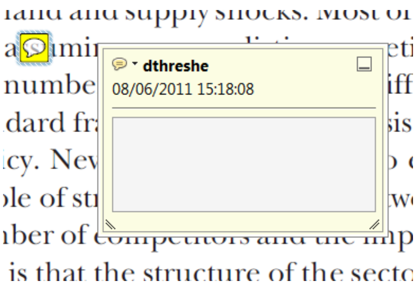
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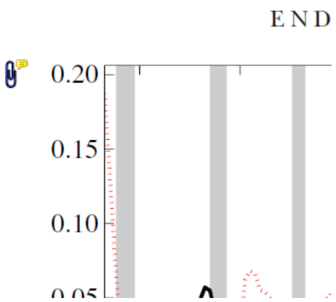

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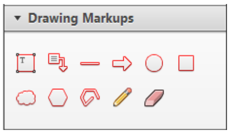

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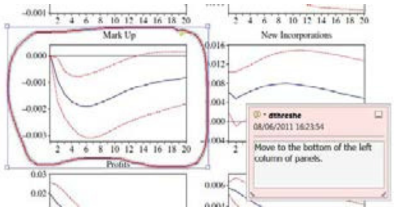

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[Palaeontology, 2015, pp. 1–16]

GIVING TAXONOMIC SIGNIFICANCE TO MORPHOLOGICAL VARIABILITY IN THE BIVALVE *PTYCHOMYA* AGASSIZ

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Abstract: The taxonomic significance of the morphological variability present in the genus *Ptychomya* Agassiz has remained obscure due to inadequacy of the traditional qualitative approach to account for complex patterns of variation. In this work, we focus on solving the distinction between intra- and interspecific variability in *Ptychomya* from Hauterivian marine beds of the Neuquén Basin (west-central Argentina), a longstanding and unresolved palaeontological issue, using the quantitative framework provided by geometric morphometrics and multivariate statistics. The species *Ptychomya koeneni* Behrendsen and several subspecies had been recognized in the studied succession by former authors based on qualitative morphological descriptions. We tested two hypotheses: (1) that there exist morphological discontinuities between hypothesized species that mark their boundaries; and (2) that morphology follows a pattern of geographical variation, suitable for recognition of subspecies. We find two morphological groups in the studied sample, with a significant morphological gap between them, supporting the idea that there are at least two species in the studied succession, *Ptychomya* cf *koeneni* and *Ptychomya coihuicoensis* Weaver. On the other hand, we found no correspondence between morphological and geographical distributions, leading to the conclusion that the named subspecies are invalid taxa. This study highlights the potential of this methodological approach for solving the taxonomy of this notorious genus of pandemic distribution during the Cretaceous.

Key words: *Ptychomya*, Argentina, Lower Cretaceous, geometric morphometrics, taxonomy.

PTYCHOMYA Agassiz, 1845 (Veneroida, Astartidae) is a genus of extinct marine burrowing bivalves. It comprises about 20 species and several subspecies, and can be found in marine deposits from all regions across the world except for Oceania and Antarctica. *Ptychomya* is restricted to the Cretaceous, being especially abundant in the Lower Cretaceous (Freneix 1972; but see Hallam *et al.* 1986), and it can be an abundant component of shallow marine bivalve assemblages. It is characterized by a medium-sized, oval to subrectangular, posteriorly elongated shell, slightly to markedly truncated towards the posterodorsal end, with a row of crenulations on the dorsal margin alongside its opisthodetic ligament. However, its most conspicuous trait is the external divaricate ribbing pattern. This can be strikingly complex, showing two clearly differentiated regions: one anterior with rounded oblique and the other posterior with subradial ribs (Fig. 1). Two series of chevrons can be recognized, the first one running dorso-ventrally and pointing towards the umbo (anterior chevron series), while the second runs from the umbo to (and points towards) the posterior shell margin (posterior chevron series). In addition, ribs may show a zigzag arrangement that can vary in intricateness depending on the region of the shell (Chavan 1952; Freneix 1972). Internally, the shell is far less eye-catching, bearing a typical astartoidean heterodont hinge, isomyarian adductor muscles and entire pallial line (Freneix 1972).

Whereas the features described above make *Ptychomya* a very distinctive, easily recognizable morphology-based taxon, they also make the recognition of taxonomically and palaeobiologically meaningful entities below the genus level a daunting task. In addition to variation in shell outline, members of this genus also show variability in the ornamentation pattern that includes differences in number, coarseness, separation, orientation and zigzagging of the ribs, which may range from subtle to marked even between members of the supposedly same species. Traditional, qualitative taxonomic descriptions are not

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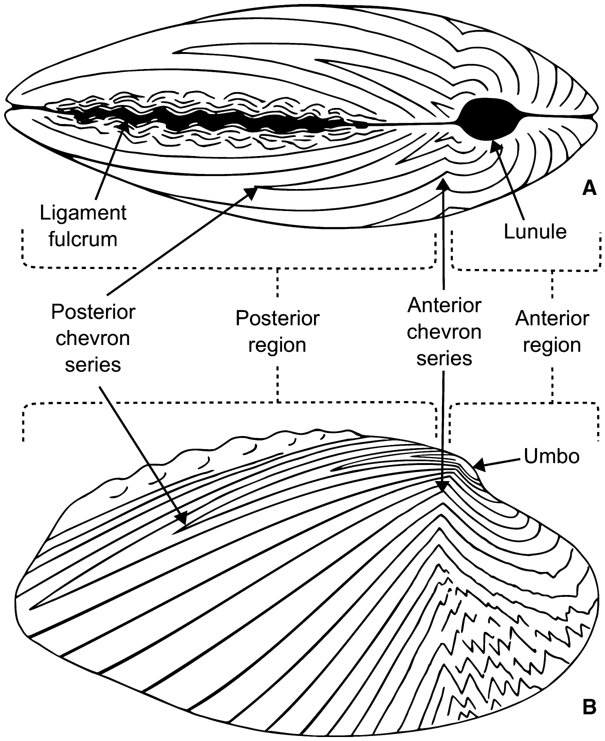


FIG. 1. Scheme of the external morphology of *Ptychomya* Agassiz. A, dorsal view. B, right lateral view.

well suited to reflect and make use of the complex morphological variability displayed by *Ptychomya*. For example, a common practice is to describe a species and then arbitrarily assign subspecies in order to formalize morphological variation not considered initially (e.g. Weaver 1931; Dietrich 1938; Mullerried 1948). This practice could have obscured the distinction between intra- and interspecific variability in this genus, at both local and regional scales.

Hence, we consider that a quantitative approach focused on discriminating intra- from interspecific variability is needed to address this problem. In this regard, the combination of geometric morphometrics and multivariate statistics constitutes a powerful tool that has been successfully used for the study of morphological variation in a variety of organisms, including extant and fossil bivalves (e.g. Crampton and Maxwell 2000; Márquez *et al.* 2010; Leyva-Valencia *et al.* 2012; Echevarría 2014). Thus far, no attempt of using this rigorous quantitative framework has been made for the study of *Ptychomya*, despite the advantages it offers.

However, application of this quantitative approach alone is not sufficient to solve this problem. Well-defined operative criteria stemming from strong conceptual foundations are also needed for proper analysis and interpretation of the data. There are a myriad of species concepts and definitions (Mayden 1997), but only few of them are applicable to fossil diversity. In palaeontology, the primary source of information is morphology of hard parts, and as such, species are mainly delimited using morphological criteria (i.e. they are morphospecies). As natural populations always exhibit some degree of variability in their attributes, the sole recognition of morphological differences is not sufficient to delimit new species. In order to do so, it is necessary to observe morphological discontinuities separating them (Darwin 1859; Simpson 1937; Mallet 1998; Zapata and Jiménez 2012). In this context, the validity of subspecies as taxonomic entities lies in the concept of geographical variation (i.e. the organization of morphological variation following a geographical pattern; Wilson and Brown 1953; Drooger 1954; Mayr 1982; Thorpe 1987; Mallet 2007). Otherwise, subspecies become just arbitrary sections in a continuum of variation.

The main goals of this work were to explore the clustering structure and taxonomic significance of the morphological variation of *Ptychomya* within the quantitative framework provided by geometric morphometrics and multivariate statistics. In particular, we investigated the case of *Ptychomya* from upper Hauterivian marine successions of the Neuquén Basin, in west-central Argentina. Two hypotheses were tested: (1) that there exist morphological discontinuities between hypothesized species; and (2) that morphology follows a pattern of geographical variation among hypothesized subspecies. After thorough morphometric and statistical analysis, we present a new systematic proposal for the genus *Ptychomya* from Argentina.

The genus Ptychomya *in the Lower Cretaceous of Argentina*

In Argentina, *Ptychomya* has been recorded from Valanginian–Barremian marine beds of the Neuquén Basin in the Quintuco (Weaver 1931), Chachao (Damborenea *et al.* 1979), Mulichinco (Leanza *et al.* 2001) and Agrio (Weaver 1931) Formations. To date, their only known representative is *Ptychomya koeneni* Behrendsen, 1892. (Haupt (1907) also recorded *Ptychomya germani* 3
Pictet and Campiche, 1866 in the Valanginian of Cerro Lotena. However, the actual taxonomic status of this record is difficult to assess because the material was not been figured and could not be retrieved from the collections of the Steinmann Institut für Geologie, Mineralogie und Paläontologie (Universität Bonn). Therefore, it was not taken into account in this work.). The type specimens were collected by W. Bodenbender from lower–upper Valanginian deposits of the Pilmatué Member of the Agrio Formation at Arroyo Truquicó section (Neuquén Province) and then donated to O. Behrendsen for study. They are currently housed in the Geoscience Centre of the University of Göttingen, Museum, Collections and Geopark, Göttingen (Germany).

This species has been also recorded abundantly in upper Hauterivian deposits of the Agua de la Mula Member from the Agrio Formation (Weaver 1931), where it exhibits a remarkable variability in external morphology of the valves including differences in ribbing pattern and geometry of shell outline (Weaver 1931; Aguirre-Urreta *et al.* 2011). This variability was taxonomically formalized by Weaver (1931) on qualitative grounds with the nomination of six subspecific varieties of *P. koeneni*. These varieties are as follows: (1) *P. koeneni* var. *agrioensis* Weaver, 1931 (Fig. 2A), characterized by its suboval, posteriorly acuminated shell, with fine straight posterior ribs and fine anterior ribs with an intricated anteroventral zigzag arrangement; (2) *P. koeneni* var. *coihuicoensis* Weaver, 1931 (Fig. 2B–C), which has an oval shell with very coarse, curved posterior ribs with a marked zigzag arrangement, and fine anterior ribs with an intricated anteroventral zigzag arrangement; (3) *P. koeneni* var. *groeberi* Weaver, 1931 (Fig. 2D–F), characterized by an acuminated, ventrally projected posteroventral end, with straight ribs in the posterior region; (4) *P. koeneni* var. *windhauseni* Weaver, 1931 (Fig. 2G–H), characterized by an acuminated, projected posteroventral end, and very coarse, straight posterior ribs that may show a strong zigzagging near the anterior chevron series, and coarse anterior ribs which may show a zigzag arrangement; (5) *P. koeneni* var. *vacaensis* Weaver, 1931 (Fig. 2I–L), which possesses an elongated shell, slightly truncated in the posterior end, with straight to slightly curved posterior ribs and fine anterior ribs which may show a subtle anteroventral zigzagging; and (6) *P. koeneni* var. *leufuensis* Weaver, 1931, which has an oval, elongated shell, with thin, straight ribs. From this point onwards, we treat these varieties as subspecies, which is the appropriate term to refer to subspecific taxa according to the International Commission on Zoological Nomenclature (ICZN, 1999).

However, the true taxonomic significance of the morphological variability of *Ptychomya* in the upper Hauterivian of the Neuquén Basin remains uncertain, and allocation of newly collected specimens to any of the Weaver's (1931) *Ptychomya* subspecies is often extremely difficult. The observed within-bed variation is wide-ranged and subtly gradational, implying that specimens from the same bed can be either very similar or very different. Thus, assessing the presence of a morphological gap from a qualitative point of view becomes a very difficult task. Moreover, the hypothesis of geographical variation had never been tested, so the validity of the subspecies nominated by Weaver (1931) had remained to be confirmed.

GEOLOGICAL SETTING

The Neuquén Basin is located in the Andes of west-central Argentina and corresponds mostly to the territories of Mendoza and Neuquén Provinces. It originated in the Late Triassic and had a nearly continuous sedimentation until the Early Palaeogene, accumulating deposits of several thousand metres that includes continental and marine deposits (Howell *et al.* 2005).

This basin has one of the richest and most complete fossil records of marine invertebrates for the Jurassic and Cretaceous in the Southern Hemisphere. In particular, the ammonoid faunas of the Neuquén Basin are of great importance and have allowed the establishment of high-resolution biostratigraphical schemes for western Gondwana and their correlation to the Tethys region (e.g. Aguirre-Urreta *et al.* 2005, 2007, 2011; Lazo *et al.* 2009; Riccardi *et al.* 2011; Aguirre-Urreta and Rawson 2012).

The material studied in this work is derived from the Agua de la Mula Member of the Agrio Formation. The latter outcrops across much of the basin and varies considerably in thickness, lithology and fossiliferous content. It spans from the lower Valanginian to the upper Hauterivian or lowest Barremian and was divided into three members, namely Lower or Pilmatué, Middle or Avilé and Upper or Agua de la Mula (Leanza *et al.* 2006). The latter has been interpreted as a shallow offshore marine deposit, which shows a marked shallowing tendency towards the top of the succession. Five ammonoid biozones have been recognized in this member, namely *Spitidiscus riccardii, Crioceratites schlagintweiti, Crioceratites diamantensis, Paraspiticeras groeberi* and *Sabaudiella riverorum,* ranging from upper Hauterivian to lowest Barremian (Aguirre-Urreta *et al.* 2007; Aguirre-Urreta and Rawson 2012). Recently, Aguirre-Urreta *et al.* (2015) provided U–Pb zircon radioisotopic ages of two tuffs from the Agua de la Mula Member, identifying the base of the upper Hauterivian in the *Spitidiscus riccardii* biozone (*c.* 129.5 Ma) and the Hauterivian/Barremian boundary near the top of the *Paraspiticeras groeberi* biozone (*c.* 127 Ma).

MATERIAL AND METHODS

The material for this study is based on 48 unpublished specimens of *Ptychomya* housed in the Palaeontological Collection of the University of Buenos Aires (CPBA), carefully selected from an original pool of 184 specimens taking into account that external ornamentation and shell outline needed to be well preserved for quantification. In addition, 12 syntype specimens of the subspecies of Weaver (1931; Fig. 2), housed in the Burke Museum of Natural History and Culture (BMNHC), USA, were included. The syntype of *P. k. leufuensis* Weaver, 1931, recorded in the Pilmatué Member of the Agrio Formation, was not considered in this work as it is older than the rest of the subspecies. The studied specimens are derived from the Agua de la Mula Member of the Agrio Formation, *Crioceratites*

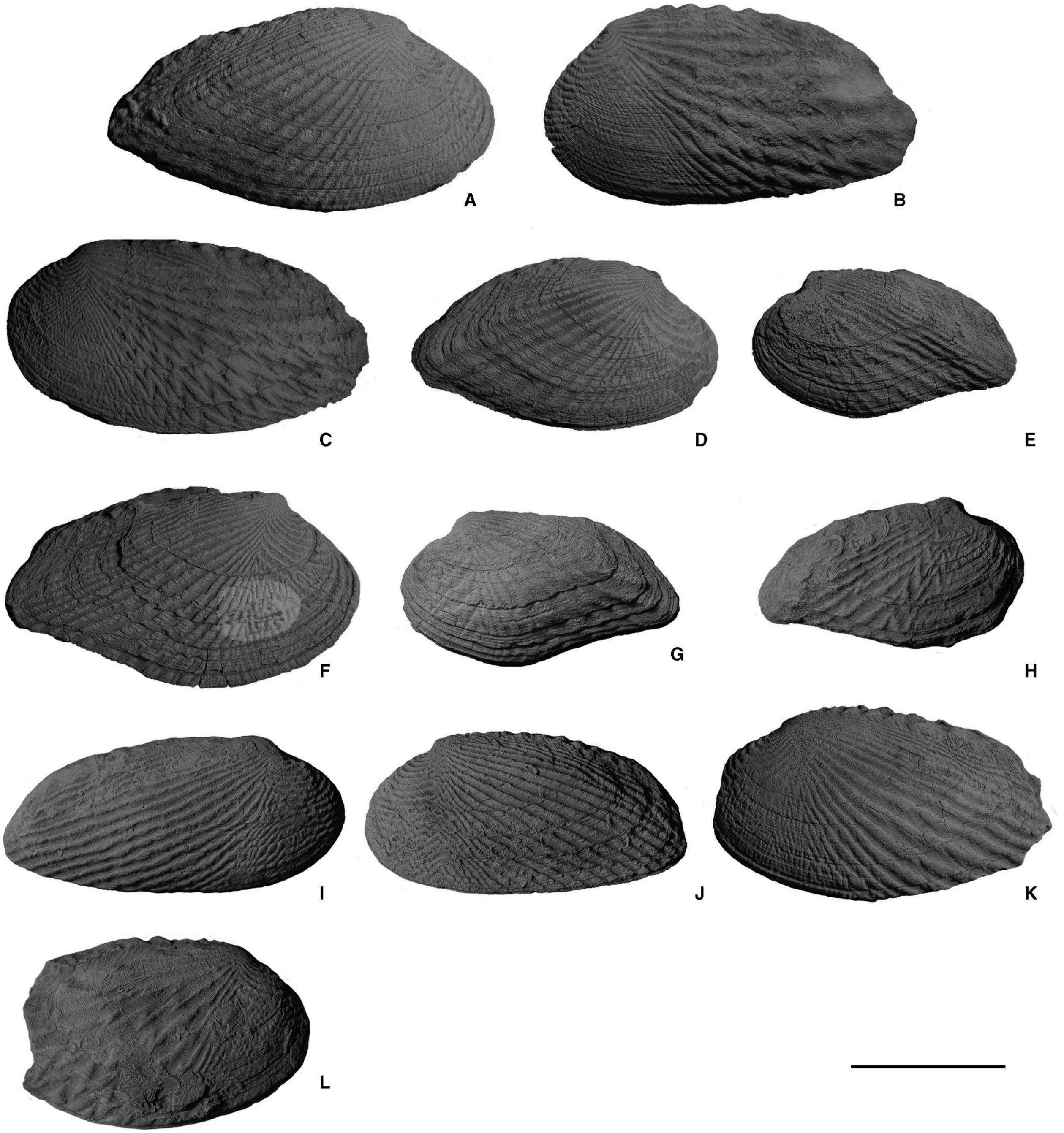


FIG. 2. Weaver's (1931) subspecies syntypes analysed in this work, from the Agua de la Mula Member of the Agrio Formation, late Hauterivian, Neuquén Basin. A, *Ptychomya koeneni agrioensis* BMNHC-28545, right lateral view. B–C, *P. k. coihuicoensis*; B, BMNHC-28549, left lateral view; C, BMNHC-28547, left lateral view. D–F, *P. k. groeberi*; D, BMNHC-235, right lateral view; E, BMNHC-234, left lateral view; F, BMNHC-233, right lateral view. G–H, *P. k. windhauseni*; G, BMNHC-232, left lateral view; H, BMNHC-231, right lateral view. I–L, *P. k. vacaensis*; I, BMNHC-28555, right lateral view; J, BMNHC-28553, left lateral view; K, BMNHC-28552, left lateral view; L, BMNHC-28554, right lateral view. All specimens were collected from Cerro Vaca Muerta, with the exception of specimen BMNHC-28549, collected from Arroyo Coihueco. Scale bar represents 3 cm.

diamantensis ammonoid zone, upper Hauterivian, Neuquén Basin, west-central Argentina (Aguirre-Urreta and Rawson 2012; see *Ptychomya* localities in Table 1 and Fig. 3).

Morphometric analysis

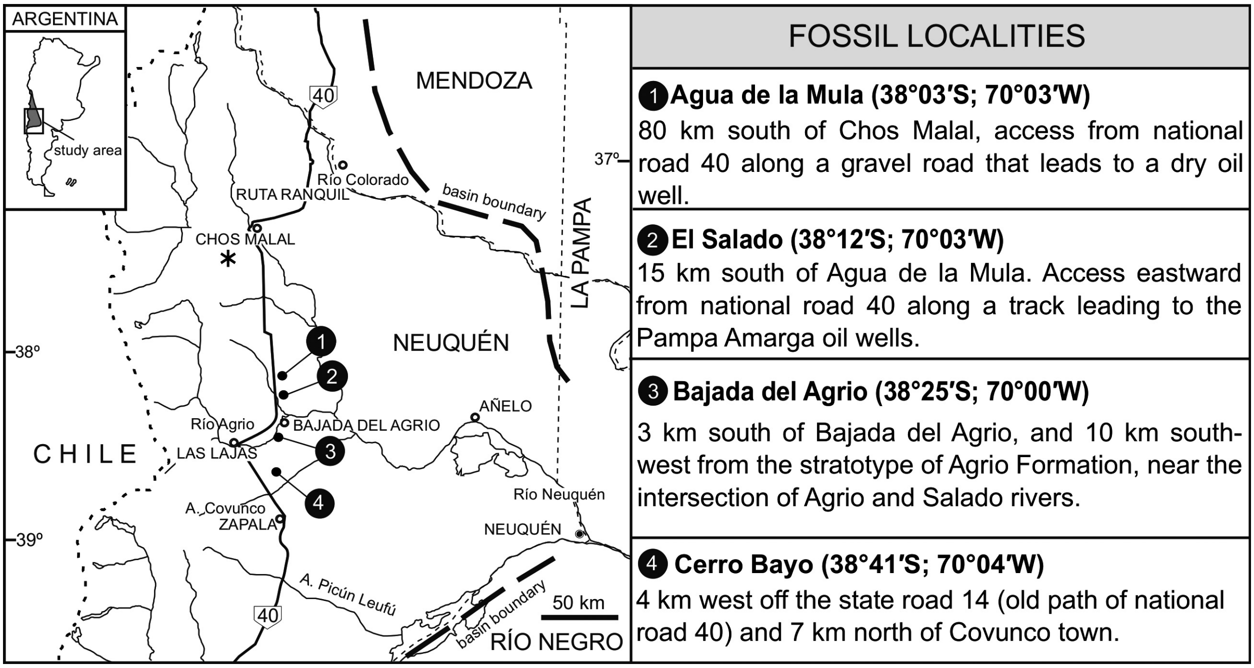
The external morphology of the left valve of all the studied specimens was digitalized in 2D with a Canon Powershot

TABLE 1. Sample size and procedence of the material of *Ptychomya* considered for this work.

Locality	Bed	Sampled specimens	Studied specimens
Agua de la Mula	24	3	0
	26	1	0
	28	40	5
	29	6	0
Bajada del Agrio	3	1	1
	4	5	4
	9	4	2
	10	84	17
	11	10	2
	14	10	2
Cerro Bayo	–	17	12
El Salado	–	3	3
Total	–	184	48

S50 camera fixed in a tripod, keeping constant the distance from camera to sample, camera settings and sample orientation. Morphometric analysis was carried out through Elliptic Fourier Analysis (EFA; Kuhl and Giardina 1982), a procedure that allows the quantification of geometry of closed contours. In EFA, a given number of harmonics (sine and cosine functions) are employed to imitate a set of target contours; the degree of fit depends upon the number of harmonics used, with more complex contours requiring a higher number of harmonics for a more accurate description. Each harmonic has four coefficients, which are used as shape descriptors of the target contours. Then, a principal component analysis of the variance–covariance matrix of coefficients of all harmonics is made. Resulting principal components represent different aspects of the geometric variation present in the set of studied contours (MacLeod 2012). Scores of specimens on principal components were used as the input variables for all subsequent statistical analyses.

Two different traits pertaining to the external morphology of the valves were quantified: shell outline and ribbing pattern, each one sampled using a different set of contours. The perimeter of the shell in lateral view (Fig. 4A–B) was used to capture its outline (growth lines were used when necessary and possible, i.e. when the shell margin was chipped). The contour used to describe ribbing pattern had a higher degree of complexity. The posterior rib forming the larger posterior chevron and the anterior rib converging with it were taken as reference (Fig. 4A). These reference ribs along with the third converging ventral posterior and anterior ribs from it and the margin of the shell were used to define this contour (Fig. 4C). Adopting this arbitrary but structurally constant criterion allowed to sample the complex design of ribbing pattern in a standardized and comparable way. For selection of the number of harmonics needed for proper description, the most geometrically complex contours of each set were reconstructed using different



FOSSIL LOCALITIES

❶ Agua de la Mula (38°03′S; 70°03′W)
80 km south of Chos Malal, access from national road 40 along a gravel road that leads to a dry oil well.

❷ El Salado (38°12′S; 70°03′W)
15 km south of Agua de la Mula. Access eastward from national road 40 along a track leading to the Pampa Amarga oil wells.

❸ Bajada del Agrio (38°25′S; 70°00′W)
3 km south of Bajada del Agrio, and 10 km southwest from the stratotype of Agrio Formation, near the intersection of Agrio and Salado rivers.

❹ Cerro Bayo (38°41′S; 70°04′W)
4 km west off the state road 14 (old path of national road 40) and 7 km north of Covunco town.

FIG. 3. Map of the Neuquén basin showing the fossil localities sampled for this work, with their corresponding GPS coordinates and access routes. Arroyo Truquicó, where the type material of *Ptychomya koeneni* of Behrendsen 1892 is derived from, is marked in the map with an asterisk.

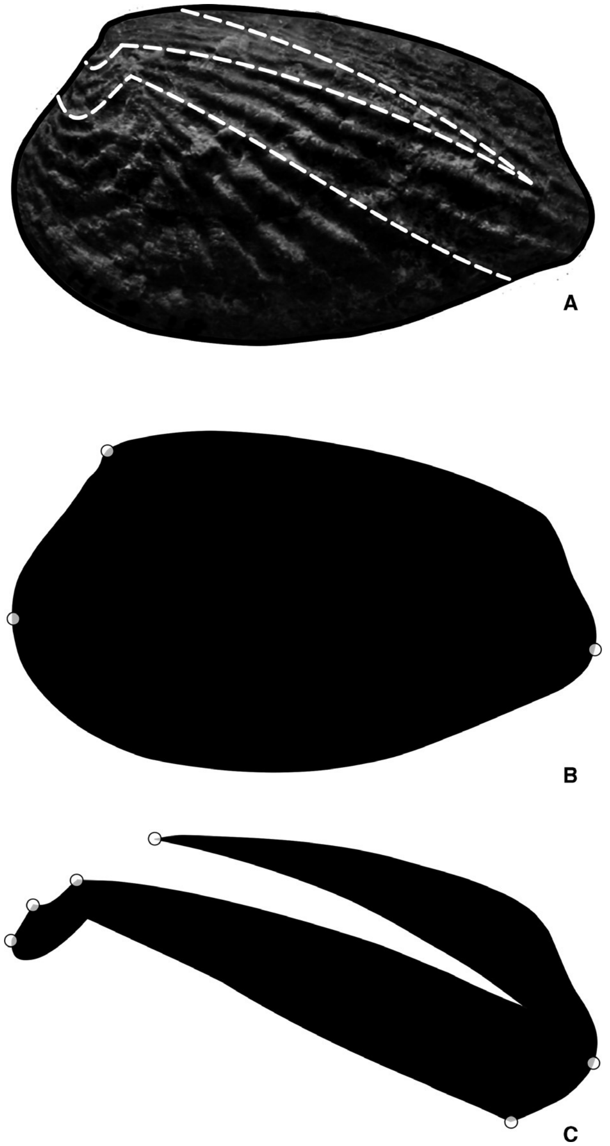


FIG. 4. The two sets of contours sampled in each specimen. A, *Ptychomya* specimen in left lateral view, with both contours drawn upon its surface (solid black line: shell outline contour; dashed white line: ribbing pattern contour). B, shell outline contour. C, ribbing pattern contour. Open circles indicate the landmarks used for Generalized Procrustes Analysis (GPA) normalization of each set of shapes.

numbers of harmonics. The lowest number of harmonics that deviates on average less than 0.01% of the centroid size from the best possible shape (calculated using the highest possible number of harmonics) was used to describe the shapes of each set of contours. In this way, seven and 40 harmonics were selected for description of shell outline and ribbing pattern contours, respectively.

One key feature of geometric morphometrics analyses is normalization, that is the removing of rotation, translation and scaling differences from the studied objects, leaving only the shape variation between them to be analysed (MacLeod 2009). There are a number of ways for achieving normalization of contour data (e.g. Kuhl and Giardina 1982; Ferson *et al.* 1985; Rohlf 1990; Dryden and Mardia 1998). In the present work, we performed a Generalized Procrustes Analysis (GPA; Gower 1975; Rohlf and Slice 1990) prior to EFA, after placing landmarks on the contours (Fig. 4). GPA superimposes the studied shapes by minimizing the squared distance between corresponding landmarks across the entire data set (MacLeod 2009), and it is a method proven to be superior to standard contour normalization procedures, both mathematically and biologically (Frieß and Baylac 2003). Both GPA and EFA were carried out in the R environment (R Core Team 2014) using the package Momocs v. 2.0-6 (Bonhomme *et al.* 2014).

Statistical analysis

Probable number of morphological groups in the data set was explored using the gap statistic (Tibshirani *et al.* 2001). This is a standard method for estimating the optimal number of clusters in a given data set by comparing the within-cluster dispersion to the expectation under a null reference distribution with no obvious clustering structure. For this analysis, Euclidean distances were selected as our dissimilarity measure, as they work well for continuous data. Three different clustering algorithms were used to determine the optimal number of groups: k-means non-hierarchical clustering, and hierarchical clustering based on Ward and centroid linkage algorithms. Classification of specimens was determined using partitioning around medoids (PAM), a non-hierarchical clustering method that is insensitive to both noise and outliers (Singh and Chauhan 2011). This classification was used as the input *a priori* categories for a linear discriminant analysis (LDA) with leave-one-out cross validation, to generate the canonical axes, identify the shape variables involved in-group distinction and produce a robust reclassification of the specimens. *A priori* probabilities for groups were set to be equal.

The degree of overlapping (i.e. the magnitude of the morphological gap) between the identified groups was tested following the procedure proposed by Zapata and Jiménez (2012) for quantitative continuous characters. Under this approach, it is assumed that phenotypic variation was the result of the effect of several genes and that there was random mating among conspecifics from a geographical region, thus making intraspecific morphological variation reasonably described by a normal distribution. Evidence for morphological discontinuity between hypothesized species arises in assessing the overlap of the tails of their probability distributions. This is achieved estimating tolerance regions for the distribution of each hypothesized species at an *a priori* nonzero frequency cut-off (i.e. certain percentage of the hypothesized species is encompassed within these regions; see Wiens and Servedio 2000) and assessing their overlap. Accordingly, tolerance regions for normal distribution were calculated for the distribution of each group along the canonical axes resulting from LDA. Univariate normal distribution of each group was tested with a Shapiro–Wilks normality test. An *a priori* frequency cut-off of $\beta = 0.1$ was selected (i.e. the tails of the distributions of hypothesized species may only overlap in an area comprising the 10% or less of their populations; otherwise, they are considered to be overlapped). Statistical significance was evaluated through a permutation test (1000 random permutations).

Finally, the validity of the subspecies nominated by Weaver (1931) was assessed by testing the hypothesis of geographical variation. Under this hypothesis, the morphological variability is expected to be organized following a geographical pattern. An LDA was performed in order to produce a classification of the samples to the subspecies established by Weaver (1931) and to generate a morphospace in which those categories were maximally separated, following the same procedure described above for LDA on PAM grouping. Then, correspondence between the morphological and geographical patterns was evaluated through visual inspection by plotting the specimens in a morphospace and distinguishing them according to their locality of origin.

All the analyses were performed in the R environment (R Core Team 2014) using the packages cluster (Maechler *et al.* 2014), NbClust (Charrad *et al.* 2014), MASS (Venables and Ripley 2002), sm (Bowman and Azzalini, 1997, 2015) and tolerance (Young 2010).

RESULTS

Three and seven principal components (i.e. shape variables) resulting from EFA were retained after principal component analysis for each set of contours, describing shell outline (O1–3) and ribbing pattern (R1–7), respectively (Figs 5–6, Table 2). Only components representing at least 1% of the original variation were retained. The first shell outline variable (O1; 92% of explained variation in shell outline) describes variation in concavity of the dorsal and posteroventral margins, the shape of the anterior end of the shell and the acumination of the posterior end, with higher values corresponding to shells with slightly concave dorsal and posteroventral margins, dorsally oriented anterior end and broader posterior end, while lower values of O1 correspond to shells with straight dorsal margin, slightly convex posteroventral margin, ventrally oriented anterior end and narrower posterior end. The second shell outline variable (O2; 4% of explained variation in shell outline) mainly describes changes in the height/length ratio of the shell. Higher values of O2 correspond with elliptic, relatively elongated shells, while lower values of O2 correspond to more subtrapezoidal shells.

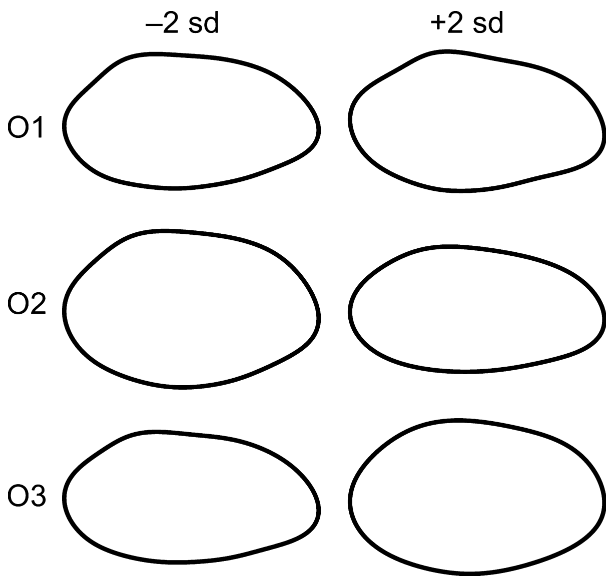


FIG. 5. The three shell outline variables retained from Elliptic Fourier Analysis (EFA) of the shell outline contours, depicted at −2 and +2 standard deviations.

On the other hand, variation in depth of the posterior chevron series, zigzagging and curvature of the posterior portion of the ribs, and development of the anterior portion of the ribs were somehow accounted by nearly all ribbing variables, reflecting the high degree of variability present on these characters. The first and second ribbing variables (R1 and R2; 38% and 32% of explained variation in ribbing pattern, respectively) mainly reflect variation in depth and relative width of the posterior chevron series and zigzagging of the posterior portion of the ribs (although with opposite trends). Higher values of R1 indicate deeper posterior chevrons pointing posteroventrally and straight, densely packed (or coarser) posterior ribs, while lower values correspond to shorter posterior chevrons, with more separated (or finer) posterior ribs showing zigzag arrangement. For the case of R2, higher values correspond to shorter posterior chevrons, posterior ribs posterodorsally curved with zigzag arrangement and more developed anterior portion of the ribs, while lower values indicate deeper posterior chevrons pointing posteriorly, straight posterior ribs with no zigzag arrangement and less developed anterior portion of the ribs. It is important to keep in mind that, although it may seem that some of these variables are representing essentially the same patterns of morphological variation, they are in

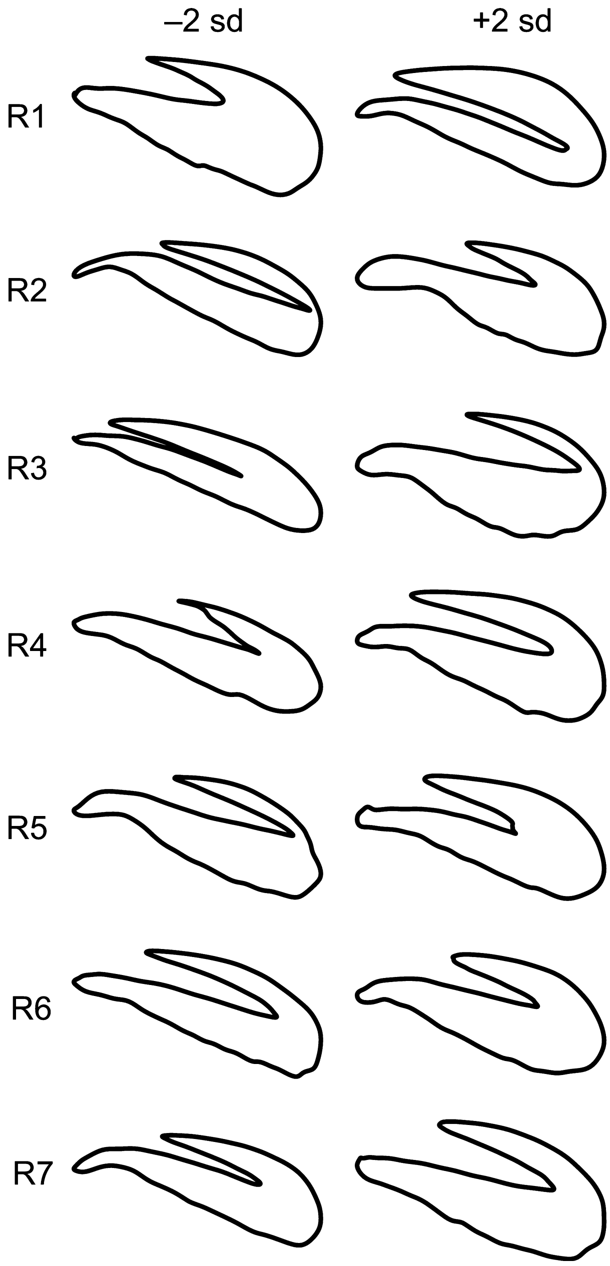


FIG. 6. The seven ribbing variables retained from Elliptic Fourier Analysis (EFA) of the ribbing pattern contours, depicted at −2 and +2 standard deviations.

TABLE 2. Percentage of variation explained by each morphological variable (O1–3, shell outline; R1–7, ribbing pattern), as well as loadings of each one on the canonical axis resulting from linear discriminant analysis (LDA) of groups A and B (see text for details).

Elliptic Fourier Analysis Variables	Explained (%)	Explained (cumulative %)	Loadings on Canonical axis 1
O1	92.406	92.406	0.192
O2	4.407	96.813	−0.331
O3	1.161	97.974	−0.007
R1	38.190	38.190	−0.753
R2	32.507	70.697	−0.090
R3	15.576	86.273	0.5988
R4	4.193	90.466	0.113
R5	2.485	92.951	−0.197
R6	1.741	94.692	−0.027
R7	1.153	95.845	0.071

fact mathematically orthogonal. Attention must be paid to the subtle geometrical differences expressed by each variable in order to make a proper assessment of the morphological variability present in the sample. Distributions of the specimens in the morphospaces defined by the first two variables of each set are depicted in Figures 7–8.

All clustering algorithms tested found that the best number of clusters in the data set was two (gap statistic (k-means) = −0.610; gap statistic (Ward) = −0.561; gap statistic (centroid) = −0.788), hereafter referred as groups A and B (see Discussion for a morphological description of these groups). Their probability distributions along the canonical axis resulting from the LDA separating them are essentially unimodal and Gaussian (Fig. 9; Shapiro–Wilks test of normality: W = 0.966, p = 0.248; W = 0.971, p = 0.806, respectively). Tolerance regions calculated for the two recognized morphological groups did not overlap (Fig. 9; permutation test, p < 0.001), providing statistical evidence for the existence of a morphological gap between them. Loadings of the shape variables on the canonical axis (Table 2) show that discrimination of groups A and B were mainly achieved using differences in the ribbing pattern, with variables R1 and R3 (posterior rib zigzag arrangement, broadness of the posterior chevron series) having the higher loadings. Shell outline variable O2 also contributed to separation of groups, although to a lesser extent.

LDA of subspecies of Weaver (1931) produced four canonical axes. The subspecies do not show a marked separation in the morphospace defined by the first two canonical axes (Fig. 10), with the exception of *P. k. coihuicoensis*, which appear distinctively separated from the rest of specimens mainly along the first canonical axis. Loadings of shape variables on the canonical axes (Table 3) indicate a similar pattern of differentiation to that observed for groups A and B: higher loading of variables R1 and R3 on the first canonical axis (72% of explained variation) followed by O2. On the other hand, variable R5 (shape of the posterior chevron series, upward concavity of the posterior ribs) had the highest loading on the second canonical axis (19% of explained variation), followed by R1 and R4 (shape of the posterior chevron series). No correspondence between geographical

COLOR

FIG. 7. Distribution of specimens in the morphospace formed by the first two shell outline variables.

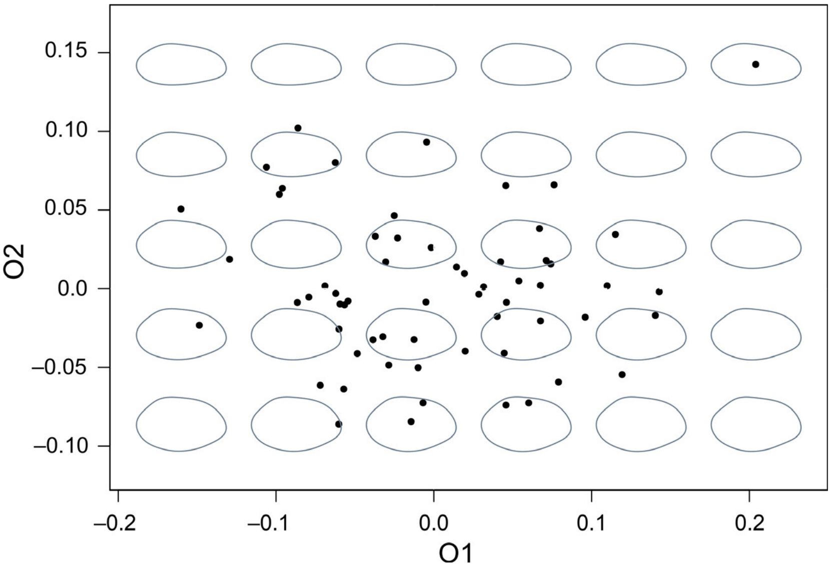


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FIG. 8. Distribution of specimens in the morphospace formed by the first two ribbing pattern variables.

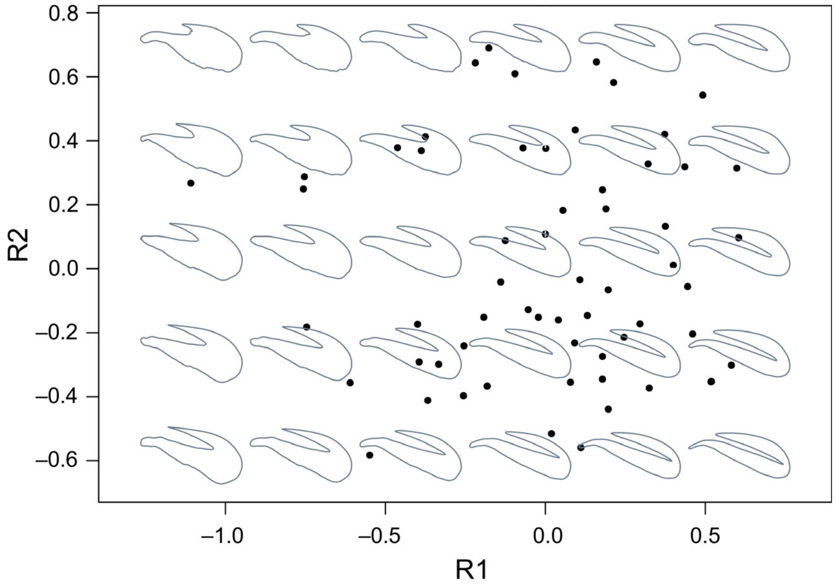
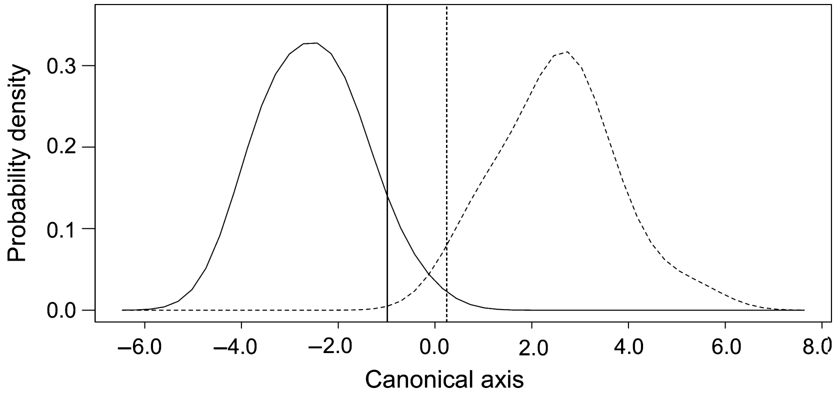


FIG. 9. Probability distributions for Group A (solid line) and B (dashed line) along the canonical axis, and the upper (solid vertical line) and lower (dashed vertical line) limits of the tolerance regions (β = 0.1) of each group, respectively. Note that the distribution of the groups does not overlap within their tolerance regions.



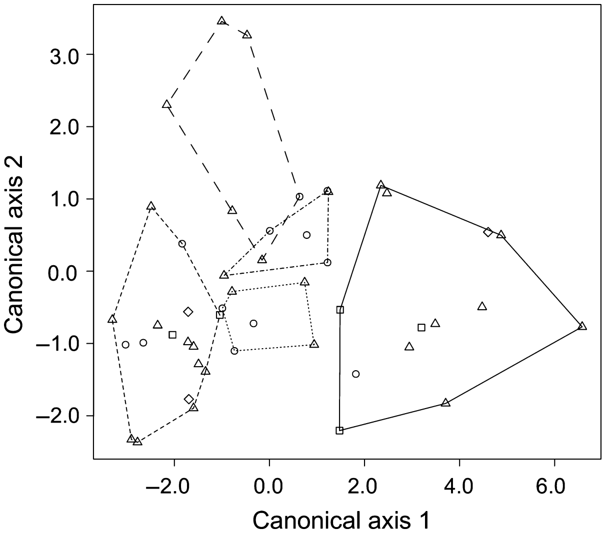


FIG. 10. Distribution of the 60 analysed specimens in the morphospace formed by the first two canonical axes resulting from the linear discriminant analysis (LDA) of Weaver's (1931) subspecies: *P. k. coihuicoensis* (solid line), *P. k. agrioensis* (short-dashed line), *P. k. windhauseni* (long-dashed line), *P. k. groeberi* (dotted line) and *P. k. vacaensis* (dotted-dashed line). Specimens are identified by its locality of provenance: Bajada del Agrio (triangles), Cerro Bayo (circles), El Salado (diamonds) and Agua de la Mula (squares).

TABLE 3. Loadings of the shape variables (O1–3, shell outline; R1–7, ribbing pattern) on the canonical axes resulting from linear discriminant analysis (LDA) of subspecies *sensu* Weaver (1931).

Variable	LD1	LD2	LD3	LD4
O1	0.243	−0.075	−0.038	0.183
O2	0.268	0.195	0.573	−0.065
O3	0.151	−0.004	0.190	−0.184
R1	−0.547	−0.216	0.225	0.448
R2	0.019	−0.162	−0.314	−0.180
R3	0.379	0.128	−0.103	0.819
R4	0.259	−0.213	−0.698	−0.040
R5	−0.202	0.815	−0.162	0.029
R6	−0.122	−0.205	−0.109	0.182
R7	−0.190	−0.072	−0.466	0.116

and morphological distributions was observed, as none of the subspecies were representative of a single locality.

DISCUSSION

The morphological variability displayed by this genus is organized into two main groups, which are separated by a statistically significant morphological discontinuity. This same pattern arose when subspecies were used as reference for grouping (Fig. 10), with one group constituted by specimens belonging to *P. k. coihuicoensis* and another group formed by the rest of Weaver's (1931) subspecies. This evidence supports the idea that there are at least two species present in the studied sample, as the morphological gaps are used to infer the action of some evolutionary force that prevented the lineages from phenotypic homogenization (Mallet 2008; Zapata and Jiménez 2012).

An alternative explanation to this phenomenon is that of temporal variation within a single lineage (i.e. anagenetic evolution), and the gap in morphology was produced by the loss of intermediate stages due to bias in the fossil record. However, the analyses performed here were carried out using material coming from a single biozone, the *Crioceratites diamantensis* ammonoid zone (the average duration of a single biozone in this section is 400 kyr), minimizing the sampled stratigraphical interval and therefore the sampled anagenetic variation. Moreover, specimens from either group were in some cases collected from the same bed. This criterion, known as the 'ancestral survival' criterion, was proposed by Gould (2002) to infer cladogenesis in fossil successions. Thus, the origin of observed morphological variation is regarded as cladogenetic rather than anagenetic.

When examining the classification of type specimens, one thing that stands out is that group B corresponds almost perfectly with those specimens nominated as *P. k. coihuicoensis* by Weaver (1931). This is the most easily recognizable of Weaver's (1931) subspecies, being characterized by 'its oval-elongate outline, very evenly rounded but narrow and somewhat attenuate posterior end, corrugated posterior dorsal border, small but deep and slightly elongate lunule, posterior slope of the umbonal line separating the anterior and posterior sculpture, very coarse heavy ribs on the posterior surface which show the zigzag arrangement and the fine ribbing on the anterior end with the intricate zigzag pattern' (Weaver 1931, p. 345).

The only mismatch found between group B and *P. k. coihuicoensis* was the assignment of one *P. k. vacaensis* syntype specimen (BMNHC-28554, Fig. 2L), along with the two *P. k. coihuicoensis* syntype specimens (BMNHC-28547 and -28549, Fig. 2B–C) included in the analyses, into the same morphological category. However, this specimen is remarkably similar to the syntypes of *P. k. coihuicoensis*, and its morphology coincides with the description that Weaver (1931) gave for *P. k. coihuicoensis*, rather than with the one made for *P. k. vacaensis* based on syntypes BMNHC-28552, -28553 and -28555. Moreover, the external view of this particular syntype of *P. k. vacaensis* was never included in any of the original plates of Weaver (1931; although the internal view was illustrated in pl. 37, fig. 225). Hence, we regarded

P. k. coihuicoensis from Weaver (1931) and our group B to be the same taxonomic entity. As noted above, this same conclusion was supported by the results from LDA of Weaver's (1931) subspecies: specimens belonging to *P. k. coihuicoensis* form a morphologically distinct group, separated from the rest of the subspecies. Thus, we adopt the subspecific name coined by Weaver (1931) to refer to this species, from now on *Ptychomya coihuicoensis* (Fig. 11).

The original description of *P. coihuicoensis* by Weaver (1931) is consistent with the morphometric characterization based on our results. As it can be inferred from results of LDA and EFA, *P. coihuicoensis* has a rather ovate, relatively high shell outline, with rounded transitions between dorsal, anterior and ventral margins (Fig. 12A). Posterior ribs are coarser and more separated, posterodorsally curved and show zigzag arrangement, while the posterior chevron series is broader and shorter (Fig. 12B).

The rest of the subspecies defined by Weaver (1931) fell into our group A, forming a continuum of morphological variation. Even though Weaver (1931) regarded them as belonging by default to *Ptychomya koeneni*, more research is needed before we can assure it is the same lineage as described by Behrendsen (1892) from the lower–upper Valanginian of Arroyo Truquicó in Neuquén. For this reason, we refer to them as *Ptychomya* cf. *P. koeneni*. We found no support for the geographical variation hypothesis, as the four subspecies of *P.* cf. *P. koeneni* from the upper Hauterivian of the Neuquén Basin failed to represent local geographical variants. Instead, they constitute arbitrary sections from the continuum of morphological variation they belong to. Thus, we conclude that there are no reasons to justify the establishment of subspecies of *P.* cf. *P. koeneni* in the upper Hauterivian of Neuquén Basin, ultimately converting *P. k. agrioensis, P. k. groeberi, P. k. vacaensis* and *P. k. windhauseni* into invalid taxa. *Ptychomya* cf. *P. koeneni* in the upper Hauterivian of Neuquén is characterized by its relatively elongated shell outline, with a more acuminated posterior end (Fig. 12C). Posterior ribs are fine and straight showing no zigzag arrangement, and form a deep posterior chevron series (Fig. 12D). This species shows great morphological variability that mainly involves aspects of shell outline as can be seen in the assessment of the dispersion of the shape variables relative to *P. coihuicoensis* (Fig. 13). Variables O1

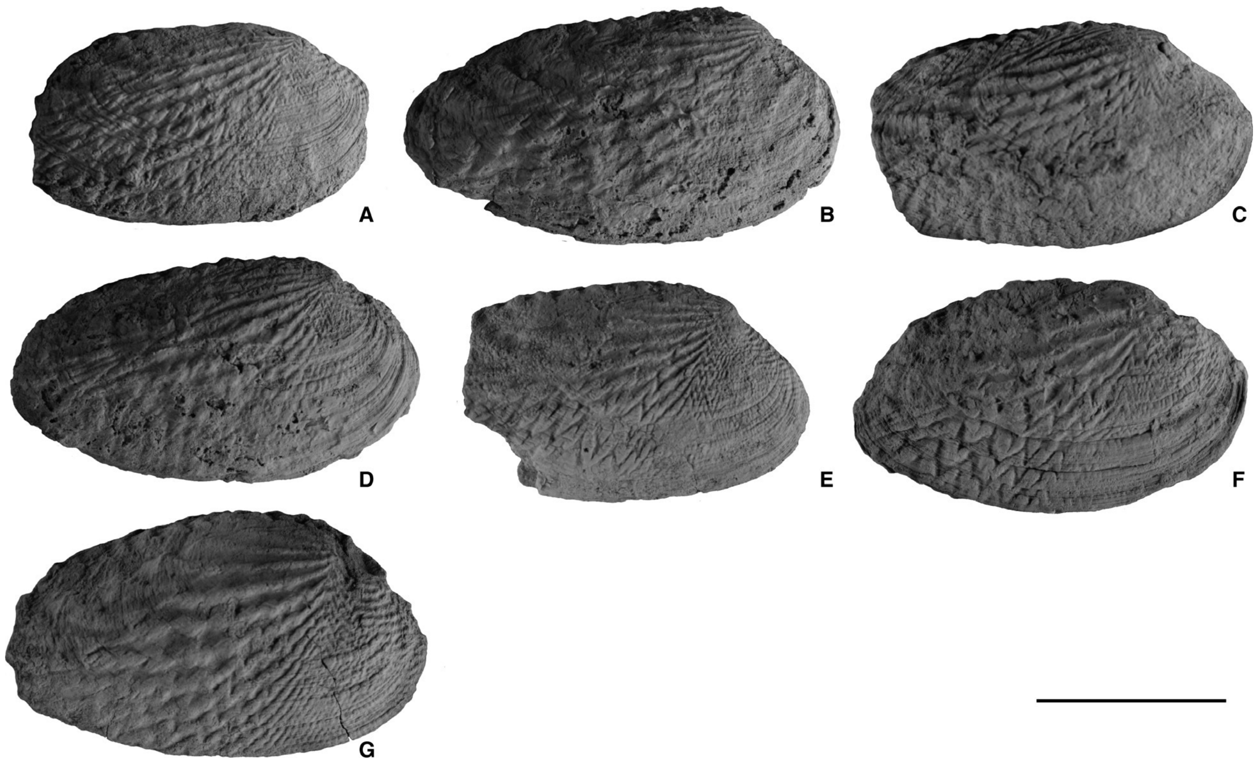


FIG. 11. *Ptychomya coihuicoensis* Weaver, 1931 from the Agua de la Mula Member of the Agrio Formation, late Hauterivian, Neuquén Basin. A, CPBA-21113.1. B, CPBA-21113.2. C, CPBA-21113.3. D, CPBA-21113.5. E, CPBA-21113.6. F, CPBA-21114.1. G, CPBA-21115.1. CPBA-21113.1–6 were collected from bed 10 of Bajada del Agrio; CPBA-21114.1 was collected from bed 28 of Agua de la Mula; CPBA-21115.1 was collected from bed 11 of Bajada del Agrio. All specimens are shown in right lateral view. Scale bar represents 3 cm.

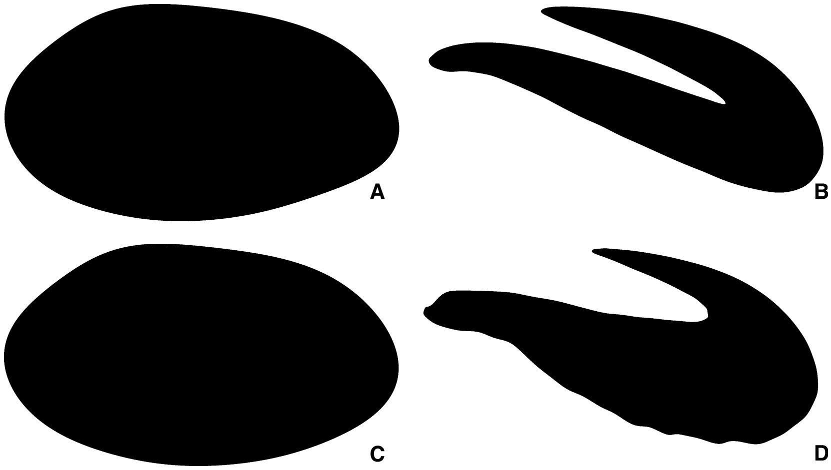


FIG. 12. Mean shapes of the two identified species, resulting from Elliptic Fourier Analysis (EFA). A–B, mean shell outline and ribbing pattern shapes for *Ptychomya* cf. *P. koeneni*. C–D, mean shell outline and ribbing pattern shapes for *Ptychomya coihuicoensis*.

and O3 have the highest relative dispersion, meaning that this variation is focused on the concavity of the dorsal and anteroventral margins, the shape of the anterior end, the relative elongation of the valve and the acumination of the posterior end. Ribbing variables show a somewhat greater dispersion in *P. coihuicoensis* (Fig. 13). This is probably due to the effect of a more variable ontogeny, as the initially straight posterior ribs of *P. coihucoensis* become more strongly zigzaged as the animal grew larger, while posterior ribs of *Ptychomya* cf. *P. koeneni* remain essentially straight through the entire ontogeny. This was confirmed by calculating the residuals of simple linear regressions between size (calculated as centroid size) and the morphological variables, and reassessing their dispersion. While *Ptychomya* cf. *P. koeneni* still showed greater dispersion for shell outline variables, dispersion of the ribbing variables for both species becomes similar (results not shown).

The integrative approach adopted here proved to be very effective in distinguishing between inter- and intraspecific variation and recognizing palaeobiologically meaningful taxa within this genus. Generally speaking, it consisted of the following: (1) the choice of the systematic hypotheses to be tested; (2) quantification of the morphological variation using geometric morphometrics; (3) exploration of the grouping structure present in the data set; and (4) quantitative assessment of the taxonomic level of variation in accordance with the chosen criteria. Although for each step there are several alternatives (e.g. different criteria for species–subspecies delimitation, the use of landmarks and semilandmarks to quantify the variability, or the use of different clustering algorithms), we

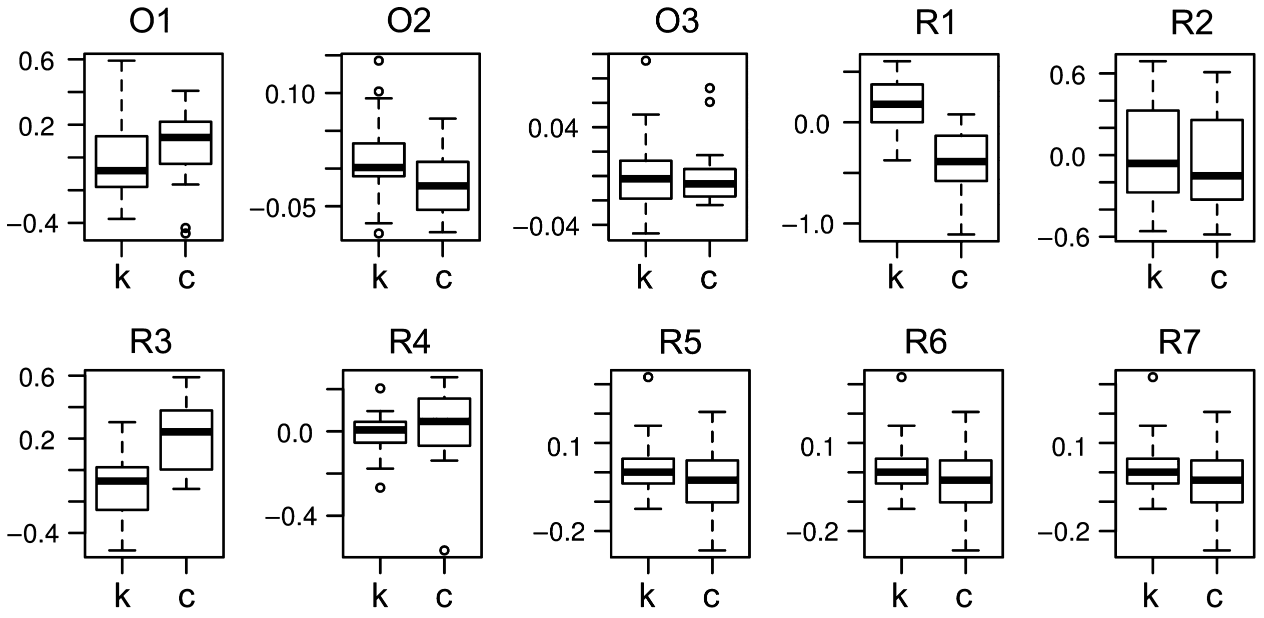


FIG. 13. Box plots of the shape variables analysed. k, *Ptychomya* cf. *P. koeneni*; c, *Ptychomya coihuicoensis*.

think the particular choices we have made were the best suited to deal with the study case of *Ptychomya*. This approach is easily applicable to the occurrence of this genus in other regions, as the only major restrictions are the quality of preservation and sample size. However, these restrictions are attenuated by the possibility of using the growth lines of the shell and the relatively low sample size needed to identify the same pattern reported here in a reduced data set of 44 individuals (Milla Carmona 2014). Additional considerations to keep in mind are geographical and stratigraphical resolution. The former needs to be set depending on the taxonomic scale of the study; for example, if the only purpose is identification of morphospecies, it is not necessary (although desirable) to sample a wide geographical distribution. As for the latter, care must be taken to avoid confusion between anagenetic and cladogenetic variation. In this regard, the 'ancestral survival' criterion (Gould 2002) can be useful to identify certain cases of cladogenesis.

Institutional abbreviations. CPBA, Colección de Paleontología, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires Pabellón II, Ciudad Universitaria, 1428 Buenos Aires, Argentina; BMNHC, Burke Museum of Natural History and Culture, Seattle, USA.

SYSTEMATIC PALAEONTOLOGY

Family ASTARTIDAE d'Orbigny, 1844
Subfamily PTYCHOMYINAE Freneix, 1972

Genus PTYCHOMYA Agassiz, 1845
Ptychomya coihuicoensis Weaver, 1931
Figures 2B–C, 2L, 11

v. 1931 *Ptychomya koeneni* var. *coihuicoensis* Weaver, pl. 37, figs 214–216, 224.
v. 1931 *Ptychomya koeneni* var. *vacaensis* Weaver, plate 37, fig. 225 (not pl. 37, figs 212–213; pl. 38, fig. 230).
v. 2011 *Ptychomya koeneni* var. *coihuicoensis* Weaver; Aguirre-Urreta *et al*., p. 481, fig. 10g.

Diagnosis. Oval-elongate outline, very evenly rounded but narrow and somewhat attenuate posterior end, corrugated posterior dorsal border, small but deep and slightly elongate lunule, posterior slope of the umbonal line separating the anterior and posterior sculpture, very coarse heavy ribs on the posterior surface which have fine ribs on the anterior surface with an intricate zigzag pattern (modified from Weaver 1931).

Age. Late Hauterivian, *Crioceratites diamantensis* ammonoid zone (Aguirre-Urreta *et al*. 2007).

Type material. Three articulated specimens and one right valve from Cerro Vaca Muerta, Agua de la Mula Member, Agrio Formation (BMNHC-28547, -28548, -28549 and -28550).

Additional material. Six articulated specimens from Bajada del Agrio, bed 10 (CPBA-21113.1-6), one articulated specimen from Bajada del Agrio, bed 11 (CPBA-21114.1), one articulated specimen from Agua de la Mula, bed 28 (CBPA-21115.1) and one right valve from Cerro Vaca Muerta (BMNHC-28554). All these specimens are derived from the Agua de la Mula Member, Agrio Formation.

Description. Medium-sized, moderately inflated shell, equivalve and inequilateral, with oval, anteroposteriorly elongated shell outline and a narrow and slightly truncated posterior end. Umbo prosogyrate and low, located at one-fifth of the total length of the shell from the anterior end, marking the highest point of the shell. Dorsal margin posterior to the umbo straight, with a row of crenulations (around 11 in the largest specimens). The transition between dorsal and posterior margins rounded, making an angle of approximately 200°. Posterior margin rounded, slightly truncated, passing imperceptibly towards the ventral margin, which is convex, and gradually towards the anterior end. Anterior margin rounded and short.

Ornamentation divided into two well differentiated regions: an anterior region with thin, oblique, markedly curved, concave-upward ribs, which continue in a posterior region where they become thick, subradial, asymmetrical in transversal view (with the highest angle pointing dorsally), curving posterodorsally along their course. Ribs exhibit a marked zigzag arrangement in posterior region. Transition between anterior and posterior regions of the sculpture abrupt, with ribs changing their orientation in approximately 250°, forming a series of chevrons that point to the umbo (anterior chevron series). These chevrons are aligned, forming a straight line slightly inclined posteroventrally, which runs dorsoventrally from the umbo to the ventral margin and separates the anterior and posterior regions of the ornamentation.

In both anterior and posterior regions of the shell, dorsal and ventral subregions can be recognized. Dorsal subregion of the anterior region bears well-defined rounded oblique ribs, while ribs from ventral subregion of the same region show an intricate zigzag arrangement. Dorsal subregion of the posterior region shows ribs that are oriented subradially and slightly curved posterodorsally, and then abruptly change their direction in an angle of approximately 30°, towards the dorsal margin. The change in orientation of these ribs defines a second series of chevrons that points towards the posteroventral end (posterior chevron series). Ribs from ventral subregion of the posterior region are similar to the dorsal ribs, but they do not change their direction and culminate in the posterior or, in the case of most ventral ribs, the ventral margin.

Affinities. This species was previously thought to be a subspecies of *Ptychomya koeneni* Behrendsen, 1892 (e.g. Weaver 1931, Aguirre-Urreta *et al*. 2011). Once we established their specific rank, *P. coihuicoensis* and *P. koeneni* constitute the only two representatives of the genus *Ptychomya* in the upper Hauterivian and, provisionally, in the Lower Cretaceous of Argentina, at least until further studies can clarify the taxonomic status of *P.* cf. *P. koeneni*. Both species could be considered to be more closely related to each other than with any other fossil bivalve taxon of the area, although this still has to be confirmed through a rigorous phylogenetic analysis. Freneix (1972) recognized four groups of *Ptychomya* species, based on the external morphology of their valves. The *Ptychomya koeneni* group is characterized by its suboval to subtrapezoidal shell outline, with an obliquely truncated posterior end, and irregular posterior ribs. This group includes endemic forms from South America (*Ptychomya crispa* Philippi, 1899 and *Ptychomya buchiana* Karsten, 1886) and South Africa (*Ptychomya renniei* Freneix, 1972). *Ptychomya coihuicoensis* would belong to this group, being more akin to the Gondwanan species than to European, Asian or North American representatives.

Within this group, *P. crispa* is the species that most resembles to *P. coihuicoensis,* mainly due to the presence of coarse ribs with zigzags in the posterior region of the shell, and their suboval (although apparently more elongated) shell outline. However, the material depicted in Philippi (1899, pl. 35, fig. 9) is not just incomplete, but also poorly illustrated, making it difficult to assess in detail its morphology. On the other hand, *P. buchiana* from the Aptian of Colombia (Dietrich 1938, pl. 22, fig. 3) has a suboval shell outline and lacks the zigzag arrangement in the posterior region. Finally, *P. renniei* from the Early Cretaceous of South Africa (Rennie 1936, pl. 49, figs 1–2) can be distinguished from *P. coihuicoensis* by its fine posterior ribs that lack zigzags, and by its shell outline which slightly broadens posteriorly, giving it a rectangular shape.

Remarks. *Ptychomya coihuicoensis* differs from *P.* cf. *P. koeneni* in external valve morphology. Although both of them show the characteristic divaricate pattern, ribs from the posterior region of *P. coihuicoensis* are coarser, curved posterodorsally and exhibit a conspicuous zigzag arrangement, while in *P.* cf. *P. koeneni*, they are thin, straight to slightly curved posterodorsally and lack zigzags. Posterior chevron series of *P. coihuicoensis* is less acute and broader than the one of *P.* cf. *P. koeneni*. Shell outline also differs, having *P.* cf. *P. koeneni* relatively more elongated, subtrapezoidal shells, and bearing in some cases a posteroventral projection that is absent in *P. coihuicoensis*. The quantitative study carried out in the present work brought evidence of a morphological discontinuity between these two taxa, justifying their establishment as separate species.

CONCLUSION

The genus *Ptychomya* in the upper Hauterivian of the Neuquén Basin, Argentina, was previously thought to be represented by a single species, *Ptychomya koeneni* Behrendsen, with six subspecies nominated by Weaver (1931). Our results do not support this scenario. Instead, this genus is composed of, at least, two coexisting species: *P.* cf. *P. koeneni* and *P. coihuicoensis*, which are separated by a significant morphological gap. Subspecies recognized by Weaver (1931) do not define geographical groupings and instead appear to define arbitrary sections of a continuum of morphological variation. Hence, with the exception of *P. k. coihuicoensis*, found to be here a separate species by virtue of the aforementioned morphological discontinuity, we regarded the subspecies of Weaver (1931) as invalid taxa.

The integration of morphological criteria, biological concepts and quantitative framework adopted in this work proved to be very effective at solving the distinction between inter- and intraspecific variation and identifying palaeobiologically meaningful taxa in *Ptychomya*. This general approach can be easily extended to the study of other occurrences of *Ptychomya*, in order to give taxonomic significance to the complex morphological variability known in this genus using a rigorous quantitative protocol.

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DATA ARCHIVING STATEMENT

Data for this study are available in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.58k0t.

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