

LIMITS OF SINGLE-LOCUS BARCODING IN THE *ASPERGILLUS FLAVUS* COMPLEX FROM OCULAR INFECTIONS

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Introduction

Accurate identification of pathogenic *A. flavus* complex remains challenging in routine laboratory. Traditional identification, relying on macroscopic colony characteristics (e.g., color, texture) and microscopic features (e.g., conidiophore structure), is often insufficient to resolve species within this complex.

Material & Method

I. 3 isolates of *A. flavus*

Morphologically identified from keratitis patients

II. Cultivation for mycelium

III. DNA Exaction with kit

(© Vivantis Technologies, MY)

IV. PCR amplification using Master Mix

ITS^a: ITS1, ITS4, ITS5, V9G, LS266

BenA^a: Bt2a, Bt2b

CaM^a: cmd5, cmd6

V. Sanger sequencing

3 ITS, 3 BenA, 3 CaM

VI. BLASTN: ITS/BenA/CaM

Type or reference strains from GenBank

VII. 3 Datasets

19 ITS & 20 BenA & 12 CaM

(*A. flavus* isolates + type+ ref)

VIII. Alignment

ClustalW algorithms

IX. Editing

BIOEDIT v.7.2.5^b

X. Neighbor - joining phylogenetic tree

MEGA X^c

XI. Species identification

By % clustering and similarity

Result

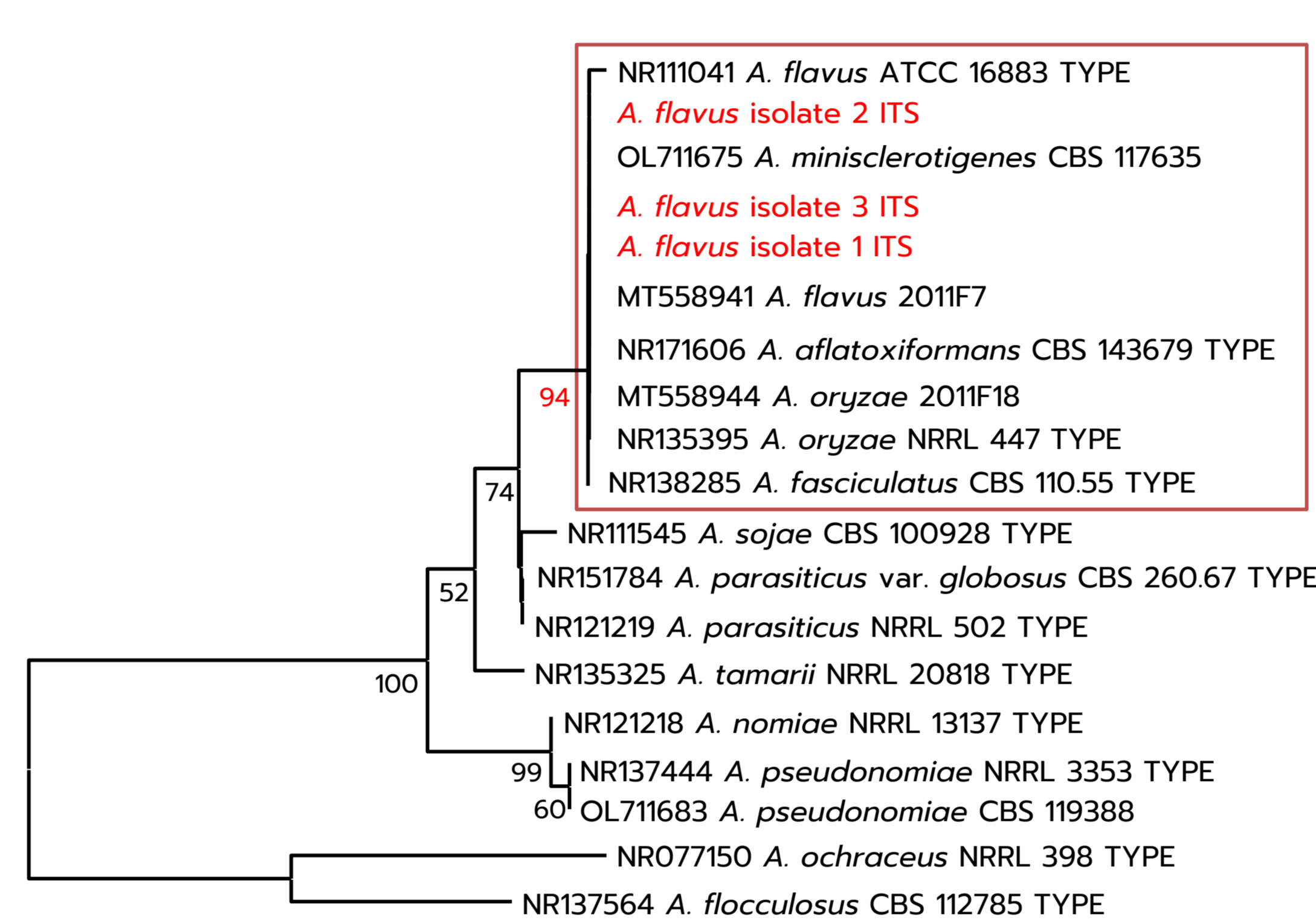


Fig 1. NJ tree of ITS: 19 *Aspergillus* spp., clustalW aligned, K2+G model, ≥ 50% 1,000 Bootstrap.

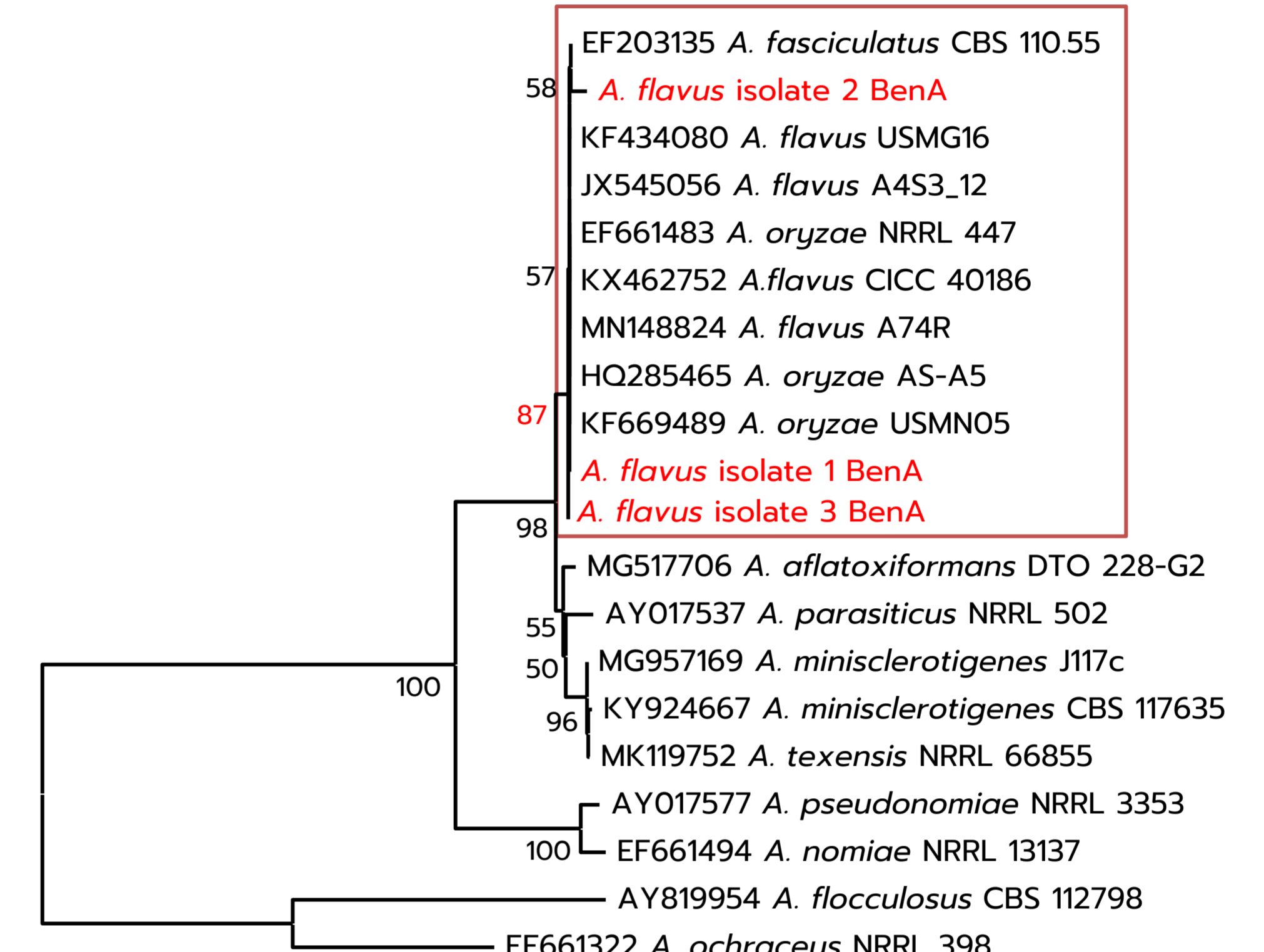


Fig 2. NJ tree of BenA: 20 *Aspergillus* spp., clustalW aligned, K2+G model, ≥ 50% 1,000 Bootstrap.

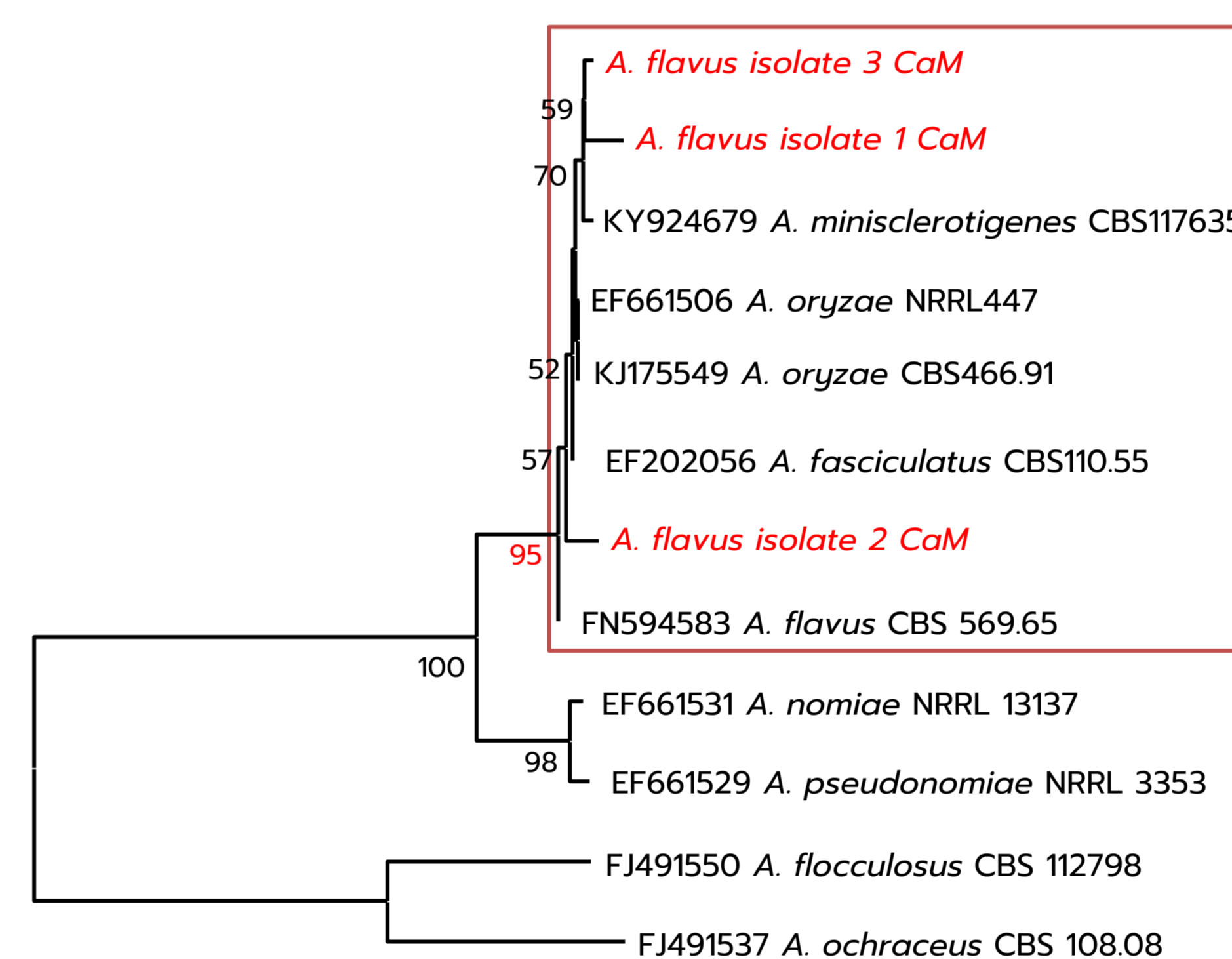


Fig 3. NJ tree of CaM: 12 *Aspergillus* spp., clustalW aligned, K2+G model, ≥ 50% 1,000 Bootstrap.

1. ITS Identification (Fig 1.)

Three strains were clustered with *A. flavus*, *A. oryzae*, *A. fasciculatus*, *A. minisclerotigenes* and *A. aflatoxiformans* at 100% similarity with 94 % bootstrap support.

2. BenA Identification (Fig 2.)

Three strains were clustered with *A. flavus* and *A. oryzae* at 99-100% similarity with 87 % bootstrap support.

3. CaM Identification (Fig 3.)

Three strains were clustered with *A. flavus*, *A. oryzae* and *A. fasciculatus* at 97-99% similarity with 95 % bootstrap support.

ITS, BenA and CaM did not separate the isolates at species level within the *A. flavus* complex.

For routine clinical, reported as *A. flavus* complex is recommended.

References

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