Rapid report

Early Middle Ordovician evidence for land plants in Argentina (eastern Gondwana)

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Summary

• The advent of embryophytes (land plants) is among the most important evolutionary breakthroughs in Earth history. It irreversibly changed climates and biogeochemical processes on a global scale; it allowed all eukaryotic terrestrial life to evolve and to invade nearly all continental environments. Before this work, the earliest unequivocal embryophyte traces were late Darriwilian (late Middle Ordovician; *c.* 463–461 million yr ago (Ma)) cryptospores from Saudi Arabia and from the Czech Republic (western Gondwana).

• Here, we processed Dapingian (early Middle Ordovician, *c.* 473–471 Ma) palynological samples from Argentina (eastern Gondwana).

• We discovered a diverse cryptospore assemblage, including naked and envelope-enclosed monads and tetrads, representing five genera.

• Our discovery reinforces the earlier suggestion that embryophytes first evolved in Gondwana. It indicates that the terrestrialization of plants might have begun in the eastern part of Gondwana. The diversity of the Dapingian assemblage implies an earlier, Early Ordovician or even Cambrian, origin of embryophytes. Dapingian to Aeronian (Early Silurian) cryptospore assemblages are similar, suggesting that the rate of embryophyte evolution was extremely slow during the first *c.* 35–45 million yr of their diversification. The Argentinean cryptospores predate other cryptospore occurrences by *c.* 8–12 million yr, and are currently the earliest evidence of plants on land.

Introduction

Embryophytes are a monophyletic group of photosynthetic eukaryotes that share the following characters: a diplobiontic life cycle; a terrestrial growth habit; a cuticle; antheridia; archegonia (Kenrick & Crane, 1997a; Karol *et al.*, 2001). Another important character of embryophytes is the ability to synthesize sporopollenin, an extremely resistant polymer

that is a major component of the outer cell wall (exine) of spores and pollen grains. The presence of sporopollenin in exine was presumably a decisive factor during the early terrestrialization of plants because it allows long-range dispersal of spores, and provides structural support and partial protection against UV light, desiccation and micro-organism attack. The production of spores covered by sporopollenin is generally accepted to indicate unambiguous embryophyte affinities (Kenrick & Crane, 1997b; Wellman *et al.*, 2003; Gensel, 2008). Sporopollenin is also found in *Coleochaete* (Charophyta; Delwiche *et al.*, 1989), but in this case it is deposited on the fertilization product (the zygote), and not on the meiosis products (zoospores).

Colonization of the land by plants presumably occurred in a step-wise fashion starting during the Early Paleozoic with plants at a bryophyte, most likely liverwort, grade of organization (Steemans *et al.*, 2009). It resulted in acceleration of weathering processes and in the formation of modern terrestrial environments, including structured soils and complex microbial communities (Algeo *et al.*, 2001); it also profoundly affected carbon cycling, changed the atmosphere composition and irreversibly altered climates (Algeo *et al.*, 2001; Berner, 2001; Graham *et al.*, 2004; Beerling & Berner, 2005). Despite outstanding recent advances, the timing and locations of origination of all major embryophytes traits (alternation of generations, spores, cuticle and stoma, vascular tissue, leafs, wood, seeds, etc.) that lead to their dominant position in almost all terrestrial ecosystems are the subject of active controversies, especially concerning the early evolution of sporopollenin-coated propagules (Steemans *et al.*, 2010). The propagules of the earliest embryophytes are cryptospores (i.e. spores that lack haptotypic features such as a trilete or a monolete mark) (Steemans, 2000). Cryptospores may be preserved in monad, dyad or tetrad configurations, with different types of contact areas between individual spores and diverse external features: cryptospore outer walls are smooth or ornamented; the cryptospores themselves are naked or enclosed within an envelope; the envelopes are smooth or ornamented. Before this work, the oldest uncontroversial records of embryophytes were late Darriwilian/Llanvirn (*c.* 463–461 million years ago (Ma)) cryptospores from the Czech Republic and from Saudi Arabia, on the western margin of the Gondwana paleocontinent (Vavrdová, 1990; Strother *et al.*, 1996). Analysis of wall ultrastructure of slightly younger cryptospores from Oman suggests that they were produced by early liverworts (Wellman *et al.*, 2003). In this paper, we report the discovery of a moderately diverse cryptospore assemblage from an early Middle Ordovician locality in Argentina (see the Supporting Information, Fig. S1).

Materials and Methods

Palynological samples were collected from the Rio Capillas outcrop, southern part of the Sierra de Zapla of the Sierras
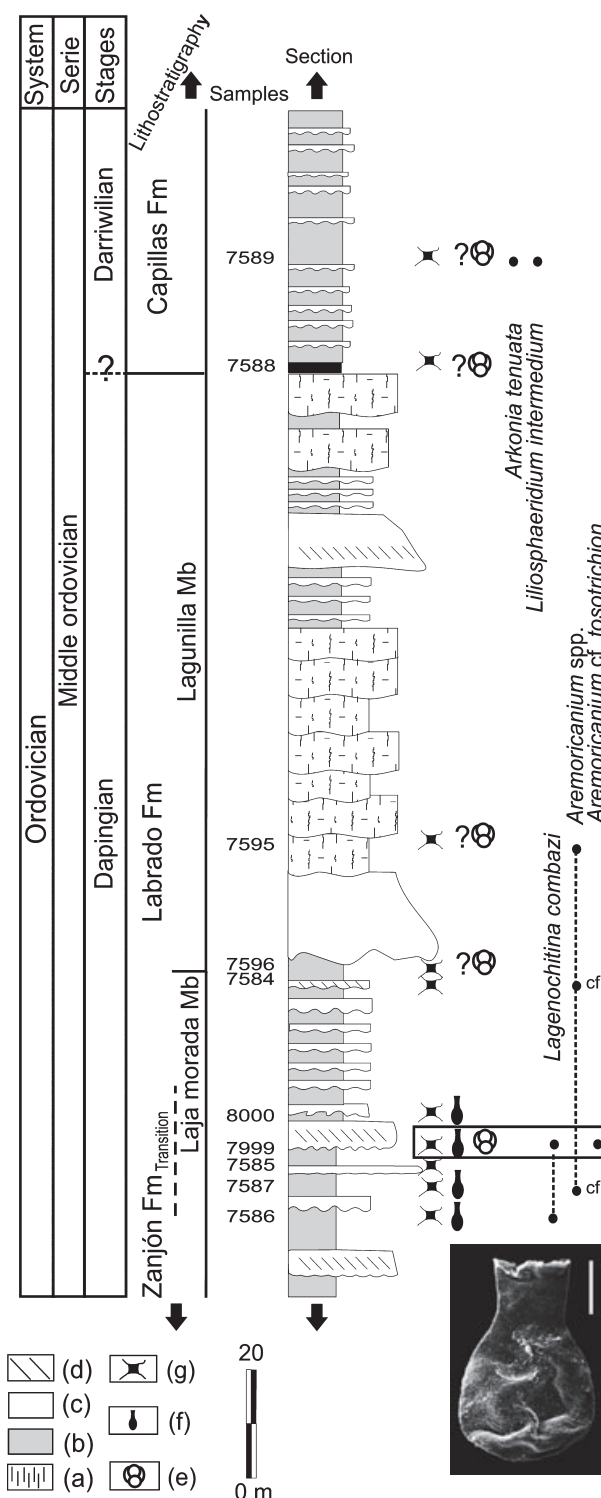

Fig. 1 Middle Ordovician units exposed in the Río Capillas showing stratigraphy, lithology and location of samples. The biostratigraphic range of some important palynomorphs is provided. The sample studied here is from level 7999. A specimen of the chitinozoan biostratigraphic marker, *Lagenochitina combazi,* is illustrated. Bar, 50 μm. Lithology: (a) bioturbation, (b) siltstone/mudstone, (c) sandstone, (d) cross-bedded sandstone. Palynomorphs: (e) cryptospores, (f) chitinozoans, (g) acritarchs.

Subandinas (Subandean Ranges), Central Andean Basin, north-western Argentina (Fig. S2). The Ordovician section exposes in stratigraphic order the upper part of the Zanjón Formation, the Labrado Formation and the lower part of the Capillas Formation (Fig. 1). Based on sedimentological and ichnological data, the Zanjón Formation is interpreted as a succession of tidal parasequences that shallow up into heterolithic and muddy intervals and subaerial exposure features (Astini, 2003; Astini & Marengo, 2006). Accordingly, the Zanjón Formation is interpreted to represent mostly estuarine environments.

Five samples were collected from upper part of the Zanjón Formation and its transition to the Laja Morada Member of the Labrado Formation (Fig. 1) that are assigned an early to middle Dapingian age (early Middle Ordovician) on the basis of the presence of the chitinozoan biostratigraphic marker *Lagenochitina combazi* (Chen *et al.*, 2009; F. Paris, pers. comm.) (Fig. 1; Note S1). This age is consistent with the presence of the acritarch genus *Aremoricanium*, which has a first appearance datum in the Dapingian (Brocke *et al.*, 2000). Sample 7999 (Fig. 1) from the Zanjón Formation includes a moderately diverse cryptospore assemblage described here.

Samples were carefully cleaned before processing in order to prevent contamination from modern material. The samples were processed at the laboratory of the Paleopalynology Unit, IANIGLA, CCT CONICET – Mendoza, Argentina, using standard palynological HCl–HF–HCl acid maceration techniques. A duplicate sample was processed at the palynological laboratory of the University of Liège, Belgium. Each sample was crushed and *c.* 30 g were demineralized in HCl–HF. The residue was oxidized in HNO_3 and $KClO_3$ and sieved through a 12 μm mesh. The palynological characteristics of preparations from both laboratories were identical and included cryptospores.

Results

The cryptospores are relatively well preserved, allowing the identification of several specimens at generic or specific level (Figs 2, S3). The assemblage comprised monads and tetrads. The assemblage includes the following taxa. (1) *Chomotriletes*? sp. (Fig. 2a,b; Note S2) is an ornamented hilate monad consisting of a subcircular cryptospore distally sculptured by convolute muri mimicking finger prints. The specimens are close to *Chomotriletes*? sp. A from the Late Silurian of the Urubu area, Brazil (Steemans *et al.*, 2008). (2) *Gneudnaspora* (*Laevolancis*) *divellomedia* (Balme, 1988) or *Laevolancis chibrikovae* (Steemans *et al.*, 2000) (Fig. 2c,d) is a monad with subcircular amb and an equatorial and distal patina delimitating a thin proximal laevigate hilum. The relatively poor preservation of the specimens does not allow a secure identification of the species. (3) *Sphaerasacus glabellus* (Steemans *et al.*, 2000) (Fig. 2e,f) is a circular monad with a thick laevigate wall, enclosed within a thin and narrow envelope. Specimens are similar to those observed in the Late Ordovician (Wellman, 1996) and in the Llandovery (Steemans *et al.*, 2000). (4) *Tetrahedraletes* cf. *medinensis* (Strother & Traverse, 1979) (Fig. 2g) is a tetrad of subcircular, laevigate spores, with a very thin exine. (5) A new genus (Fig. 2h) consists of a quadraedric tetrad of subcircular laevigate spores with equatorial crassitude,
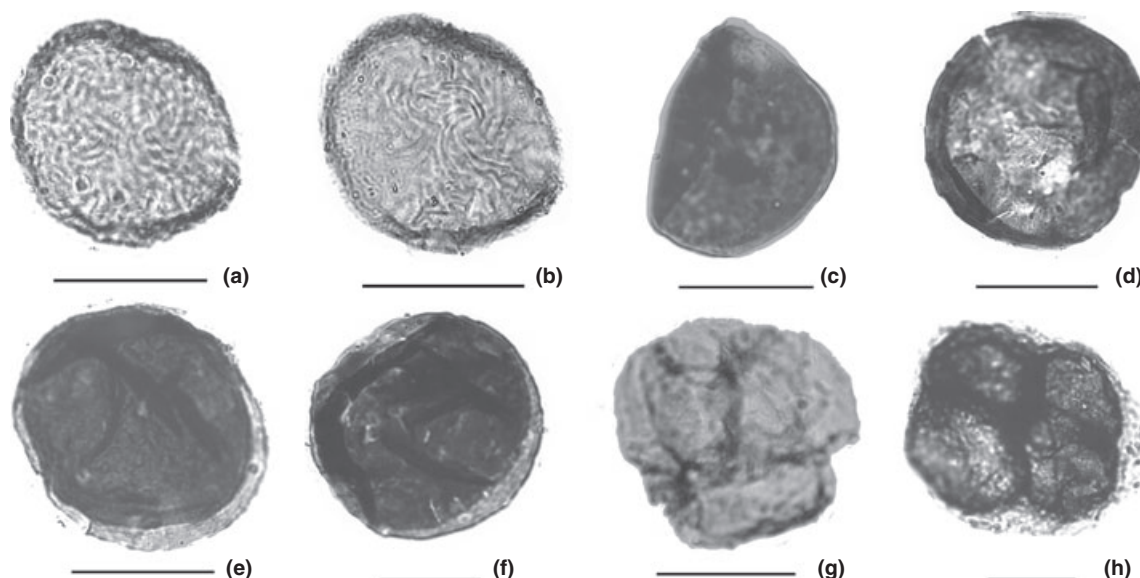

Fig. 2 Cryptospore plate from upper part of the Zanjón Formation. (a) Distal face of *Chomotriletes*? sp.; (b) proximal face of *Chomotriletes*? sp.; (c,d) *Gneudnaspora* (*Laevolancis*) *divellomedia* or *Laevolancis chibrikovae*; (e,f) *Sphaerasacus glabellus*; (g) *Tetrahedraletes* cf. *medinensis*; (h) new genus. Bar, 20 μm. (See the Supporting Information Fig. S3 for color plate.)

enclosed within a thin, loose, apparently smooth envelope. Identical tetrads have been found at a Llandovery locality from Paraguay (Mendlowicz Mauller, 2004) and at Middle to Late Ordovician localities from Saudi Arabia Commission Internationale de la Microflore du Paléozoïque (CIMP; http://www.cimp.ulg.ac.be/) working group on Saudi Arabia, work in progress).

Discussion

The cryptospore assemblage described here is of Dapingian age (*c.* 473–471 Ma), thus predating by *c.* 8–12 million yr the previous earliest known cryptospores from the Czech Republic (Vavrdová, 1990) and from Saudi Arabia (Strother *et al.*, 1996) (Fig. S1). There is a growing body of evidence that terrestrial life originated on the Gondwana paleocontinent. The early cryptospore record (Vavrdová, 1990; Strother *et al.*, 1996), the earliest plant fragments (Wellman *et al.*, 2003) and the earliest trilete spores (Steemans *et al.*, 2009), most probably produced by the earliest vascular plants or their immediate ancestors, were all described from localities on the western margin of Gondwana. On the basis of the discovery of this Argentinean cryptospore assemblage and according to the paleogeographic configuration (Fig. S4) of the Gondwana during Dapingian times (Scotese, 2003), we suggest that the early steps of the terrestrialization of plants could rather have occurred on the eastern margin of the continent, some six thousand kilometers more to the east, and that plants only subsequently reached the western part of Gondwana. Interestingly, localities from Argentina and from the Czech Republic/Saudi Arabia were all located at *c.* 35°S latitude (Scotese, 2003) during Middle Ordovician times (Fig. S4).

The classification of green organisms (green algae and embryophytes) is still actively debated, but it is generally accepted that embryophytes originated from charophycean green algae, with Charales being their most probable closest living relatives (Karol *et al.*, 2001; Qiu *et al.*, 2007), and that liverworts are the most basal lineage in embryophyte phylogeny (Qiu *et al.*, 1998, 2007). The estimated time of divergence of Charales and embryophyte ranges from *c.* 805 Ma to *c.* 438.8 Ma, depending on the study considered (Magallón & Hilu, 2009). The cryptospores described here are *c.* 470 million yr old. Their occurrence may be considered a reliable estimate of the latest possible date for the origin of embryophytes. It also provides a solid calibration point for molecular evolutionary models used to estimate the divergence time of the major lineages of embryophytes. Our discovery of Dapingian cryptospores further supports the basalmost position of the liverworts in embryophyte phylogeny.

During Ordovician times, the Sierras Subandinas were the outermost region of the proto-Andean foreland basin, positioned at the eastern Gondwana margin. The Zanjón Formation that yielded the cryptospore assemblage described

here has been interpreted as mostly representing estuarine environments (Astini, 2003; Astini & Marengo, 2006). Unfortunately, this does not give any information on the actual origin of the cryptospore-producing plants, as small particles such as cryptospores can be transported in water over very long distances (Traverse, 2007). Nevertheless, the presence of early cryptospores in nearshore or estuarine environments suggests that their mother plants did not live in remote upland areas disconnected from major hydrographical networks, as had previously been suggested for much younger, earliest Devonian cryptospores (Steemans, 2000).

The assemblage described here includes five cryptospore genera. Both from morphological and systematic points of view, this Dapingian assemblage is not different from younger cryptospore occurrences, including Aeronian (Early Silurian, *c.* 439–436 Ma) assemblages. This seems to indicate that the evolutionary rate of the earliest embryophytes was extremely low, or that selective pressures did not act on the morphology of propagules.

The Dapingian cryptospore assemblage from Argentina currently represents the earliest unequivocal fossil evidence of the presence of plants on emerged lands (Fig. S1). It suggests that the earliest steps of plants in terrestrial habitats may have occurred on the eastern margin of the Gondwana paleocontinent (Scotese, 2003) (Fig. S4). Although moderate, its diversity (five different genera) implies that the very origin of land colonization by plants is presumably older, and that it may have occurred during Early Ordovician times (488–472 Ma) or even Cambrian (*c.* 542–488 Ma) times (Strother & Beck, 2000).

References

Algeo TJ, Scheckler SE, Maynard JB. 2001. Effects of the Middle to Late Devonian spread of vascular land plants on weathering regimes, marine biotas and global climate. In: Gensel PG, Edwards D, eds. *Plants invade the land. Evolutionary & environmental perspectives*. New York, NY, USA: Columbia University Press, 213–236.

Astini RA. 2003. Ordovician basins of Argentina. In: Benedetto JL, ed. *Ordovician fossils of Argentina*. Córdoba, Argentina: Universidad Nacional de Córdoba, 1–74.

Astini RA, Marengo L. 2006. Paleoambientes y estratigrafía secuencial de depósitos marinos marginales del Ordovícico de la sierra de Zapla (Sierras Subandinas, noroeste argentino) y su relación con la Cuenca Andina Central. *Revista Geológica de Chile* **33**: 247–276.

Balme B. 1988. Miospores from Late Devonian (Early Frasnian) strata, Carnarvon Basin, Western Australia. *Palaeontographica Abt B* **209**: 109–166.

Beerling DJ, Berner R. 2005. Feedbacks and the coevolution of plants and atmospheric CO_2. *Proceedings of the National Academy of Sciences, USA* **102**: 1302–1305.

Berner R. 2001. The effect of the rise of land plants on atmospheric CO_2 during the Paleozoic. In: Gensel PG, Edwards D, eds. *Plants invade the land: evolutionary and environmental approaches*. New York, NY, USA: Columbia University Press, 173–178.

Brocke R, Li J, Wang Y. 2000. Upper Arenigian to lower Llanvirnian acritarch assemblages from South China: a preliminary evaluation. *Review of Palaeobotany and Palynology* **113**: 27–40.

Chen XH, Paris F, Wang XF, Zhang M. 2009. Early and Middle Ordovician chitinozoans from the Dapingian type sections, Yichang area, China. *Review of Palaeobotany and Palynology* **153**: 310–330.

Delwiche CF, Graham LE, Thomson N. 1989. Lignin-like compounds and sporopollenin in Coleochaete, an algal model for land plant ancestry. *Science* **245**: 399–401.

Gensel PG. 2008. The earliest land plants. *Annual Review of Ecology Evolution and Systematics* **39**: 459–477.

Graham LE, Kodner RB, Fisher MM, Graham JM, Wilcox LW, Hackney JM, Obst J, Bilkey PC, Hanson DT, Cook ME. 2004. Early land plant adaptations to terrestrial stress: a focus on phenolics. In: Hemsley AR, Poole I, eds. *The evolution of plant physiology. From whole plants to ecosystems*. London, UK: Elsevier Academic Press, 155–169.

Karol KS, McCourt RM, Cimino MT, Delwiche CF. 2001. The closest living relatives of land plants. *Science* **294**: 2351–2353.

Kenrick P, Crane PR. 1997a. *The origin and early diversification of land plants. A cladistic study*. Washington and London, USA and UK: Smithsonian Institution press.

Kenrick P, Crane PR. 1997b. The origin and early evolution of plants on land. *Nature* **389**: 33–39.

Magallón S, Hilu KW. 2009. Land plants (Embryophyta). In: Hedges SB, Kumar S, eds. *The timetree of life*. Oxford, UK: University Press, 133–137.

Mendlowicz Mauller P. 2004. *Caracterização Estratigráfica e Bioestratigráfica do Intervalo Llandoveriano (Siluriano) da Bacia do Paraná, no Paraguai Oriental, com base nos furos de sondagem 269-R1 e 269-R2*. Master's degree, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil.

Qiu YL, Cho Y, Cox JC, Palmer JD. 1998. The gain of three mitochondrial introns identifies liverworts as the earliest land plants. *Nature* **394**: 671–674.

Qiu YL, Li LB, Wang B, Chen ZD, Dombrovska O, Lee J, Kent L, Li RQ, Jobson RW, Hendry TA. 2007. A non-flowering land plant phylogeny inferred from nucleotide sequences of seven chloroplast, mitochondrial and nuclear genes. *International Journal of Plant Sciences* **168**: 691–708.

Scotese CR. 2003. Paleomap Project. URL http://www.scotese.com/Default.htm.

Steemans P. 2000. Miospore evolution from the Ordovician to the Silurian. *Review of Palaeobotany and Palynology* **113**: 189–196.

Steemans P, Higgs KT, Wellman CH. 2000. Cryptospores and trilete spores from the Llandovery, Nuayyim-2 Borehole, Saudi Arabia. In: Al-Hajri S, Owens B, eds. *Stratigraphic palynology of the Palaeozoic of Saudi Arabia*. Manama, Bahrain: GeoArabia, Special Volume 1, 92–115.

Steemans P, Le Hérissé A, Melvin J, Miller MA, Paris F, Verniers J, Wellman CH. 2009. Origin and radiation of the earliest vascular land plants. *Science* **324**: 353.

Steemans P, Lepot K, Marshall CP, Le Hérissé A, Javaux EJ. 2010. FTIR characterisation of the chemical composition of Silurian miospores (cryptospores and trilete spores) from Gotland, Sweden. *Review of Palaeobotany and Palynology*. doi:10.1016/j.revpalbo.2010.07.006.

Steemans P, Rubinstein C, Melo JHG. 2008. Siluro-Devonian miospore biostratigraphy of the Urubu River area, western Amazon Basin, northern Brazil. *Geobios* **41**: 263–282.

Strother PK, Al-Hajri S, Traverse A. 1996. New evidence for land plants from the lower Middle Ordovician of Saudi Arabia. *Geology* **24**: 55–59.

Strother PK, Beck JH. 2000. Spore-like microfossils from Middle Cambrian strata: expanding the meaning of the term cryptospore. In: Harley MM, Morton CM, Blackmore S, eds. *Pollen and spores: morphology and biology*. Kew, UK: Royal Botanic Gardens, 413–424.

Strother PK, Traverse A. 1979. Plant microfossils from the Llandoverian and Wenlockian rocks of Pennsylvania. *Palynology* **3**: 1–21.

Traverse A. 2007. Paleopalynology, 2nd edn. In: Landman NL, Jones DS, eds. *Topics in geobiology*. Dordrecht, the Netherlands: Springer, 1–813.

Vavrdová M. 1990. Early Ordovician acritarchs from the locality Myto near Rokycany (late Arenig, Czechoslovakia). *Casopis pro Mineralogii a Geologii* **35**: 239–250.

Wellman CH. 1996. Cryptospores from the type area for the Caradoc Series (Ordovician) in southern Britain. *Palaeontology* **55**: 103–136.

Wellman CH, Osterloff PL, Mohiuddin U. 2003. Fragments of the earliest land plants. *Nature* **425**: 282–285.

Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Ordovician chronostratigraphic scale.

Fig. S2 Location map and regional geological map of the studied area along the Rio Capillas.

Fig. S3 Color plate of cryptospores from upper part of the Zanjón Formation.

Fig. S4 Schematic Middle Ordovician paleogeographic map.

Note S1 Additional stratigraphic data.

Note S2 About the biological affinities of *Chomotriletes*.

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