Pleiocarpon gardiennetii (*Nectriaceae*), a new holomorphic species from French Guiana

Christian LECHAT
Jacques FOURNIER

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Abstract: *Pleiocarpon gardiennetii* sp. nov. is described and illustrated based on a collection on a dead pyrenolichen, *Astrothelium* sp. (*Trypetheliaceae*), in French Guiana. The cylindrocarpon-like asexual morph of this fungus was obtained in culture and a rDNA ITS sequence was likewise obtained from this fungus. Based on morphological characteristics of both asexual and sexual morphs, this fungus belongs to the aggregate formerly known as *Neonectria s. l.* (*Nectriaceae*), which is supported by our phylogenetic data. Our phylogenetic analysis places it in the monotypic genus *Pleiocarpon*, but distinct from *P. streliziae*, the type species. *Pleiocarpon gardiennetii* is therefore proposed as a new species; it is characterized by orange red to red ascomata turning purple in 3% KOH and yellow in lactic acid, rough-walled, not collapsing upon drying, with a discoid apex; ascospores are hyaline, echinulate, 9–10 × 4–4.5 µm µm; microconidia in culture are 7–13(–15) × 3.5–5 µm and macroconidia are 1–3-septate and up to 52–55 × 6 µm.
Keywords: Ascomycota, cylindrocarpon-like, *Hypocreales*, lichenicolous fungi, ribosomal DNA, taxonomy.

Résumé : *Pleiocarpon gardiennetii* sp. nov. est décrit et illustré d'après une récolte sur un pyrénolichen mort, *Astrothelium* sp. (*Trypetheliaceae*), en Guyane française. La forme asexuée de type cylindrocarpon a été obtenue en culture et une séquence ITS a également été obtenue de ce champignon. En se fondant sur les caractères morphologiques des stades sexué et asexué, ce champignon fait partie du groupe connu précédemment comme *Neonectria s. l.* (*Nectriaceae*), ce qui est confirmé par nos résultats phylogénétiques. Notre analyse phylogénétique le situe dans le genre monotypique *Pleiocarpon*, mais distinct de *P. streliziae*, l'espèce type. *Pleiocarpon gardiennetii* est donc proposé comme espèce nouvelle ; il est caractérisé par des ascomes rouge orange à rouge foncé devenant violets dans la potasse à 3 % et jaunes dans l'acide lactique, à paroi rugueuse, ne s'aplatissant pas au séchage, dotés d'un sommet discoïde ; ses ascospores sont hyalines, échinulées, 9–10 × 4–4.5 µm ; les microconidies en culture mesurent 7–13(–15) × 3.5–5 µm et les macroconidies possèdent 1–3 cloisons et mesurent jusqu'à 52–55 × 6 µm.
Mots-clés : ADN ribosomal, Ascomycota, champignons lichénicoles, cylindrocarpon, Hypocréales, taxinomie.

Introduction

In the course of a survey of fungi in Saül, French Guiana, in August 2018, a nectriaceous fungus was collected on a dead pyrenolichen, *Astrothelium* sp. Morphological characteristics of the sexual morph and the cylindrocarpon-like asexual morph obtained in culture clearly suggested affinities with genera formerly assigned to *Neonectria s. l.* (Rossman *et al.*, 1999; Mantiri *et al.*, 2001; Chaverri *et al.*, 2011; Salgado-Salazar *et al.*, 2016). Comparison of an ITS sequence from this fungus with those of species in genera assigned to the *Neonectria* clade showed closest affinities with the recently described monotypic genus *Pleiocarpon* L. Lombard & D. Aiello (Aiello *et al.*, 2017). The comparison with the ITS sequence and asexual morph of *P. streliziae* L. Lombard & D. Aiello led us to propose our fungus as a distinct species, *P. gardiennetii* sp. nov. This paper presents morphological, cultural and molecular evidence supporting the description of this new species.

Materials and methods

Dry specimens were rehydrated and examined using the method described by Rossman *et al.* (1999). Microscopical observations and measurements were made in water. The holotype specimen was deposited in LIP herbarium (Lille, France) and living cultures at CIRM-CF (Centre International des Resources Microbiennes, Marseille, France). Cultures of the living specimen were plated on PDA (Potato Dextrose Agar) with 5 mg/l of streptomycin in Petri dishes 5 cm diam., incubated at 25°C. DNA extraction, amplification, and sequencing were performed by ALVALAB (Santander, Spain) as follows: total DNA was extracted from dry specimens blending a portion using a micropestle in 600 µL CTAB buffer (CTAB 2%, NaCl 1.4 M, EDTA pH 8.0 20 mM, Tris-HCl pH 8.0 100 mM). The resulting mixture was incubated for 15 min at 65 °C. A similar volume of chloroform: isoamylalcohol (24:1) was added and carefully mixed with the samples until their emulsion. It was then centrifuged for 10 min at 13,000 g, and the DNA in the supernatant was precipitated with a volume of isopropanol. After a new centrifugation of 15 min at the same speed, the pellet was washed in 70% cold ethanol, centrifuged again for 2 min and dried. It was finally resuspended in 200 µL ddH_2O. PCR amplification was performed with the primers ITS1F and ITS4 (White *et al.*, 1990; Gardes & Bruns, 1993) for ITS. Chromatograms were checked searching for putative reading errors, and these were corrected.

Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 6 (Tamura *et al.*, 2013). Nomenclature follows Mycobank (Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands).

Taxonomy

Pleiocarpon gardiennetii Lechat & J. Fourn., *sp. nov.* Fig. 2
Mycobank: MB 829828

Diagnosis: *Pleiocarpon gardiennetii* differs morphologically from *P. strelitziae* in having larger microconidia, and longer 1–3-septate macroconidia and by its lichenicolous habitat.

Holotype: French Guiana: Saül, sentier des Gros Arbres, on a dead pyrenolichen *Astrothelium* sp. on bark, 24 Aug. 2018, *leg.* A. Gardiennet, CLLG18038 (LIP), ex-type culture: BRFM 2782 (CIRM-CF, Marseille, France), ITS GenBank sequence: MK499444.

Etymology: Named in honour of our friend and colleague A. Gardiennet, who collected this species.

Ascomata superficial, scattered or in small groups on substrate, non-stromatic, tightly attached to the substrate, rugose, reddish orange to dark red, widely obpyriform to subglobose, (240–)260–280(–300) µm high, 230–250 µm diam. (Me = 275 × 240 µm, n = 10), not collapsing or laterally pinched when dry, turning purple in 3% KOH, yellow in lactic acid. **Perithecial apex** discoid, flattened, slightly constricted beneath the disc, composed of globose to narrowly ellipsoidal, thick-walled cells with dark orange wall 2.5–3 µm thick, with a minute, lighter coloured, obtuse papilla. **Ascomatal wall** 30–40 µm thick, composed of two intergrading regions; outer

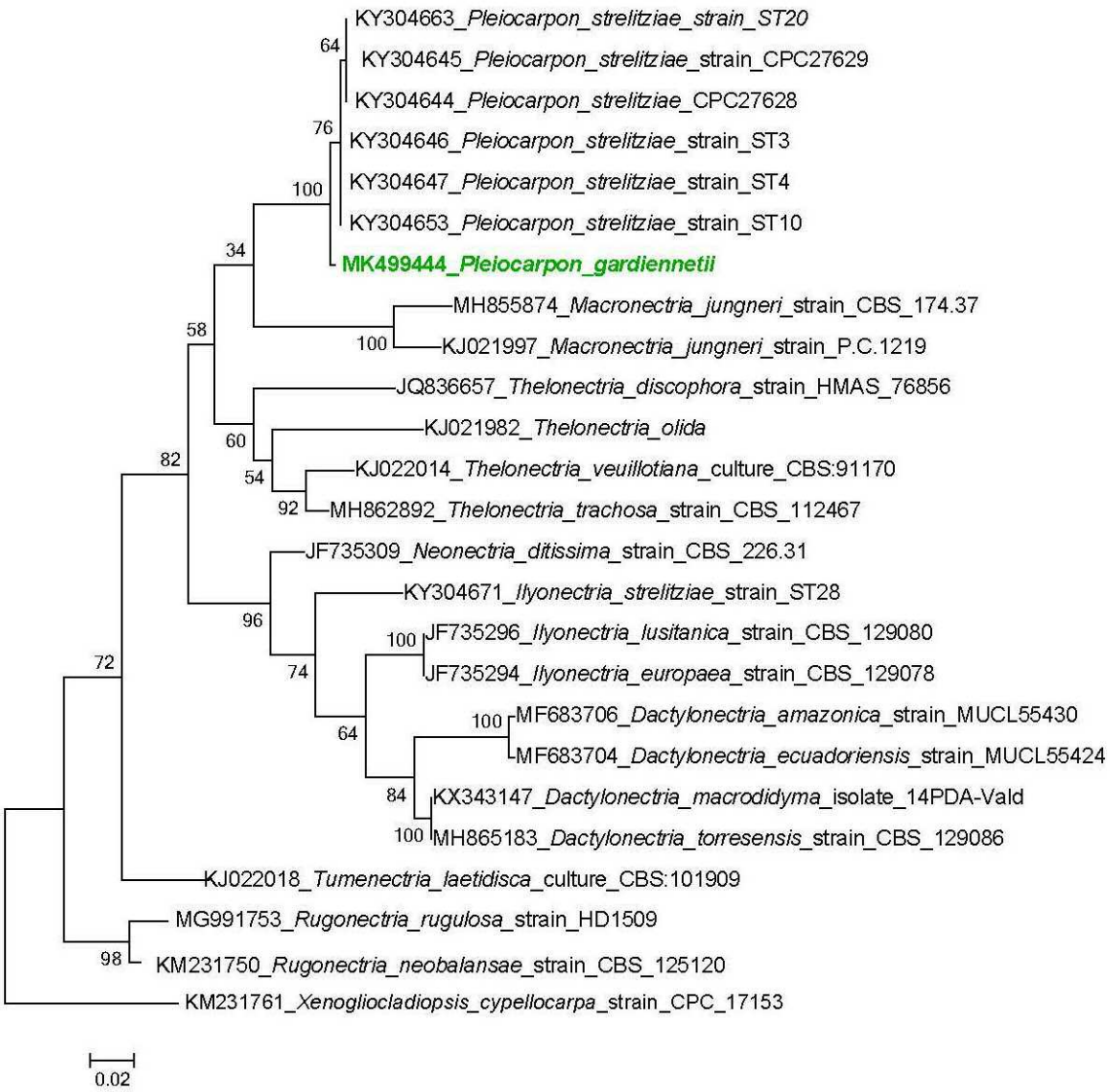

Fig. 1 – Maximum likelihood phylogeny (-lnL = 3008.47131) of *Pleiocarpon gardiennetii* inferred by PhyML 3.0, model HKY85 from a 560 bp matrix of ITS sequences, rooted with *Xenogliocladiopsis cypellocarpa*.

region 10–15(–20) µm thick, composed of subglobose to ellipsoidal thick-walled cells 10–20 × 8–15 µm, with orange walls 2–2.5(–3) µm thick, some protruding outwardly; inner region 20–25 µm thick, composed of globose to ellipsoidal, orange, thick-walled cells, becoming subhyaline, elongated, flattened, 10–18 × 3–4 µm inwardly. **Ascomatal surface** composed of subglobose to ellipsoidal, subangular, thick-walled cells up to 20 µm in greatest dimension, with wall 2.5–3 µm thick. **Asci** (65–)75–85(–90) × 7–10(–12) µm (Me = 80 × 8.5 µm, n = 20), short-stipitate, cylindrical to narrowly clavate, apex with a thin slightly refractive disc, containing 8 obliquely uniseriate ascospores or biseriate above and uniseriate below. **Ascospores** (8–)9–10(–10.5) × 4–4.5(–5) µm (Me = 9.5 × 4.3 µm, n = 30), ellipsoidal, equally two-celled, not to barely constricted at septum, hyaline, echinulate.

Culture characteristics: After two weeks on PDA at 25°, colony 45–50 mm diam., pale brown at inoculation point, aerial mycelium pale cinnamon in median area, off-white at margin, diffusing a rust colouration in medium, sporulating at margin. Mycelium composed of septate, hyaline, smooth hyphae 2.5–3.5 µm diam. Conidiophores simple or branched, arising from aerial hyphae, macronematous, flexuous, 60–80(–120) µm long, 3.5–4 µm diam. at base, hyaline to pale brown, producing abundant, hyaline, aseptate, ellipsoid to ovoid or subcylindrical microconidia, with rounded apices, attenuated towards base with or without a median apiculate hilum, smooth-walled, 7–13(–15) µm long, 3.5–5 µm wide (Me = 11 × 4 µm, n = 30). Macroconidia formed after three weeks, straight to slightly curved, smooth, hyaline, rounded at ends 1–3-septate; 1-septate 20–40 × 5–5.5 µm; 2-septate 37–42(–50) × 5–6 µm; 3-septate 52–55 × 6 µm. Chlamydospores abundant, single or in chains, globose to subglobose, pale brown, appearing after four weeks.

Discussion

Pleiocarpon was recently introduced by Aiello *et al.* (2017) to accommodate a single species, *P. strelitziae*, whose sexual morph is unknown. Its cylindrocarpon-like asexual morph is typical of *Neonectria s. l.* and phylogenetic analysis confirmed its placement in this clade but on a separate branch whose closest phylogenetic neighbour is *Thelonectria* P. Chaverri & C. Salgado. The main morphological difference between *Pleiocarpon* and *Thelonectria* highlighted by Aiello *et al.* (2017) is the presence of abundant microconidia in cultures of *Pleiocarpon* unlike in *Thelonectria*. Our fungus shows close phylogenetic affinities with *P. streliziae*, with which ITS sequence shows 98.6% similarity, and which occurs on a separate branch in the *Pleiocarpon* subclade (Fig. 1). Affinities with *Pleiocarpon* are corroborated by the abundant microconidia produced in culture by the new species. It is interesting to note that the same phylogenetic affinities of *Pleiocarpon* with *Thelonectria*

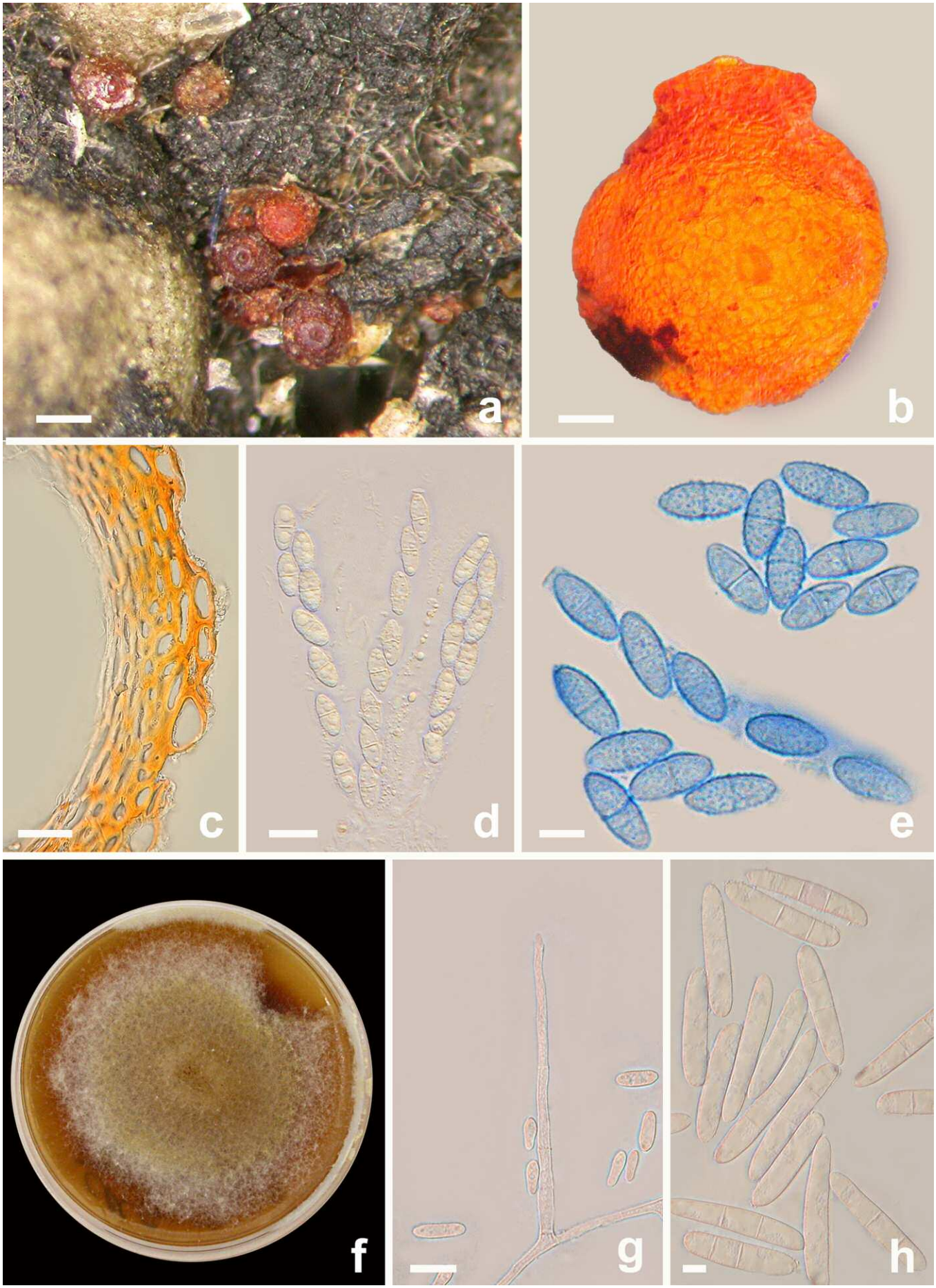

Fig. 2 – a-h: *Pleiocarpon gardiennetii* (Holotype CLLG18038); a: Ascomata on substrate; b: Ascoma in water in side view; c: Vertical section of lateral ascomatal wall; d: Asci showing slightly refractive apical discs and ascospores, in water; e: Ascus and ascospores in lactic cotton blue, ascus showing an apical disc and ascospores with echinulate ornamentation; f: Culture after three weeks; g: Conidiophore and microconidia from culture; h: Macroconidia from culture. Scale bars: a = 200 µm; b = 50 µm; c = 20 µm; d = 10; e, h = 5 µm; g = 10 µm.

demonstrated by Aiello *et al.* (2017) can be found in our results, though based on ITS only instead of a multigene dataset. Morphologically, *P. strelitziae* differs from our fungus in having smaller microconidia (6–)7–9 × 2–3 µm and shorter, 1–5-septate macroconidia (41–)42–47(–50) × 5–7 µm. Moreover, our fungus produces abundant chlamydospores in culture, unlike *P. streliziae*. This set of morphological, cultural and phylogenetic characters justifies the placement of our fungus in *Pleiocarpon*, as a distinct species different from *P. streliziae*, and the description of *P. gardiennetii* sp. nov.

Pleiocarpon streliziae is a pathogen isolated from lesions on *Strelizia reginae*, a monocot native to South Africa, but cultivated in Sicily. The ecology of *P. gardiennetii*, collected on a dead pyrenolichen in French Guiana is a further differential feature, though both can be regarded as pathogens with the pathogenicity of the latter evident from its occurrence on living or dying lichens.

A literature search for lichenicolous nectriaceous fungi possibly corresponding to *P. gardiennetii* showed that a lichenicolous *Cylindrocarpon* had been described, as *C. lichenicola* (C. Massal.) D. Hawksw. (Hawksworth, 1979). Based on molecular data, Summerbell & Schroers (2002) showed that its ellipsoid to oblong conidia were atypical and misleading, and that it was in fact a *Fusarium* of the *F. solani* complex. Sandoval-Denis & Crous (2018) recently accommodated it in *Neocosmospora*, as *N. lichenicola* (C. Massal.) Sandoval-Denis & Crous. This fungus is therefore unambiguously different from *P. gardiennetii*. Although first collected on a lichen, *N. lichenicola* proved to occur worldwide as an infrequent human or plant pathogen (Sandoval-Denis & Crous, 2018). This well-documented array of potential hosts suggests that host-specificity is not always taxonomically informative and could occur in the same way in *Pleiocarpon*.

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1: C. Lechat – 64 route de Chizé, 79360 Villiers-en-Bois, France – lechat@ascofrance.fr

2: J. Fournier – Las Muros, 09420 Rimont, France – jacques.fournier@club-internet.fr