*Phytotaxa* 220 (3): 268–276
www.mapress.com/phytotaxa/


**Article**

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

http://dx.doi.org/10.11646/phytotaxa.220.3.5

# Description of coccoid cyanoprokaryote *Nisada stipitata morphogen. et sp. nov.* from the supralittoral zone in the tropical Mexican Pacific

MICHELE GOLD-MORGAN[1], LAURA GONZÁLEZ-RESENDIZ[1,2], HILDA LEÓN-TEJERA[1] & GUSTAVO MONTEJANO[1]

[1]*Departamento de Biología Comparada, Facultad de Ciencias, Universidad Nacional Autónoma de México (UNAM). Coyoacán, Código Postal 04510, P.O. Box 70-474, México, Distrito Federal (D.F.), México*

[2] *Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México (UNAM).*

** Corresponding author (e-mail: michloug.m@ciencias.unam.mx)*

## Abstract

A distinctive morphotype consisting of an epilithic, one-layered colony of polarized, 1–3-celled pseudofilaments was recognized in the rocky shores of the state of Oaxaca in the Mexican tropical Pacific. Morphologically, it was not identifiable as any species of a previously described genus. It is similar to species from the former order Chroococcales, recently modified by Komárek *et al*. (2014), in its sessile heteropolar pseudofilaments. Specifically, it is most similar to the colonial species of the genus *Chamaesiphon*; and of *Chamaecalyx*, but the cells of the Mexican populations divide symmetrically in one or two planes, differentiating them from *Chamaesiphon* which divides asymmetrically and only in one plane, and from *Chamaecalyx*, which has multiple fission. The other defining feature is that all cells of the pseudofilament have differentiated mucilaginous structures (pad and/or stipe and cup). It has not been possible to obtain cultures of our material for further studies to complete the polyphasic approach. Nevertheless, its morphological characteristics and life cycle, plus its distinctive extreme biotope, form a unique combination of features that derive in our proposal of the morphogenus *Nisada gen. nov*., with the type species, *Nisada stipitata*, *sp. nov*. We describe the proposed taxa and the problems and current inconveniences regarding its assignment to higher taxonomic levels. We also discuss the different degrees of complexity of heteropolarity in *Nisada* and other taxa.

**Key words:** Cyanobacteria, Cyanophyta, *Chamaecalyx*, *Chamaesiphon*, Chroococcales, colonial species, *Godlewskia*

## Introduction

Numerous recent studies of the Cyanoprokaryota at the ultrastructural and molecular levels have disputed monophylly of the previously described groups, including coccoids (Brito *et al*. 2012, Strunecký *et al*. 2014). Using the polyphasic approach the Czech school of Jiri Komárek has proposed a new classification. In its current version (Komárek *et al*. 2014) many taxa, especially genera, have been ranked temporarily awaiting the necessary information for a more definitive assignment.

It is to be expected that surveys in little known types of environment, such as the tropical upper tidal zone or tropical alkaline marshes, will reveal undescribed species, perhaps belonging to new supraspecific taxa. This assumption is supported by Nabout *et al*. (2013) who estimate that 3582 species of cyanoprokaryotes (57% of the total estimated number) worldwide have yet to be described due to the lack of biodiversity studies within this group and specially in tropical/subtropical and terrestrial environments.

During the floristic studies of cyanoprokaryotes of the tropical Pacific coast of Mexico, we collected populations of a colonial biofilm in the upper tidal zone, which were not identifiable morphologically into any known cyanoprokaryote at the generic level. Our populations have conspicuous morphological and life cycle features not present in any of the species of the morphologically closest genera. We herein describe the unique morphology of the pseudofilaments that compose the colonies and discuss different degrees of complexity of sessile heteropolarity. We comment on the harsh biotope in which the Mexican populations live and finally propose the new genus and species, *Nisada stipitata*, *morphogen. et sp. nov*., in the spirit of Komárek *et al*. (2014) expressed at the end of their article: "Registration

 *Accepted by Saúl Blanco Lanza: 20 Jun. 2015; published: 24 Jul. 2015*

of cryptogenera and morphogenera has a role in advancing understanding, but are only ad interim solutions to the description of genera following polyphasic evaluation". Finally, we explain why we do not assign the proposed taxon to higher taxonomic levels. We shall refer to our material by the name *Nisada stipitata* in the remainder of this paper and to higher taxa with their current names in the classification of Komárek *et al*. (2014).

## Materials and Methods

Samples were collected during field trips made in April and December 2010 and October 2012 in San Agustín, Huatulco, Oaxaca (Fig. 1A) in the tropical Mexican Pacific. The tides are mixed with semidiurnal dominance. The Sierra Madre del Sur mountain chain ends in the coast of Huatulco, forming bays and rocky cliffs which characterize this portion of the Pacific, with a predominance of granitic and gneiss rocks (CONANP 2003). The upper tidal zone is approximately 7 m a.s.l. (Fig. 1B). (More environmental and site information in González-Resendiz *et al*. 2013). The cyanoprokaryotic growths were collected with chisel and hammer. Samples were kept dry until they were observed under the microscope after rehydration and fixation in 4% formaline in seawater, up to 6 months later. Semipermanent slides were made with glycerine jelly. Observations and micrographs were made under an Olympus BX51 microscope equipped with a DP12 digital camera. Epifluorescence micrographs were taken with an Olympus Provis AX70 microscope. Measurements were made using SigmaScan©Pro. Taxonomic determination was done following Geitler (1932) and Komárek & Anagnostidis (1998) for taxonomic position Komárek *et al.* (2014) was used.

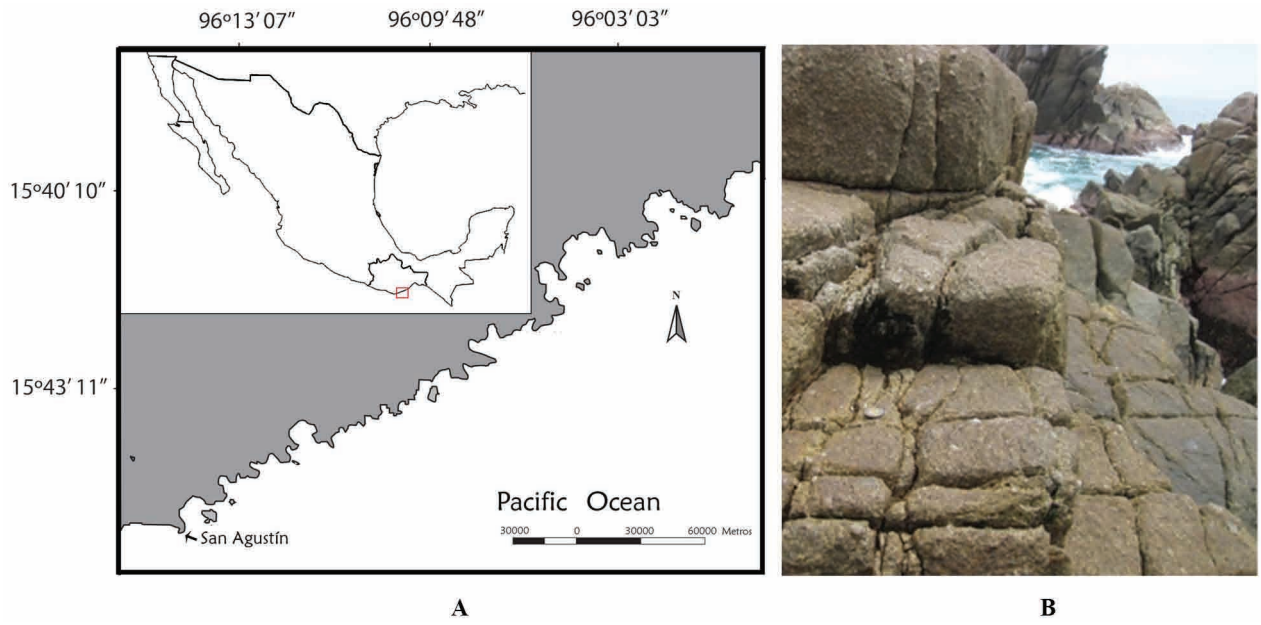


**FIGURE 1.** San Agustín Bay, state of Oaxaca, México. A. Map showing location of collection site. B. Panoramic photograph of the supralittoral zone of the site.

## Results

Class **Cyanophyceae**, *incertae sedis* (see under Discussion)

### *Nisada* Gold-Morgan, González-Resendiz, León-Tejera *et* Montejano, *morphogen. nov.* (Figs. 2–6)

Biofilm composed of a continuous and homogeneously structured mucilaginous, one-layered colony, sometimes mixed with other cyanoprokaryotes (Figs. 2A–G; 4A) made up of densely, parallelly arranged, polarized unicells to pseudofilaments (Figs. 2D, H–M). Basal and non-basal cells of the pseudofilament with differentiated mucilaginous structures: basal pad, stipe and cup-like structure (Figs. 2I–M; 4B, C) (except for the pad which is part only of the basal cell), therefore pseudofilament and each cell heteropolar.

Pseudofilaments set individually and vertically in the also mucilaginous, black, thick, rigid, well-delimited colonial sheath visible in top and lateral views (Figs. 2E, G, H). Basal/mother cell dividing symmetrically in a plane perpendicular to the longitudinal axis of the pseudofilament (Figs. 3A, B, F); first daughter cells dividing symmetrically either in a plane perpendicular to the longitudinal axis (morph 1; Figs. 3B, F) or parallel to this axis (morph 2; Fig. 3D) in pseudofilaments of the same colony; second daughter cells (present only in morph 1) dividing symmetrically in a perpendicular plane (Fig. 3B). All daughter cells reproductive and, when released (except the basal/mother cell), fix themselves in the colonial mucilage and begin a new cycle (Figs. 3E, H). The basal/mother cell remains and divides again, also initiating a new cycle. In morph 1, first daughter cells either double their size before dividing (Figs. 2K, L) and are released, or triple it and are then released without dividing (Fig. 2I). In morph 2, the two daughter cells resulting from longitudinal division of the first daughter cell are released after this division. Both morphs are present in the same colony but morph 1 is the most abundant. No morph distribution pattern is apparent within a colony.

**Type:**—*Nisada stipitata* Gold-Morgan, González-Reséndiz, León-Tejera *et* Montejano, *sp*. *nov*. (see below)

**Etymology:—***Nisada* (Zapotec) = 'Marine' (Zapotec is the language spoken by one of the ethnic groups of Oaxaca).

### *Nisada stipitata* Gold-Morgan, González-Resendiz, León-Tejera *et* Montejano, *sp*. *nov*. (Figs. 2–6)

Epilithic colonies of up to 5 mm in height. Biofilm formed by a colony of parallel, erect, short pseudofilaments of a maximum of three cells. From top view, colonies seen as composed of black round or oval cells which are pseudofilament apices: single (morph 1 in Figs. 2G and H) or in pairs (morph 2 in Figs. 2E and G). In lateral view, pseudofilaments with three types of mucilaginous structures visible: pad, stipe and cup, as cited in the generic description. Pseudofilaments enveloped by individual grayish-black, thin, firm or slightly diffluent sheaths (Fig. 2I). Stipe with rigid mucilage, straight, conical or an inverted cone, with alternating horizontal dark and light stripes or bands; top of stipe with a black, cup- or bullhorn-shaped mucilaginous structure (Figs. 2I, G; 3C; 4B, C). Cell content smooth, without granules, colorless or with an irregular brownish pattern (Figs. 2I, K; 3A, E). Cell shape diverse: spherical, hemispherical, quadrate, clavate, ovate, obovate, elongate or an inverted cone (Figs. 2L, M; 3E, F). Reproductive cells formed by binary fission. In morph 1, one or two daughter cells are released at a time; in morph 2, the two reproductive upper cells are released at a time. Dimensions: morph 1: pseudofilaments up to 7 µm (length); basal cells 0.5–3.0 × 1.0–2.5 µm (l × w); daughter cells 1.5–3.0 × 1.0–2.5 µm (l × w); in top view cells 1.0–2.5 µm (diameter). Morph 2: pseudofilaments up to 6 µm (length); basal cell 2.0–2.5 × 2.0 µm (l × w); daughter cells 3.0 × 1.5–2.0 µm (l × w); in top view 3.0 µm (diameter).

**Type:—**MEXICO. Oaxaca: San Agustín, insolated plateau of granitic cliff 7 m a.s.l., off marine tropical shore not exposed directly to wave shock, but only receiving intermittent spray, 15° 41' 17.41" N, 96° 14' 15.28" W, *González-Resendiz & León-Tejera*, *02-12-2010 (C59*, *C61)*, *03-10-2012 (C701)* (holotype FCME! C59, isotypes FCME!, C61, C701).

**Etymology**:—from Latin *stipitata*='stipitate'.

**Observations:**—The basic pseudofilament morphology in *Nisada* has many variants due to the way the cup and stipe develop and to the growth process of the pseudofilament. The cup begins as a narrow horizontal band which increases in width and each 'horn' seems to grow upwards (Figs. 2I, K) until the two join. Different stages of this apparently upwards growth can be seen in lateral view as longer or shorter horns.

Pseudofilament heteropolarity in *N*. *stipitata* is expressed shortly after a cell enters in direct contact with a substrate, as in other heteropolar coccoid species, but also after every division of a non basal cell it produces a cup and stipe, or at least a cup, before being released. Even when the division of the first daughter cell occurs lengthwise, there is a cup and stipe between the basal mother cell and the two daughter cells. This 'stronger version' of heteropolarity of *N*. *stipitata* is associated with the basal part of each cell, whether in contact with an external substrate or another cell of its pseudofilament.

*Nisada stipitata* is very stable regarding the number of cells of the pseudofilament and the presence of its differentiated mucilaginous features; but it is also morphologically quite diverse. Three factors contribute to its variability, aside from that due to the development of the cup-stipe: 1) cell growth in different length/ width proportions, between and within pseudofilaments; 2) cell size reached before division or release; and 3) the possibility of two alternative planes of division of the first daughter cells.

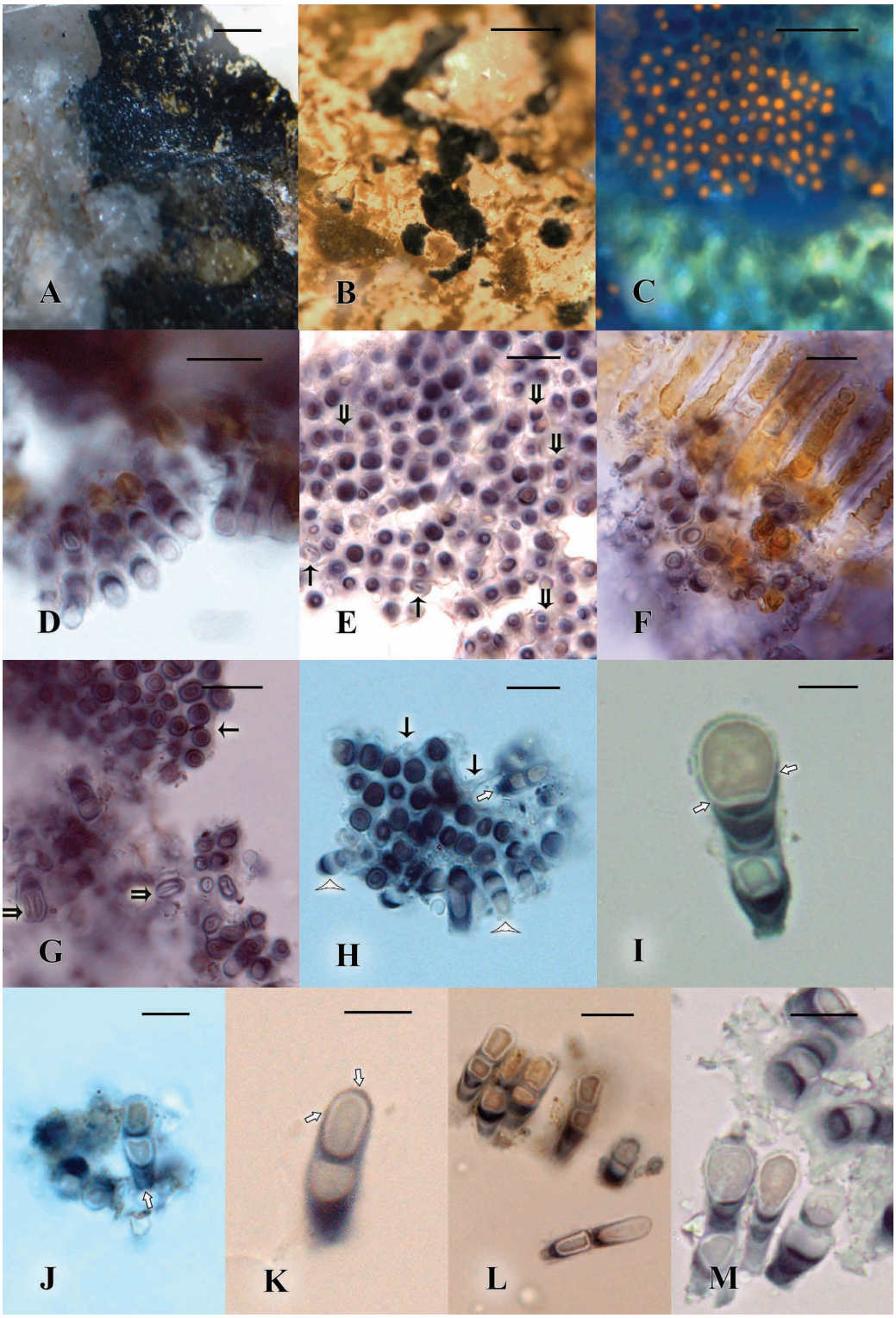


**FIGURE 2.** Morphology of *Nisada stipitata*. A. Macroscopic view of partially covered rock (black portion). B. Macroscopic view of strip of biofilm on rock. C. (tv) Pseudofilament apices in fluorescent microscopy. D. (tv) Edge of biofilm with pseudofilaments in a row. E. (tv) Apices of morph 2 before cell division (single arrow) and after division but before separation (double arrow). F. With dying filaments of *Kyrthuthrix* cf. *maculans*. G. (tv) Colonial mucilage (single arrow); apices of morphs 1 and 2 and (lv) two morphs 2 (double arrows). H. (tv) Apices of morph 1 with a few pseudofilaments in lv (arrow heads); pad of a complete pseudofilament (white arrow) and colonial mucilage (black arrow). I. (lv) Pseudofilament of morph1 with developed stipe and cups; "horns" (sheath) with synchronous growth (arrows). Daughter much larger than mother cell.J. (lv) Pseudofilament of morph 1 without pad (base of stipe, arrow). K. (lv) Pseudofilament of morph 1 showing "horns" (sheath) with asynchronous growth (arrows). L, M. Diversity of morphology of morph 1. Micrographs D–M with light microscopy. tv = top view, lv = lateral view. Scale bars: A = 1 mm, B = 200 µm, C = 25 µm, D–H, J, L = 6 µm I, K, M = 3 µm.

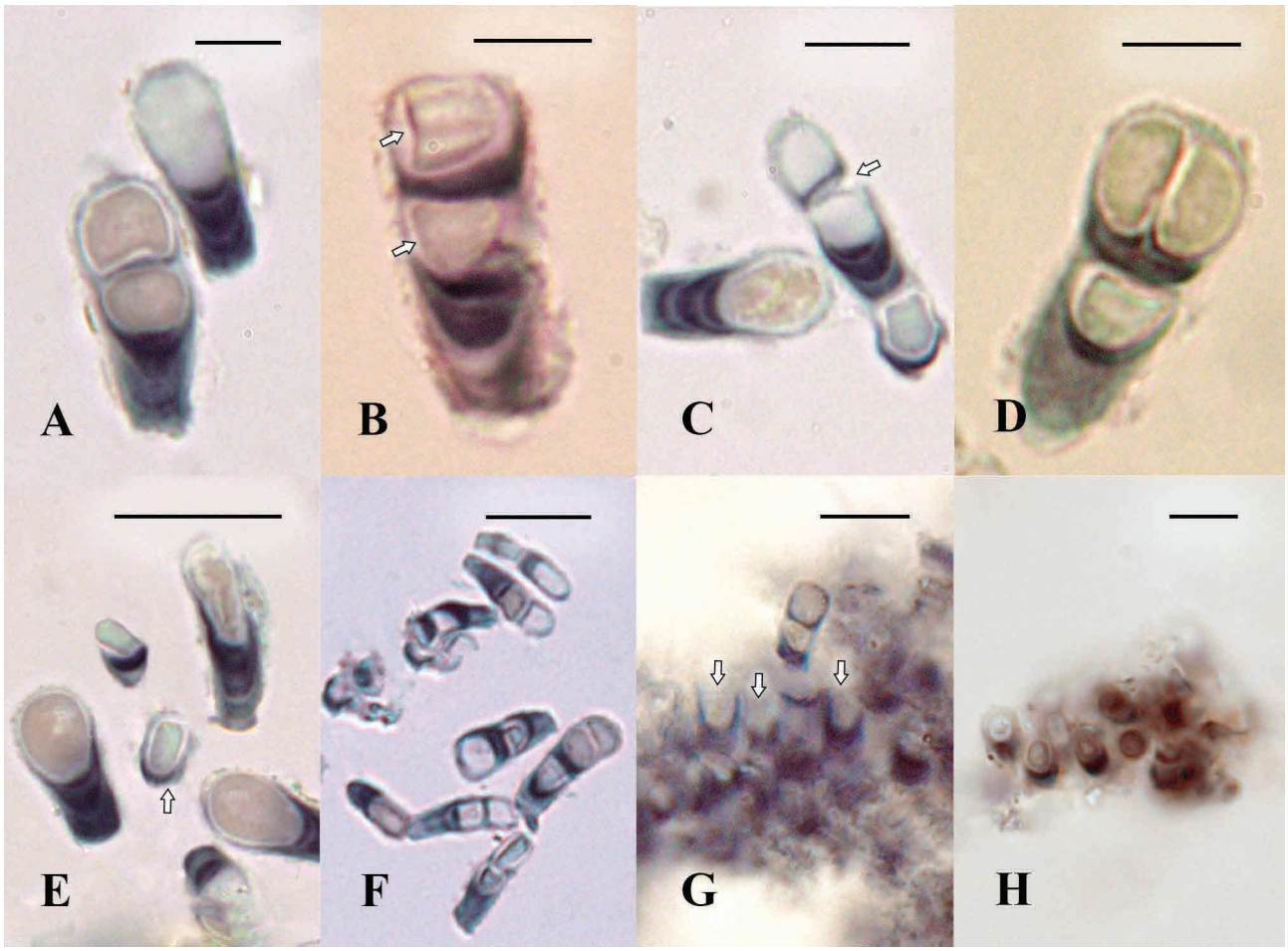


**FIGURE 3.** Stages of reproduction of *Nisada stipitata* in lateral view. A. (lv) Two pseudofilaments of morph 1 with and without cell division; perpendicular plane of division evident in left pseudofilament. B. Two-celled pseudofilament of morph 1 with beginning of perpendicular cell division of the daughter cell (top arrow); displaced basal/mother cell allowing partial view of the inside of the cup (bottom arrow). C. (lv) Two pseudofilaments of morph 1. Three-celled pseudofilament with fracture (arrow). D. (lv) Complete pseudofilament of morph 2 with division of daughter cell. E, F. Diversity of stages of the life cycle of morph 1. Pseudofilaments with all or some of the differentiated mucilaginous structures. Basal cell with incipient cup (arrow). G. (lv) Empty cups/stipes set in the colonial mucilage (arrows). H. (lv) Basal cells with different degrees of development, set in colonial mucilage. Scale bars: A–E = 3 µm, F–H = 6 µm.

## Discussion

**Taxonomic considerations:—**The taxonomy of coccoid cyanoprokaryotes is based principally on cell morphology (shape and polarity), patterns of cell division, reproduction and thallus structure (unicellular or colonial). The heteropolar coccoids are classified in three families and orders. The genus *Nisada* is most similar to the species of *Chamaesiphon* A. Braun & Grunow in Rabenhorst (1864: 177) within the Chamaesiphonaceae/Synechococcales; to *Godlewskia* Janczewski (1883: 142) *sensu* Komárek *et al*. (2014: 300, 302) in the Stichosiphonaceae/Chroococcales; and to species of *Chamaecalyx* Komárek & Anagnostidis (1986: 199) in the Pleurocapsaceae/Pleurocapsales in the classification of Komárek *et al.* (2014). Of these taxa, it is most similar to the colonial species of *Godlewskia* and several of *Chamaesiphon* in having of heteropolar cells. It also is similar to *Chamaecalyx* in having more than one plane of cell division.

The diferences between *N. stipitata* and other taxa with coccal, sessile, heteropolar pseudofilaments are: 1) *N. stipitata* undergoes symmetrical, binary cell division and therefore does not produce exocytes or baeocytes; 2) it presents two morphs and therefore two variants of the life cycle; 3) it exhibits 'strong' heteropolarity (all cells heteropolar) and therefore a more complex morphology; and 4) the morphological differentiation of its stipe versus the simple stipe in taxa with this feature. It differs from the colonial species of this group in the possibility of having two planes of division, not only one as in some *Chamaesiphon* and *Godlewskia*, and not more than two as in *Chamaecalyx*, which undergoes almost simultaneous multiple fission.

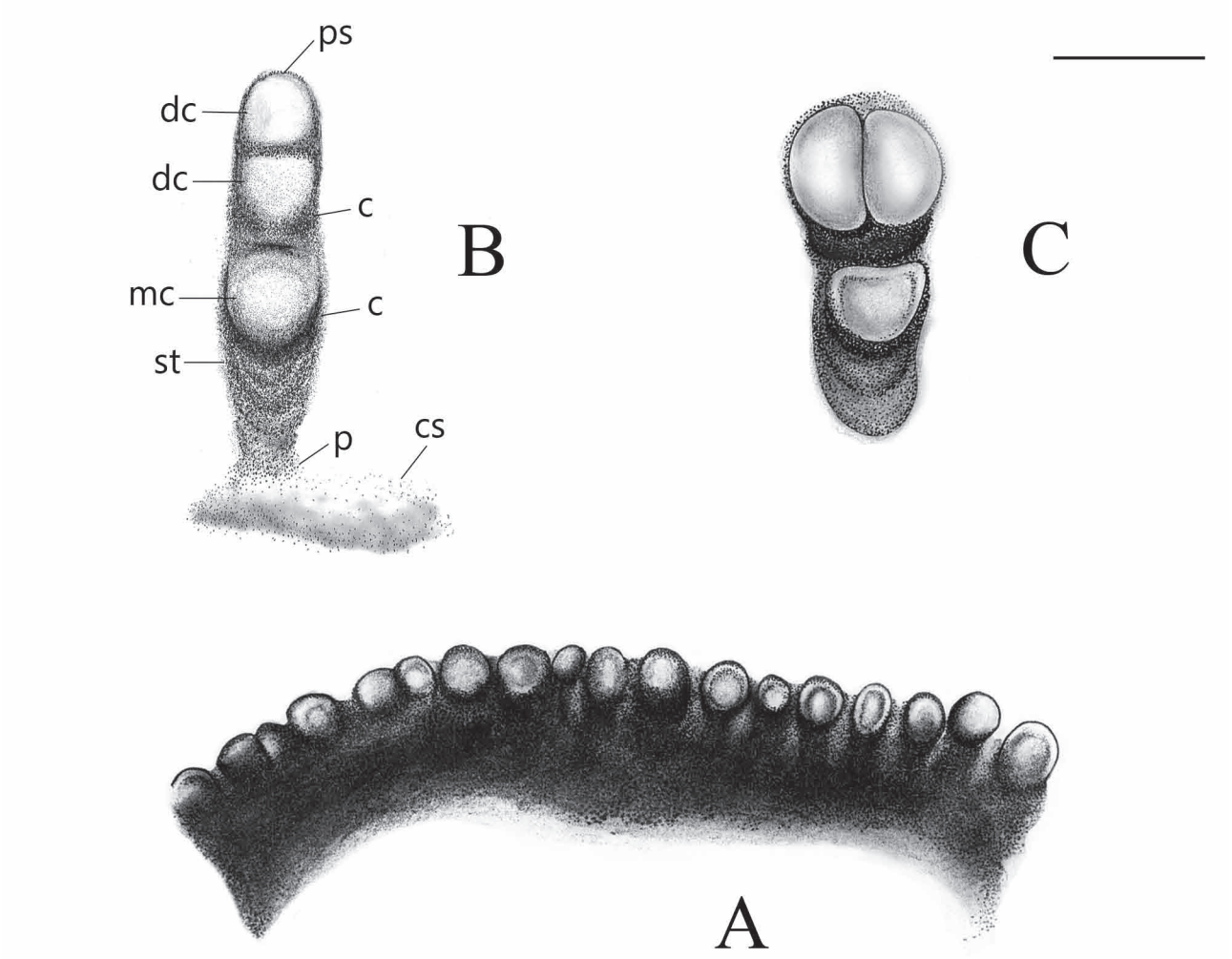


**FIGURE 4.** (lv) Line drawings of *Nisada stipitata*. A. Edge of biofilm with pseudofilaments in a row. B. Complete pseudofilament of morph 1. C. Complete pseudofilament of morph 2. Ps = pseudofilament sheath, dc = daughter cell, c = cup, mc = mother cell, st = stipe, p = pad, cs = colonial sheath. Scale bars: A = 8 µm, B, C = 6 µm.

*Nisada* also has several features in common with *Pleurocapsa* Hauck (1885: 515)/Pleurocapsaceae such as being a thin-layered, epilithic cluster of cells which form uniseriate or multiseriate rows, enveloped in a coloured, firm sheath. In this genus, a longitudinal division of the apical cell results in pseudodichotomous divarication typical of it (Komárek & Anagnostidis 1998). However, whereas *Pleurocapsa* is a crust formed by true multicelled pseudofilaments, with irregular or radial rows and enveloped by a thin sheath, *Nisada* is a mucilaginous film formed by pseudofilaments with a maximum of three cells, arranged in parallel rows, presenting a very thick colonial sheath. Longitudinal division occurs only in morph 2 of Nisada (Fig. 4C) and it does not result in divarication. There are also differences in the type of cell division and reproduction. *Pleurocapsa* has an irregular binary fission which may lead to baeocytes or nanocytes, whereas *Nisada* has symmetrical binary fission which forms two or three large reproductive cells.

An additional feature of *N. stipitata* is that each reproductive structure is liberated from its pseudofilament and henceforth grows as an independent individual, easily distinguishable from others. The consequence is the constitution of one-layered colonies, which are densely arranged but not clustered or stacked. This is different from what occurs in several colonial, sessile heteropolar pseudofilaments such as in *Godlewskia*, for example, or in the recently described *Chamaesiphon komarekii* Rott (2008:39) or *Ch. stratosus* Sant'Anna et *al*. (2011:26), in which reproductive structures (exocytes) remain attached to the sheath of the mother cell and develop a new pseudofilament *in situ* forming multilayered shrub-like colonies.

Planes of cell division and type of reproductive structures are diacritical features at the family level in the classification of Komárek *et al*. (2014), and sessile heteropolar pseudofilaments are placed in three families belonging to three orders: Chamaesiphonaceae/Synechococcales, Stichosiphonaceae/Chroococcales and Pleurocapsaceae/Pleurocapsales. *Nisada* is not in accordance with any of the three groups, which are built with insufficient information (ancient single reports or without sequence data for most taxa) or information which indicates polyphyletic groups, as explained in detail for

each order and family by Komárek *et al*. (2014). Part of these problems, in turn, are caused by the difficulty of culturing and having enough material for molecular studies. Additionally, reproductive structures in Cyanoprokaryota in general, and in coccales in particular, require further work to clarify the different types of structures that exist in different groups and the processes that produce them. For example, in some coccales there are intermediate forms of division between typical binary fission and simultaneous or sequential multiple fission, such as in *Chamaecalyx* which, although currently assigned to Pleurocapsaceae, does not produce the characteristic baeocytes of this family. Furthermore, regardless of these conceptual problems, the structures of *Nisada* are not either exocyte-or baeocyte-like, as mentioned above, as they are the result of symmetric division. The need for further studies on types of reproductive structures and the atypical characteristics regarding precisely planes of cell division and type of reproductive structures in *Nisada*, have led us to consider it premature to try to solve its placement above the genus level or to name its reproductive structures until more information is available.

*Nisada* was collected in an extreme environment: the upper littoral zone on granitic rocks subjected to high insolation, variable amounts of saline spray, rain and desiccation. This environment has not been explored by phycologists and, given its harshness, finding an undescribed taxon was predictable. The same situation occurred at a larger scale in the surveys done in the marshes the surveys done in the marshes of Belize by Komárek & Komárková-Legnerová (2007) and in the studies of the cyanoprokaryotes of central Mexico (Komárek *et al*. 1996).

**Life cycle considerations:**—Reproductive mechanism(s) are not yet totally clear because, although we have recognized the reproductive structures, which are produced after the symmetric binary fissions in both morphs, we have not observed unequivocal evidence of their process of liberation such as opening of the apical sheaths, gelatinization or pores. We have found empty stipes and cups (Fig. 3G), as well as pseudofilaments with fractures of different degrees of completion between mother and daughter cells in morph 1 (Fig. 3C), another possible mechanism of release, but it is possible that manipulations of the material originated them. On the other hand, the biofilm is quite fragile and, consequently, it may also break easily in nature. In morph 2, the two cells produced by the longitudinal division, most probably separate along this plane. In Figs. 5 and 6 we present the life cycles of morphs 1 and 2, respectively, with the current available information. As for the beginning of the life cycle, we have found (in lateral view) small, mostly spherical cells fixed in the colonial mucilage with an incipient mucilaginous band initiating the cup (Figs. 3E, H); and basal cells with well-developed stipes and cups (Figs. 3A, E).

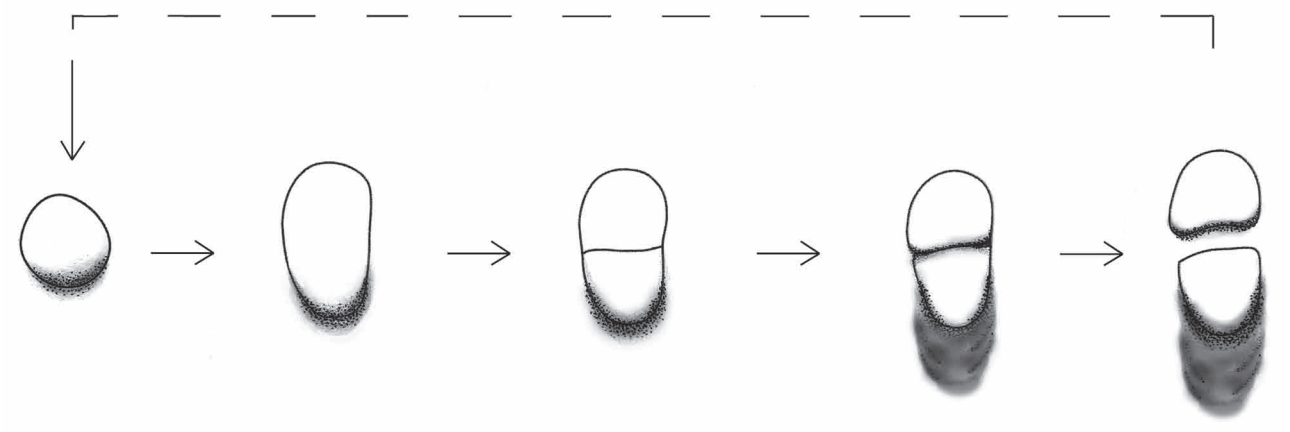

**FIGURE 5.** Line drawing of life cycle of morph 1.

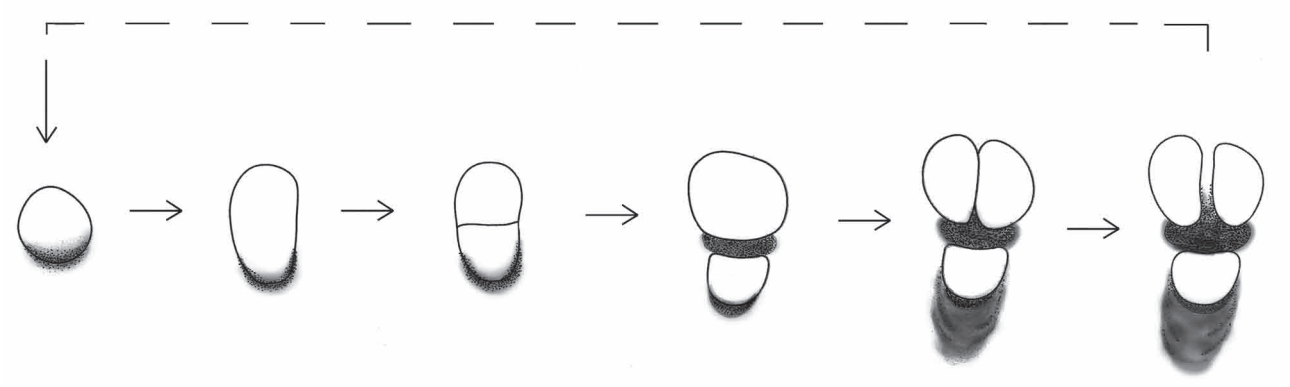

**FIGURE 6.** Line drawing of life cycle of morph 2

## Conclusion

Considering the high estimated percentage of undescribed species of cyanoprokaryotes, and the unbalanced approximation to the exploration of different geographic latitudes and types of environments, finding of a new species in the upper tidal zone of a tropical coast is in line with the implicit prediction. We also conclude that degree of heteropolarity in cyanoprokaryotes is a new and useful taxonomic criterion and heteropolarity an interesting physiological phenomenon in need of further study.


## Acknowledgments

The authors thank M. Sc. Alejandro Martínez Mena for his extraordinary technical assistance with epifluorescence images. Our special thanks to L.A.V. Mónica Peralta Caballero for image edition and L.A.V. Ana Hernández Vázquez for illustrations. The authors also thank the two anonymous reviewers for Phytotaxa for their very valuable and constructive evaluations. This paper constitutes a partial fulfillment of the doctorate degree of L. González-Resendiz, in the Graduate Program in Biological Sciences of the National Autonomous University of Mexico (UNAM). Authors acknowledge infrastructure provided by UNAM, and Project UNAM-DGAPA-PAPIIT 223614.


## References


Brito, A., Ramos, V., Seabra, R., Santos, A., Santos, C.L., Lopo, M., Ferreira, S., Martins, A., Mota, R., Frazao, B., Martins, R., Vasconcelos, V. & Tamagnini, P. (2012) Culture-dependent characterization of cyanobacterial diversity in the intertidal zones of the Portuguese coast: a polyphasic study. *Systematic and Applied Microbiology* 35: 110–119. http://dx.doi.org/10.1016/j.syapm.2011.07.003

CONANP (2003) *Programa de manejo Parque Nacional Huatulco*. CONANP (Comisión Nacional de Áreas Naturales Protegidas), México, 205 pp.

Geitler, L. (1932) Cyanophyceae. *In*: Rabenhorst, L. (Ed.) *Kryptogamen Flora von Deutschland*, *Vol. 14*. Akademische Verlagsges, Leipzig, 1196 pp. http://dx.doi.org/10.1038/130292a0

González-Resendiz, L., León-Tejera, H.P., Díaz-Larrea, J., Alba-Lois, L. & Segal-Kischinevzky, C. (2013) *Hassallia littoralis*, sp. nov. (Cyanobacteria, Microchaetaceae) from Mexico's marine supralittoral based on morphological and molecular evidence. *Phytotaxa* 137 (1): 35–47. http://dx.doi.org/10.11646/phytotaxa.137.1.4

Hauck, F. (1885) Die Meeresalgen Deutschlands und Österreichs. *In*: Rabenhorst, L. (Ed.) *Kryptogamen-Flora von Deutschland*, *Österreich und der Schweiz*. *Zweite Auflage*. Eduard Kummer, Leipzig, pp. 513–575.

Janczewski (1883) Nowy rodzaj sin. *Rozprawy Akademii umiejetnosci, Wydzial matematyczno-przyrodniczy, Akademia umiejetnosci, Krakow* 11: 142–144.

Komárek, J. & Anagnostidis, K. (1986) Modern approach to the classification system of cyanophytes, 2–Chroococcales. *Algological Studies* 43: 157–226.

Komárek, J. & Anagnostidis, K. (1998) *CyanoDb.cz a database of cyanobacterial genera.* Available from http://www.cyanodb.cz (22 May 2015)

Komárek, J. & Anagnostidis, K. (1998) Cyanoprokaryota 1. Teil: Chroococcales. *In*: Ettl, H., Gärtner, G., Heynig, H. & Mollenhauer, D. (Eds.) *Süsswasserflora von Mitteleuropa 19/1*. Gustav Fischer, Jena-Stuttgart-Lübeck-Ulm, 548 pp.

Komárek, J. & Komárková-Legnerová, J. (2007) Taxonomic evaluation of the cyanobacterial microflora from alkaline marshes of northern Belize. 1. Phenotypic diversity of coccoid morphotypes. *Nova Hedwigia* 84: 65–111. http://dx.doi.org/10.1127/0029-5035/2007/0084-0065

Komárek, J., Montejano, G., Gold-Morgan, M. & Tavera, R. (1996). Taxonomic identity and distribution of tropical cyanoprokaryotes (Cyanophyta, Cyanobacteria): An example from central Mexico. *Nova Hedwigia* 112: 49–54.

Komárek, J., Kaštovský, J., Mareš, J. & Johansen, J.R. (2014) Taxonomic classification of cyanoprokaryotes (cyanobacterial genera) 2014, using a polyphasic approach. *Preslia* 86: 295–335.

Nabout, J.C., Silva-Rocha, B., Carneiro, F.M. & Sant'Anna, C.L. (2013) How many species of cyanobacteria are there? Using a discovery

curve to predict the species number. *Biodiversity and Conservation* 22 (12): 2907–2918.
http://dx.doi.org/10.1007/s10531-013-0561-x

Rabenhorst, L. (1864) *Flora europaea algarum aquae dulcis et submarinae*. *Sectio I*. *Algas diatomaceas complectens*, *cum figuris generum omnium xylographice impressis*. Apud Eduardum Kummerum, Leipzig, 359 pp.
http://dx.doi.org/10.5962/bhl.title.7029

Rott, E. (2008) *Chamaesiphon komarekii* species nova, a new benthic freshwater chroococcalean species (Cyanophyta/Cyanobacteria) from western coniferous forest streams in British Columbia, Canada. *Algological Studies* 126: 37–46.
http://dx.doi.org/10.1127/1864-1318/2008/0126-0037

Sant'Anna, C.L., Gama, J.R., Azevedo, M.T.P. & Komarek, J. (2011) New morphospecies of *Chamaesiphon* (Cyanobacteria) from Atlantic rainforest, Brazil. *Fottea* 11: 1–6.

Strunecký, O., Komárek, J. & Šmarda, J. (2014) *Kamptonema* (Microcoleaceae, Cyanobacteria), a new genus derived from the polyphyletic *Phormidium* according to combined molecular and cytomorphological markers. *Preslia* 86: 193–207.