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Taxonomic Identity in Microbial Eukaryotes: A Practical Approach Using the Testate Amoeba *Centropyxis* to Resolve Conflicts Between Old and New Taxonomic Descriptions

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ABSTRACT. The present work focuses on 12 taxa of the genus *Centropyxis* Stein, 1857 to explore the conflict between traditional and contemporary taxonomic practices. We examined the morphology, biometry, and ecology of 2,120 *Centropyxis* individuals collected from Tiete River, Sao Paulo, Brazil; with these new data we studied the consistency of previously described species, varieties, and forms. We encountered transitional forms of test morphology that undermine specific and varietal distinctions for three species and nine varieties. Biometrical analyses made comparing the organisms at the species level suggest a lack of separation between *Centropyxis aculeata* and *Centropyxis discoides*, and a possible distinction for *Centropyxis ecornis* based on spine characteristics. However, incongruence between recent and previous surveys makes taking any taxonomic–nomenclatural actions inadvisable, as they would only add to the confusion. We suggest an explicit and objective taxonomic practice in order to enhance our taxonomic and species concepts for microbial eukaryotes. This will allow more precise inferences of taxon identity for studies in other areas.

Key Words. Biometry, *Centropyxis*, morphology, taxonomy.

HIGHER-LEVEL classification has been one of the main foci of microbial eukaryotic research in the last decade (Adl et al. 2005; Baldauf 2003; Baldauf et al. 2000; Cavalier-Smith 1998; Cavalier-Smith, Chao, and Oates 2004; Nikolaev et al. 2005; Patterson 1999; Richards and Cavalier-Smith 2005; Smirnov et al. 2005; Sogin, Hinkle, and Leipe 1993); researchers have increasingly advanced towards tree-based and monophyletic classification schemes. However, there is little discussion of taxonomy and nomenclature in the least inclusive groups, the building blocks for classificatory edifices—genera and species (Patterson 1999).

Taxonomic practices in microbial eukaryotes are fundamentally different from those applied to other sets of organisms due to (1) the study and preservation of most protists being different from plants or animals and (2) the lack of a standardized approach for all organisms (Patterson and Larsen 1992). In the traditional morphological literature, few microbial eukaryotic taxa have been described in a standardized format, with statements that distinguish direct observation from interpreted data (see Berger 1978, Berger 1999; Foissner 1993; Foissner and Xu 2006; Gates 1978; Lynn 1976 for exceptions in ciliate taxonomy).

Taking advantage of new technology that yields new distinguishing characters, contemporary taxonomists are redescribing old species and discovering new species. The identity of organisms studied by earlier workers is often unclear because in most cases type material has not been designated and preserved, making it difficult for modern workers to ensure they are studying the same organism. There are rarely any attempts toward resolution of conflicts among past contributors or conflicts raised by new surveys. Furthermore, researchers describing these species rarely address the issue of species concept. Because one of the aims of nomenclatural taxonomy is stability (Corliss 1972; Patterson and Larsen 1992; Ride et al. 1999), this fundamental flaw contributes to the confusion.

The testate amoeba *Centropyxis* serves as a good example of the state of taxonomy of many protist genera. The type species *Centropyxis aculeata* (Ehrenberg 1838) has no assigned name-bearing type specimens. The genus is characterized by a flattened bilateral test that may be organic or made of mineral particles and diatom frustules agglutinated by organic matrix and that has a subterminal aperture. *Centropyxis* species usually inhabit freshwater, mosses, and soil. Species in this genus are distinguished by test morphology, since all exhibit an ellipsoid nucleus. More than 130 species and many varieties have been described to date, though many of these descriptions are inadequate (Meisterfeld 2002).

Similar species in the genus were described using characters that, when analyzed with modern morphological and biometric techniques, are ambiguous: the diagnostic character for *Centropyxis discoides* Pènard, 1890 is a ''shell more compressed anteriorly'' (Deflandre 1929). The high variability in virtually all test features (i.e. form and size of test; form and size of aperture; form, size, and number of spines) in species attributed to the genus *Centropyxis* has long been known (Cash and Hopkinson 1905; Chardez 1956, 1966a; Deflandre 1929; Leidy 1879; Medioli and Scott 1983, 1985; Netzel 1972, 1975; Ogden 1988; Pènard 1902; Root 1918; Wallich 1864; Wanner 1999). Still, many varieties and forms often based on small sample sizes, were distinguished using these characters. It is possible that these distinguishing characters fall into the natural variation of a single species. However, the possibility of continuous variation has never been examined. For example, Root (1918) concluded that *C. aculeata* is capable of producing spineless individuals in culture—spines being the only discriminatory feature of *Centropyxis ecornis*—yet no efforts have been made since to otherwise distinguish these two taxa.

The present work explores a closely related group of 12 taxa in the testate amoeba genus *Centropyxis* Stein, 1857, based on a large number of specimens collected from a natural fresh water environment. Its aim is to outline an objective approach for dealing with multiple and unclear taxonomic concepts.

MATERIALS AND METHODS

Centropyxis individuals were sampled from two localities in the Ecological Park of Tiete River, Sao Paulo, Brazil in February (summer) and August (winter) 2004. The first locality was in the river itself (23°29.374′S, 46°31.500′W), a flowing water environment. The second was in a marginal lake (23°29.055′S, 46°30.939′W), 100 m away from the river. In each locality, samples were taken separately from the sediment and from roots of floating aquatic plants. Sampling methods, biometry, morphological analysis, and scanning electron microscope (SEM) work follow Lahr and Lopes (2006). Specimens, preserved in 70% ethanol, and SEM stubs used for ultrastructural analyses are deposited at Laboratorio de Malacologia at Instituto de Biologia, Universidade de Sao Paulo (IBUSP).

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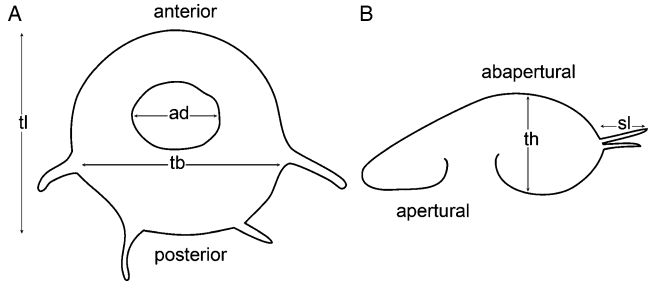

Fig. **1.** Schematic outline of test of *Centropyxis aculeata*, showing position of measured axis and polarity. **a.** Apertural view. **b.** Lateral view. tl, test length; tb, test breadth; th, test height; ad, aperture diameter; sl, spine length.

Terminology used in morphological descriptions follows Foissner and Korganova (2000), Lahr and Lopes (2006, 2007), and Lüftenegger et al. (1988). The 2,120 *Centropyxis* individuals were examined, identified, and measured using a light microscope; 50 individuals, representing a wide range of variability, were chosen for examination with the SEM.

Six morphometric characters were measured: test length (tl), test breadth (tb), test height (th), aperture diameter (ad), spine length (sl), and spine number (sn) (Fig. 1). Statistical analyses were performed using the programs STATISTICA 6.0 (StatSoft Inc. 1995) and SYSTAT 10.2 (Wilkinson 2000).

Specimens were classified to putative taxa using morphometric characters (Fig. 1) in conjunction with original and subsequent taxonomic descriptions (Bovee 1985; Chardez 1966a, b; Daday 1905; Decloitre 1978; Deflandre 1926, 1929; Hedley, Ogden, and Mordan 1976; Netzel 1972, 1975; Ogden 1988; Ogden and Hedley 1980; Štěpánek 1952, 1963, 1965; Van Oye 1949, 1956, 1958, 1959; Velho, Lansac-Tôha, and Serafim-Junior 1996; Vucetich 1972, 1973; Zapata, Alvarez, and Cea 2002). Using these data, the numbers of specimens unambiguously assigned to each taxon as well as the number assignable to multiple taxa were tallied (Table 1). Analyses were made regarding the three nominal morpho-species *C. aculeata*, *C. discoides*, and *C. ecornis.*

The diagnostic utility of the morphometric characters was evaluated using box plots and multivariate statistics. Distributions of characters for the three species of *Centropyxis* were compared using box plots. Principal component analysis (PCA) was used to determine how specimens classified to the three species related in multivariate space (Pimentel 1979). Principal component analysis was run using a correlation matrix, standardized loadings on PCs were examined to determine the relative contribution of each morphometric character to each component, and factor scores were plotted to examine how species partitioned in morphospace (Pimentel 1979). Because spine number data were collected for only $\sim 42\%$ of specimens, a PCA was run including all 2,120 specimens but excluding spine number and a second PCA was run including spine number, but including only the 882 specimens for which we had those data. Both analyses gave the same qualitative results, so only results from the latter (including all variables) are presented or discussed further. A PCA including spine number but not spine length was also performed and also gave similar qualitative results, so is not discussed any further.

Table **1.** List of diagnosis and morphometric amplitude for the nominal taxa studied from Tiete River, Sao Paulo.

Nominal taxa	Description	Diagnosis	Morphometric amplitude					NT	NU
			tl	tb	ad	sn	sl		
Centropyxis aculeata	Deflandre, 1929	Bilateral test, aperture subterminal, variable number of spines	120–150	?	31–60	2–8	18–50	12	246
Centropyxis aculeate var. *tropica*	Deflandre, 1929	Longer spines than type species, relative to test length	70–150	?	ST	ST	20–60	96	426
Centropyxis aculeata var. *grandis*	Deflandre, 1929	Bigger than type species	150–200	?	ST	ST	ST	426	107
Centropyxis aculeate var. *oblonga*	Deflandre, 1929	Test oblong, never circular	61–140	60–120	ST	ST	7	29	319
Centropyxis aculeata var. *minima*	Van Oye, 1956	Smaller than type species	65–85	70	40	?	?	0	3
Centropyxis aculeata var. *intermedia*	Van Oye, 1956	Between *C. aculeta* and *C. discoides*	161	153	62	ST	ST	3	107
Centropyxis aculeata var. *gigantea*	Decloitre, 1978	Larger than *C. aculeata* var. *grandis*	300	?	?	?	?	5	2
Centropyxis discoides	Penard, 1890	Larger than *C. aculeata*, discoid lateral view	70–450	?	?	?	?	887	14
Centropyxis discoides var. *grandistoma*	Chardez, 1966b	Aperture larger than type species, relative to test length	147–155	?	115–118	4–5	ST	10	4
Centropyxis ecornis	Deflandre, 1929	Absence of latero-posterior spines	125–275	125–275	ST	NA	NA	56	66
Centropyxis ecornis var. *deflandrei*	Thomas, 1957	Larger than type species	236–303	239–276	65–90	NA	NA	4	13
Centropyxis ecornis var. *leidyi*	Thomas, 1957	Aperture smaller than type species, relative to test length	170–216	?	66–76	NA	NA	0	53

Amplitudes are based on the original description and expanded by subsequent researches, listed in the text. Measures in μm. Characters as designated in Fig. 1. NT, number of specimens assigned to this taxon alone; NU, number of specimens that could be assigned to this and other taxa; ?, not stated in any taxonomic work; NA, non applicable; ST, same as parent taxon; tl, test length; tb, test breadth; ad, aperture diameter; sn, number of spines; sl, spine length.

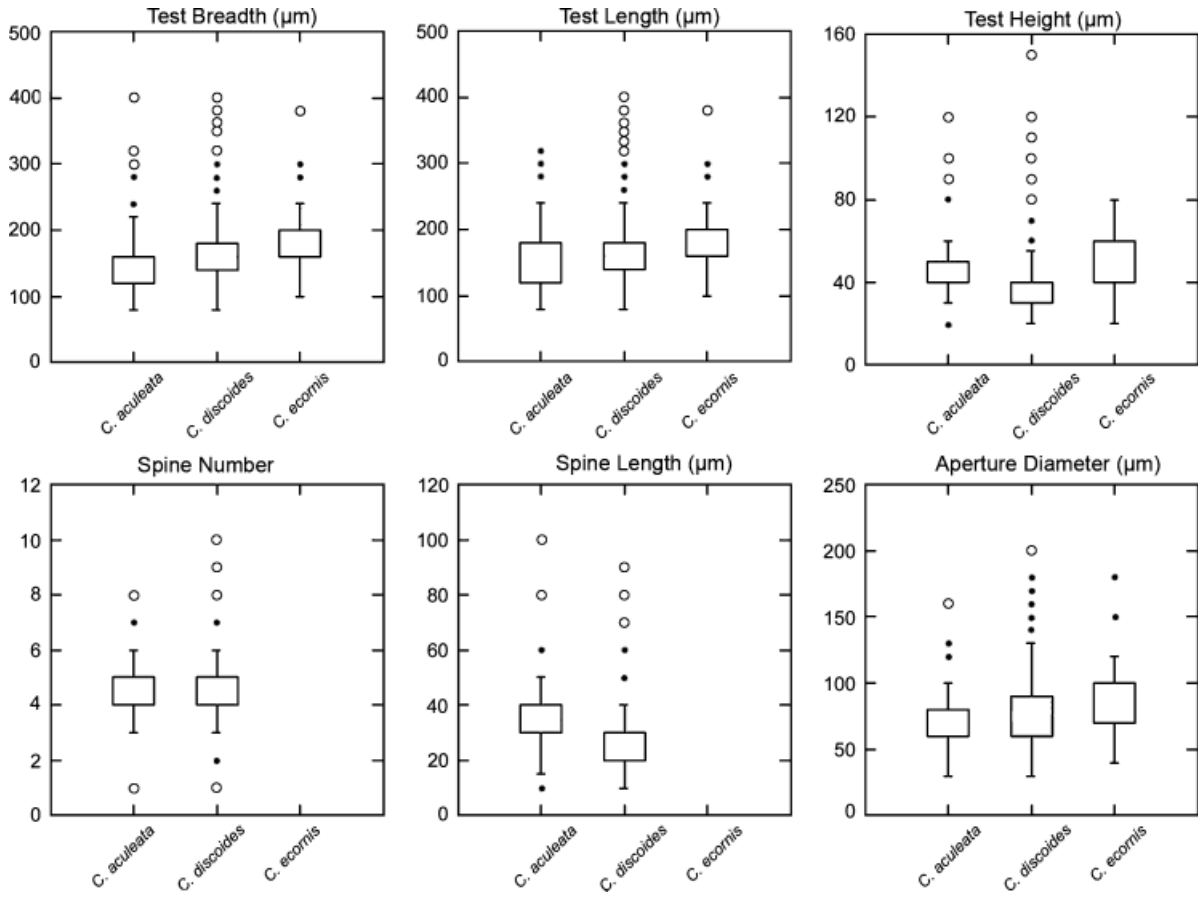

Fig. **2.** Box plots for the three species recognized in the *C. aculeata* complex (i.e. *Centropyxis aculeata*, *Centropyxis discoides*, *Centropyxis ecornis*), for all measured characters. Boxes contain the center 50% of values, box edges are at the first and third quartiles. Whiskers contain values within 1.5 midranges. Asterisks represent outside values and empty circles represent extreme outliers.

RESULTS

Most morphometric variables show little differentiation among the three species: *C. aculeata*, *C. discoides*, and *C. ecornis*. All 2,120 specimens examined could be assigned to one of these species, but many can be assigned to multiple species based on previous descriptions (Table 1). When considered individually, test length, test breadth, and test height, as well as aperture diameter showed almost complete overlap in ranges for the three species and their constituent varieties (Fig. 2). The number of spines and spine length also do not allow differentiation between specimens of *C. aculeata* and *C. discoides*; however, *C. ecornis* lacks spines, and can be differentiated based on this character (Fig. 2).

Principal component analysis of all morphometric variables allows differentiation of *C. ecornis* from *C. aculeata* and *C. discoide* in morphospace defined by the first two, but not differentiation of the latter two species (Fig. 3). There is no differentiation among the three species using the third principal component (not shown). Examination of PC loadings again indicates that spine characters (spine length and spine number) define the major axes of differentiation among species of *Centropyxis*. Specifically, all four test characters load highly positively on principal components (PC1) and are offset by negative loadings on both spine characters (Table 2). Both spine number and spine length load highly positively on PC2 (Table 2), which best differentiates *C. ecornis* from the other two species (Fig. 3). In contrast, PC3 does not differentiate among species well, and both spine and test characters load highly on it, also making this PC difficult to interpret (Table 2).

Centropyxis ecornis also differs from *C. aculeata* and *C. discoides* in its apparent habitat occurence. All three species predominate in river as opposed to lake habitats in the study area (Table 3). Within the river, approximately two-thirds of *C. ecornis* occur in sediment as opposed to being associated with the roots of floating aquatic plants. This contrasts with the other two species of *Centropyxis*, which occur more commonly in river root habitats as opposed to sediment (Table 3).

From our morphometric analysis, presented above, it is clear that, for a large sample of specimens, *C. aculeata* and *C. discoides* are not differentiable. *Centropyxis ecornis* is only differentiated from the other species based on spine characteristics and, to a lesser degree, on habitat characteristics. It is unclear from our analyses whether these differences are genetic or can be accounted for by phenotypic plasticity (Root 1918). Hence, we provide below a single description for all three species and their varieties, but do not synonymize them.

Centropyxis aculeata species complex (Ehrenberg 1838)

Synonyms:

Arcella aculeata—Ehrenberg, 1838: 133, pl IX, Fig. VI
Centropyxis aculeata—Pènard, 1902: 303, Fig. 1

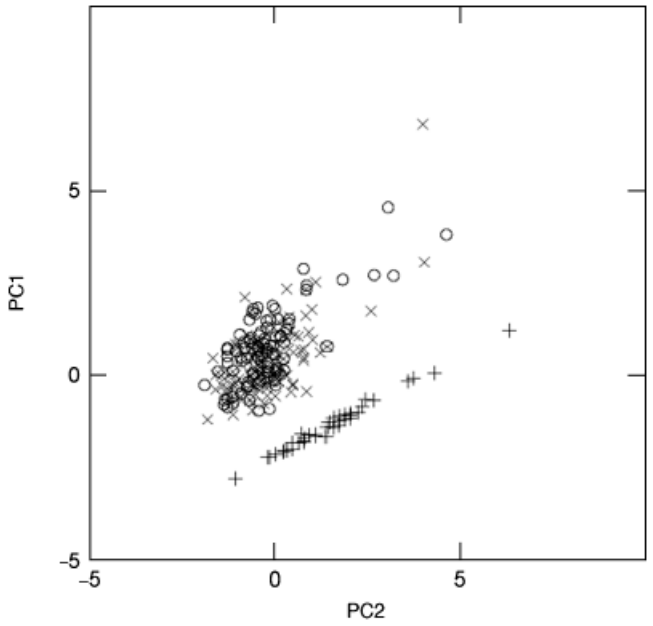

Fig. **3.** Plot of principal component 1 (PC1) versus principal component 2 (PC2), showing a sharp distinction for data referring to *Centropyxis aculeata*. Symbols are: ○ , *Centropyxis aculeata*; × , *Centropyxis discoides*; +, *Centropyxis ecornis*.

Table **2.** Standardized component loadings and their standard errors (SE) for principal component analysis of all morphometric variables ($n = 882$), showing how each variable contributes to each of the first three principal components (PCs).

Character	PC1	SE	PC2	SE	PC3	SE
tb	**0.5228**	0.0086	0.1850	0.0218	0.1286	0.0233
tl	**0.5202**	0.0087	0.1758	0.0227	0.1592	0.0242
th	**0.3563**	0.0188	0.2226	0.0490	**−0.7599**	0.0409
ad	**0.4633**	0.0124	0.0900	0.0329	**0.3963**	0.0309
sl	−0.2047	0.0268	**0.7148**	0.0254	−0.2336	0.0642
ns	−0.2692	0.0244	**0.6051**	0.0329	**0.4111**	0.0607
% var	53.8		21		12	

Also shown is the percent variance explained by each principal component (% var). Loadings greater than 0.35 are in bold (arbitrarily).
PC, principal component.

Table **3.** Occurrence according to habitat of the *Centropyxis* species studied from Tiete River.

Habitat	River		Lake	
	Roots	Sediment	Roots	Sediment
All taxa ($n = 2,120$)	52.64	26.65	18.92	1.79
Centropyxis aculeata ($n = 1,093$)	56.27	24.52	16.93	2.29
Centropyxis discoides ($n = 901$)	51.72	24.97	22.20	1.11
Centropyxis ecornis ($n = 126$)	27.78	57.14	12.70	2.38

A river and a lake were sampled, and within each, two different microhabitats: the sediment and the roots of floating aquatic plants. Numbers in %. *n*, number of specimens.

Previously described indistinct taxa:

Centropyxis aculeata var. *tropica*—Deflandre, 1929: 348, Fig. 94, 95
Centropyxis aculeata var. *grandis*—Deflandre, 1929: 349, Fig. 93
Centropyxis aculeata var. *oblonga*—Deflandre, 1929: 349, Fig. 96–103
Centropyxis aculeata var. *minima*—Van Oye, 1956: 97–98, pl. I, Fig. 8
Centropyxis aculeata var. *intermedia*—Van Oye, 1949: 338, Fig. 12
Centropyxis aculeata var. *gigantea*—Decloitre, 1978: 64
Centropyxis discoides—Deflandre, 1929: 351–353, Fig. 104–107
Centropyxis aculeata var. *discoides*—Pènard, 1890, 1902: 306, Fig. 1–7
Centropyxis discoides var. *grandistoma*—Chardez, 1966b
Centropyxis ecornis—Leidy, 1879, Fig. 20–34
Centropyxis ecornis var. *deflandrei*—Thomas, 1957
Centropyxis ecornis var. *leidyi*—Thomas, 1957

Morphology. Test is irregularly circular in apertural view (Fig. 4–8), flattened in lateral view, and varying from an evenly flat disc shape (Fig. 9) to falciform (Fig. 10), rounded at the posterior end and compressed anteriorly, to a variable degree. The test possesses 0–10 conical hollow spines projecting laterally and/or posteriorly in a single longitudinal line (Fig. 8–10). Spines are variable in form (Fig. 11–16), sometimes with a small piece of quartz inserted at the distal end (Fig. 11).

The apertural region is composed solely of proteinaceous material in most specimens (Fig. 17–19); the abapertural region is composed of agglutinated grains of quartz and/or diatom shells (Fig. 20,21), held together by an underlying layer of organic cement matrix (Fig. 22,23). Exceptions to this condition are: a test composed completely of agglutinated particles cemented together, and in this case, the apertural region is covered with an organic layer); and a test completely without agglutinated particles. The organic cementing matrix is made up of several interlaced strings composing a thick tissue (Fig. 24). This pattern can be seen on the inner side of the test; at the outside, a pattern of small depressions is seen when there are no agglutinated particles.

The aperture is usually eccentric, but in some cases may be central or sub-central (Fig. 4–8). Aperture shape varies from circular to irregular polygonal with its outline shaped like an outward protruding ridge (Fig. 17); larger specimens (> 150 μm) have four cylindrical hollow pillars that project to the abapertural region of the test, where it can be seen as a depression (Fig. 20). Usually, the

Fig. **4–24.** Morphology of the *Centropyxis aculeata* complex. **4–10.** Scanning electron micrographs (SEMs) showing different forms of the *C. aculeata* complex. Apertural view: **4.** *C. aculeata*; **5.** *C. aculeata* var. *grandis*; **6.** *Centropyxis discoides*; **7.** *Centropyxis ecornis*; **8.** *C. discoides*. Side view: **9.** *C. aculeata*; **10.** *C. discoides*. Scale bars 36 μm (3, 8); 90 μm (4, 7); 40 μm (5); 30 μm (6); 80 μm (9). **11–15.** SEMs of different types of spines present in individuals of the *C. aculeata* complex. **11–13.** Showing a terminal particle; **14–16.** Showing tips without the particle. Scale bars 5 μm (10, 13, 14, 15); 10 μm (11, 12). **17–24.** SEM images to illustrate *C. aculeata* apertural architecture and test composition. **17.** Posterior apertural pillar thinner than the anterior. **18.** Detail of posterior pillar. **19.** Detail of anterior pair of pillars. **20.** An individual showing a small amount of agglutinated particles, the four depressions on the abapertural side of the test indicate pillar insertions. **21.** An individual showing a great amount of agglutinated particles, pillar insertions are hidden beneath particles. **22.** Detail of test composition in a highly agglutinated test. **23.** Test broken to show underlying sheet of cement matrix. **24.** Detail of cement matrix, composed like a cloth. Scale bars 20 μm (16, 18); 15 μm (17); 55 μm (19); 35 μm (20); 30 μm (21, 22); 5 μm (23).

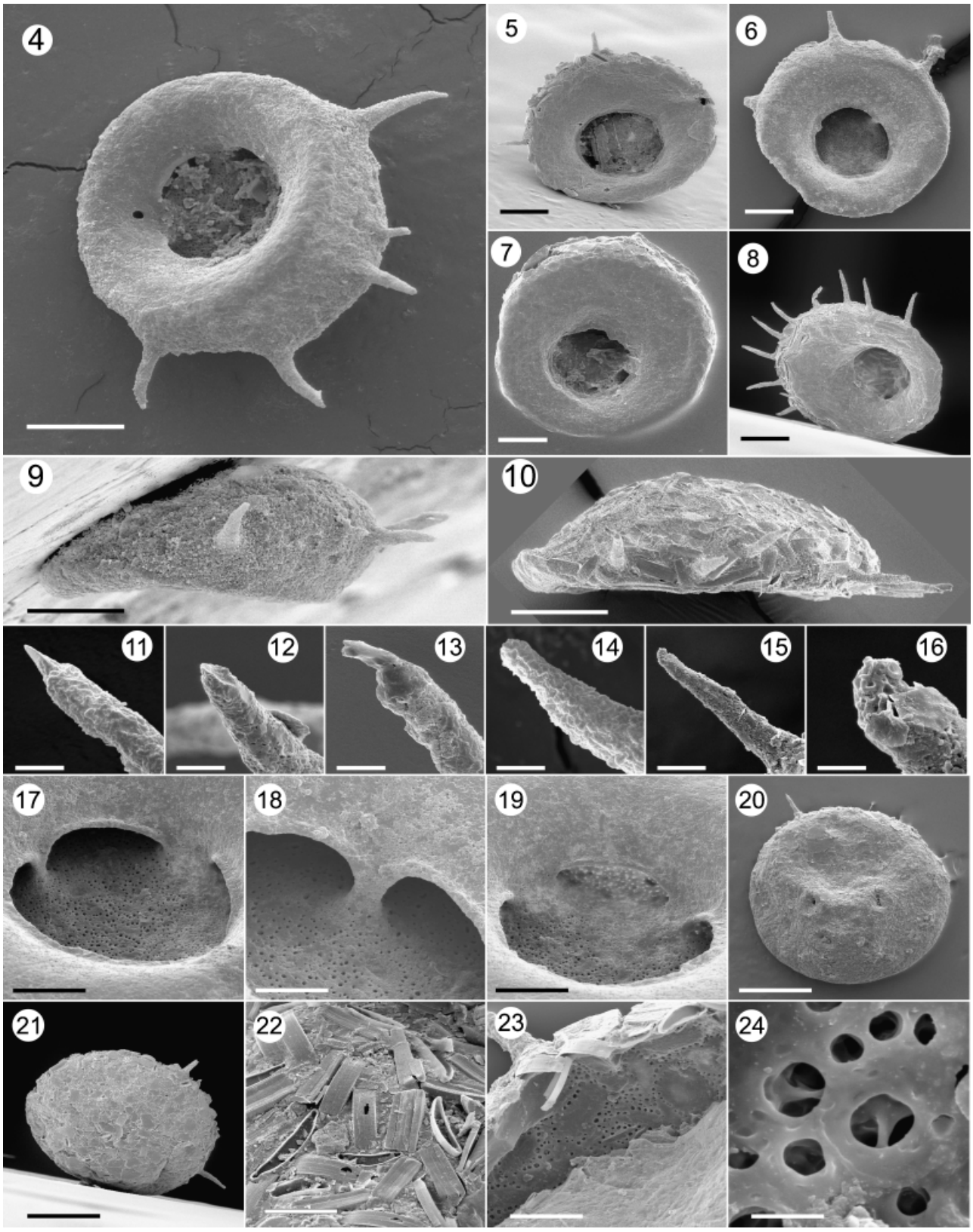
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Table **4.** Biometric characterization of the investigated *Centropyxis* species from Tiete River.

Characters	*x*	M	Minimum	Maximum	SD	SE	CV	*n*
All taxa								
tb	159.4	160.0	60.0	400.0	41.72	0.91	26.2	2,120
tl	157.3	160.0	80.0	400.0	41.92	0.91	26.7	2,109
th	41.5	40.0	20.0	150.0	11.85	0.26	28.6	2,120
ad	71.2	70.0	30.0	200.0	20.42	0.44	28.7	2,120
sl	30.7	30.0	10.0	100.0	10.36	0.23	33.8	1,957
sn	3.8	4.0	0.0	10.0	1.99	0.07	53.0	882
Centropyxis aculeata								
tb	149.4	150.0	80.0	320.0	33.32	1.01	22.3	1,086
tl	146.7	140.0	80.0	400.0	33.42	1.02	22.8	1,076
th	41.7	40.0	20.0	120.0	10.33	0.31	24.8	1,086
ad	66.0	60.0	30.0	160.0	17.24	0.52	26.1	1,086
sl	33.2	35.0	10.0	100.0	10.16	0.31	30.6	1,084
sn	4.4	4.0	1.0	8.0	1.27	0.07	28.6	320
Centropyxis discoides								
tb	166.1	160.0	80.0	400.0	45.87	1.53	27.6	901
tl	164.5	160.0	80.0	400.0	46.17	1.54	28.1	901
th	40.0	40.0	20.0	150.0	12.20	0.41	30.5	901
ad	75.6	75.0	30.0	200.0	21.96	0.73	29.0	901
sl	27.4	30.0	10.0	90.0	9.66	0.33	35.2	868
sn	4.3	4.0	1.0	10.0	1.40	0.07	32.6	431
Centropyxis ecornis								
tb	192.4	200.0	100.0	380.0	37.04	3.30	19.3	126
tl	189.5	200.0	100.0	380.0	36.79	3.28	19.4	126
th	47.8	50.0	20.0	80.0	12.89	1.15	27.0	126
ad	83.4	80.0	40.0	180.0	19.77	1.76	23.7	126

Characters are as designated in Fig. 1. Measurements in μm. *x*, arithmetic mean; M, median; SD, standard deviation; SE, standard error of the mean; CV, coefficient of variation in %; Min, minimum; Max, maximum; *n*, number of investigated specimens.

two posterior pillars (Fig. 18) are thinner than the two anterior ones (Fig. 19).

Biometry. All measures are highly variable (CV ranging from 26.2 to 56.0, Table 4 and Fig. 2). Size frequency distribution analysis shows that the *C. aculeata* complex has a main size class and a large size range for all characters. For test breadth (tb) and test length (tl), respectively, 56.63% and 54.6% of all measures are within 140–180 μm. For test height (th), 88.51% are within 30–50 μm. For aperture diameter (ad), 63% are within 60–80 μm. For spine length (sl), 91.54% are within 20–40 μm. The number of spines (sn) varies from 0 to 10; 57.92% of the tests are provided with four to five spines. The presence of main size classes is an important feature because most surveys do not sample extremely large numbers of individuals; hence, randomly sampled individuals are most likely to be within this main class.

A plot of PC1 and PC2 shows a sharp separation between the spineless form (*C. ecornis*) and all other individuals (Fig. 3). Because spineless forms are reported as a variation of spined individuals in cultures (Root 1918), this distinction may be attributed to phenotypic plasticity. Ratios between morphometric characteristics are given in Table 5.

Ecology. The *C. aculeata* complex occurs mostly associated with roots in a flowing water habitat, but can also be found in other microhabitats. The spineless form (*C. ecornis*) is an exception to this in that it dwells preferably in the sediment of the river (Table 3).

Remarks. The sharp separation in the PCA plot attributed to spine characters may be indicative of *C. ecornis* population being a distinct entity, and the absence of spines would be a strong diagnostic character. However, there is also evidence from culturing these protists (Root 1918) that the whole complex is polymorphic and the spineless form has preference for certain habitats, so we are being conservative in keeping *C. ecornis* in this complex of indistinct taxa. However, future molecular evidence may show that *C. ecornis* is indeed a different species.

Table **5.** Mean size ratios between morphometric characters in the studied populations of *Centropyxis* from Tiete River.

Species (*n*)	All taxa (2,120)	*Centropyxis aculeata* (1,086)	*Centropyxis ecornis* (901)	*Centropyxis discoides* (126)
tl/tb (SD)	0.99 (0.05)	0.98 (0.06)	0.99 (0.05)	0.99 (0.04)
th/tb (SD)	0.27 (0.07)	0.29 (0.07)	0.25 (0.06)	0.25 (0.06)
ad/tb (SD)	0.45 (0.07)	0.44 (0.07)	0.46 (0.06)	0.43 (0.06)
ad/th (SD)	1.78 (0.52)	1.63 (0.46)	1.96 (0.52)	1.85 (0.64)
sl/ad (SD)	0.43 (0.24)	0.53 (0.22)	0.37 (0.19)	NA
sl/th (SD)	0.72 (0.35)	0.83 (0.32)	0.69 (0.29)	NA
sl/tb (SD)	0.19 (0.09)	0.23 (0.08)	0.16 (0.07)	NA

Characters are as designated in Fig. 1. SD, standard deviation; *n*, number of studied specimens; NA, non applicable.

DISCUSSION

Our data, together with findings from previous authors, suggest that *C. aculeata* and *C. discoides* represent the same taxon, and there is some evidence that *C. ecornis* is distinct from the former taxon. Nevertheless, much work remains to ascertain the status of these taxa, particularly that of *C. ecornis*. Cytoplasmic and molecular work are still to be done, and there is a possibility that future studies of this nature will split more sibling species, as seen in other groups of microbial eukaryotes.

There is no universal concept of species and the difficulties in identifying species for putative asexual protists are particularly acute, either as category or as a taxon (Mayr 2000; Meisterfeld 1979; Schlegel and Meisterfeld 2003; Sonneborn 1957; Wanner 1999). For example, *C. ecornis* can be identified by our data as a distinct entity, but it remains unclear whether this taxon represents a form of phenotypic plasticity or a valid species. Other workers have already encountered evidence that species limits might be overestimated for other groups of unicellular eukaryotes, mainly due to enhancements of sampling efforts (Lee and Patterson 2000).

The identification of distinct entities is the primary aim of this study. However, taking all factors into account—lack of types, distant localities, different methods of study and intrinsic impediments of morphological work—synonymizing any of the three species under study or their varieties is premature and must first be supported by further data.

Therefore, we provide a single broad description for the species complex and list previously described taxa that may represent a single entity within it. We also point out possible diagnostic features (number of spines for *C. ecornis*), and assign the oldest available name to the entire species complex (*C. aculeata*). This allows for a comprehensive taxonomic revision pending collection and analysis of further data. Here, molecular data and their phylogenetic analysis may provide the most definitive answers. The morphological variability traditionally ascribed to infra-specific taxa often reflects important ecological differences (Bobrov and Mazei 2004), although, such taxa are not recognized by some (International Code of Zoological Nomenclature–Art. 46.6.3 states that varieties described after 1960 are invalid, Ride et al. 1999). With a complete list of apparently non-distinct entities, this information on previous workers' concepts will remain accessible.

Consequently, separating objective from interpreted information and clarity in regarding non-interpreted data are required. Research under this framework will result in more fruitful dis-

cussions of taxonomic and species concepts for asexual protists. Additionally, preserving previous authors' concepts will aid in identifying important forms, as shown here for *C. ecornis*.

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