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**THE BIOGEOGRAPHY, ECOLOGY AND
ENDOPHYTE MYCORRHIZA OF THE NEW
ZEALAND CORYBAS ALLIANCE (Orchidaceae)
Specifically: *Nematoceras iridescens* (Irwin et
Molloy) Molloy, D.L.Jones & M.A.Clem. (Species).**

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**Roger L. S. Watkins
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Abstract

No research on the fungal endophytes in the green New Zealand terrestrial orchids has been published. Identification of the endophyte resident in *Nematoceras iridescens* roots was accomplished by comparing hyphal septal ultra-structure and using TEM imaging of the hyphal septa, all of which indicated that the genus *Tulasnella* was involved.

The *Tulasnella* species was identified using molecular techniques focused on sequencing of the ribosomal RNA locus on the ITS1–5.8S–ITS2 nuclear ribosomal gene. The endophytic fungi, resident in the host plant *N. iridescens* and the germinating seed of this species, were identified for the first time as strains of *Tulasnella calospora*.

Scanning electron microscopy (SEM), light microscopy (LM) and Confocal Laser Scanning Microscopy (CLSM) was used to investigate spatial distribution of the endophyte hyphae within the plant. The SEM results identified four morphological types of hyphae: initiating, divaricating, intercellular and necrotised, or undergoing lysis. Peloton formation only occurred in specific areas of the root; mainly within the sub-epidermal and mid cortex cells. No hyphal involvement within the stele or the immediately adjacent cortex cells occurred.

This thesis, based on meteorological information, proposes that the centre of origin of *Nematoceras* is likely to be Papua New Guinea, with on-going dispersal being direct or from Australia to New Zealand. Wind vectors and *Nematoceras* adaptations to seed dispersal, both local and long distance, were investigated and tend to support this hypothesis.

Germination of all known orchid seed requires an obligate mycoheterotroph, generally a member of the Basidiomyceteae. For the first time, CLSM has imaged the fungal endophyte within the seed embryo and this was identified by molecular techniques and found to be a unique strain of *T. calospora*.

Three methods of orchid seed germination were trialled: symbiotic, asymbiotic and field envelopes. After a 12-month period, only field envelopes produced germinating seeds of *N. iridescens*. In all other methods the seed failed to germinate.

All *Nematoceras* spp. are solitary leaved and classified as moist mesophytes. Leaves were found to be hypostomatous. Being single leaved, protection is essential and a number of adaptations to counter herbivoury were found: raphide crystals, wax cuticle, winter maturity with summer–autumn aestivation all provide an antiherbivoury component.

The relationship between the genera of *Nematoceras* plus *Singularybas* and the associated species, of the far more ancient Hepatophyte order of Metzgeriales, were investigated. The genus *Aneura* contains peloton like vesicles of various strains of *T. calospora*. The majority of the Hepatophytes have a parallel geographic-ecological requirement. The *T. calospora* mycorrhiza of the Metzgeriale genus *Aneura* was found to have a close association with the Corybas alliance observed.

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Glossary and Abbreviations used

Δ?	Provisionally identified / diagnosed
Abaxial	Ventral aspect of a leaf, the underside
Acicular	A needle like form, especially in crystals
Acotyledonous	Without seed cotyledons
Adaxial	Dorsal aspect of a leaf, top side
Aeolian	Borne on the wind, (Aeolus, the Greek wind god)
Aestivate	A period of dormancy / quiescence
AFLPs	Amplified Fragment Length Polymorphism
Agenous	Ontogenetic pathway creating stomata without subsidiary stomatal development. An unequal mitosis occurring in protodermal cells, leading to the development of meristemoids.
Amphistomatous	Stomata found on both sides of the leaf (Generally a higher proportion are found on the Abaxial side of the leaf)
Anamorph	Describes the fungus when reproducing asexually
Anemochory	The role of wind in seed dispersal
Anomycytic	No subsidiary cells surround the guard cells.
Anomycytic stomatal complex.	Mature guard cells surrounded by epidermal cells that do not deviate morphologically from other epidermal cells in the same plant.
Anthesis	A period in which a flower is fully open and functional.
Apiculus	A long sharp pointed tip
Apomorphic	A derived type, a more contemporary state character
ASL	Above sea level
AWCGS	Allan Wilson Centre Genome Services
Biseriate	Two cell layers in depth.
CA	Corybas alliance
CLSM,	Confocal laser scanning microscopy.
Connate	Joined together, a union between two similar parts
cpDNA	Chloroplast DNA
Crustose.	Crust like, having an epidermal surface characteristic.
Cyclocytic pattern	More than four subsidiary cells surrounding a central pair of guard cells.
Cyclocytic stomatal complex.	A pattern of subsidiary cells that occur when a greater number of similar subsidiary cells radiate out from the mature guard cells outer circumference.
Defensin	Small, basic peptides that can inhibit the growth of a broad range of pathogenic fungi but seem nontoxic to either mammalian or plant cells.
Dematiaceous	Having a dark colour, usually olive, grey, or black.
Dermatogen	External cuticle of plants in a forming condition.
Diacytic	Only two subsidiary cells surrounding the stomata.
Diacytic stomatal complex	Mature guard cells surrounded by a pair of subsidiary cells with their shared wall at right angles to the long axis of the guard cells
Disjunct	A plant species that is found in two or more widely separated areas with no evidence of that species being represented between the two sites
Distichous	Two leaves growing oppositely and alternately
dNTPs	Dinucleotide triphosphates

Dolipore septum	A cross wall found in Basidiomycetes and characterized by special swellings and membranes in association with the septal pore
Druse crystals	Small open topped clusters of calcium oxalate crystals
DSE's	Dark septate endophyte
Edaphic	When produced or altered by the soil
EDAX	Energy-dispersive X-ray spectroscopy
Endemic	Taxa which occur only in a specific area
Endophyte	In the context of this dissertation the term represents a fungal endophyte that has a significant part of its lifecycle residing internally and asymptotically in various plant structures and distinguishable from mycorrhiza in not possessing external hyphae or mantels.
Endovelamen	Inner tangential wall thickening of the external velamen
Ensiform	Shaped in the form of a double edged sword.
Epivelamen	External tangential wall thickening of the velamen
e-SEM	Environmental scanning electron microscope
EtOH	Ethanol
Exo III	Exonuclease III
FIM	Fungal initiating media
GA	Gibberellic acid
Gynostemium	A fused or partially fused column of stamens and pistils.
Hemimesogenous	The meristemoid divides twice to produce a second order meristemoid.
Holoepiphytes	Epiphytes having a complete life cycle on the host tree.
Holomorph	Describes the whole shape.
Horizontal transmission	Transmission of the fungus by sexual or asexual spores.
Hyaline	Transparent, clear.
Hydrophytic	Living in /on or by water.
Hypostomatous	Leaf stomata found on the abaxial surface.
Isodiametric	Length width and height are of roughly the same dimensions.
LM	Light microscopy.
Ls	Life span.
Lotus effect	Leaf surface cleaning by water droplets running off a hydrophobic waxy cuticle.
Lysigenous development	A duct in tissue, formed following lysis of cells.
Malesia	The botanical area of Indo-malaysia and Australia
Mesogene cell	A second order meristemoid.
Mesophytic	Neither too wet nor too dry an environment.
MGS	Massey Genome Service
MMN	Melin-Norkrans agar
MMNL	MMN without agar, liquid media
Mya	Million years ago
NCBI	National Centre for Biotechnology Information
Neoendemic	Representing an evolutionarily young taxon that has not had an opportunity to disperse.
NGS	Next generation sequencing
nrDNA	Nuclear ribosomal DNA
OM	Orchid mycorrhiza
Paracytic stomatal complex.	Mature guard cells surrounded by two flanking subsidiary cells.

Palaeoendemic	Representing relicts of a once broadly dispersed taxon.
Parenthosomes	Shaped like a parenthesis symbol “(“. They are bow to dome-shaped double membranes thought to be a modified part of the endoplasmic reticulum (ER), that cover the dolipore and pore channel forming the septal pore cap.
PCR	Polymerase chain reaction
PDA	Potato Dextrose Agar, Difco
Pedicel	The stalk, internode, that arises from the peduncle and terminates at the distal end of the individual flower. Fahh (1997)
Peduncle	An inflorescence stalk bearing a solitary flower in a one-flowered inflorescence
Peloton	Intracellular coils of mycorrhizal fungal hyphae found within the root cortex cells in an endosymbiotic association, Burgeff (1936).
Perigene cells	Cells that arise during stomatal development by the division of protodermal cells around the stomatal meristemoid.
Petiole	The stalk of a leaf, attaching the blade to the stem
Phorophytes	Host trees
Plasmoptysis	The physiological state of mycorrhizal fungi after the cytoplasm has been ejected. See ptyophagy.
Plesiomorphic	An original type or primitive ancestral state character
Pseudovivipary	Production of offspring by apomictic or asexual propagules such as plantlets and bulbils.
Ptyophagy	Fungal cytoplasm is inserted into the transfer cells of roots by the specialized hyphae of the mycorrhizal mutualist.
PUA	Polyunsaturated aldehyde
rDNA	Ribosomal DNA.
RAPD	Random amplified polymorphic DNA
Raphides	Needle like crystals composed of calcium oxalate
Reniform	Kidney shaped (renal)
RFLP	Restriction fragment length polymorphism
Rosanoffian crystals	Crystals are found within a sheath, bundled within a membrane or specialist cell.
Schizogenous development.	Development of a duct by division of a common middle lamella that expands to form a lacuna.
SAP	Shrimp Alkaline Phosphatase
SEM	Scanning electron microscope
SPA	Septal pore apparatus
Teleomorph	Describes the fungus when reproducing sexually.
Tetracytic pattern	Four, roughly equal sized, subsidiary cells surround the stomatal guard cells.
Tolypophagy	Intracellular aggregates of coiled fungal hyphae that have been isolated by root or rhizoid cells prior to absorption or excretion.
TS	Transverse section
Uniseriate	One cell layer depth.
Wewelite	Calcium oxalate monohydrate crystal
Xeromorphic	Morphologically adapted for dry conditions